

# **REGISTRATION REPORT**

## **Part B**

### **Section 9**

#### **Ecotoxicology**

Detailed summary of the risk assessment

Product name(s): **INTUITY PLUS**

**(Mandestrobin 40 SC)**

Chemical active substance:

Mandestrobin, 400 g/L

Central Zone

Zonal Rapporteur Member State: Poland

**CORE ASSESSMENT**

(authorization)

Applicant: XXXX

Submission date: February 2024

MS Finalisation date: August 2025

## Version history

When	What
February 2024	Article 33 submission – Initial Applicant’s version
May 2024	- Update of the cover page with the product trade name ‘Intuity Plus’. Mandestrobin 40 SC is the internal unique name. The internal name Mandestrobin 40 SC is the one used across the dRR content. - Update of Appendix 1: studies source and owner updated
October 2024	Response to zRMS request to update aquatic risk assessment
May 2025	Response to comments of cMS AT and HU
August 2025	Final dRR by zRMS

## Table of Contents

<b>9</b>	<b>Ecotoxicology (KCP 10).....</b>	<b>6</b>
9.1	Critical GAP and overall conclusions.....	6
9.1.1	Overall conclusions.....	8
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).....	8
9.1.1.2	Effects on aquatic organisms (KCP 10.2).....	8
9.1.1.3	Effects on bees (KCP 10.3.1).....	8
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2) .....	8
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5) .....	8
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6) .....	9
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7) .....	9
9.1.2	Grouping of intended uses for risk assessment.....	9
9.1.3	Consideration of metabolites .....	10
9.2	Effects on birds (KCP 10.1.1).....	11
9.2.1	Toxicity data .....	12
9.2.1.1	Justification for new endpoints .....	12
9.2.2	Risk assessment for spray applications.....	12
9.2.2.1	Screening assessment (indicator species) .....	13
9.2.2.2	Higher-tier risk assessment.....	13
9.2.2.3	Drinking water exposure.....	13
9.2.2.4	Effects of secondary poisoning.....	14
9.2.2.5	Biomagnification in terrestrial food chains.....	15
9.2.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	15
9.2.4	Overall conclusions.....	15
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	16
9.3.1	Toxicity data .....	16
9.3.1.1	Justification for new endpoints .....	17
9.3.2	Risk assessment for spray applications.....	17
9.3.2.1	Screening assessment (indicator species) .....	17
9.3.2.2	Higher-tier risk assessment.....	17
9.3.2.3	Drinking water exposure.....	18
9.3.2.4	Effects of secondary poisoning.....	18
9.3.2.5	Biomagnification in terrestrial food chains.....	20
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	20
9.3.4	Overall conclusions.....	20
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3) .....	20
9.5	Effects on aquatic organisms (KCP 10.2).....	20
9.5.1	Toxicity data .....	22
9.5.1.1	Justification for new endpoints .....	27
9.5.2	Risk assessment .....	29
9.5.3	Overall conclusions.....	42
9.6	Effects on bees (KCP 10.3.1).....	43
9.6.1	Toxicity data .....	44
9.6.1.1	Justification for new endpoints .....	46

9.6.2	Risk assessment .....	46
9.6.2.1	Hazard quotients for bees.....	46
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies).....	49
9.6.3	Effects on bumble bees .....	49
9.6.4	Effects on solitary bees .....	49
9.6.5	Overall conclusions.....	50
9.7	Effects on arthropods other than bees (KCP 10.3.2) .....	50
9.7.1	Toxicity data .....	51
9.7.1.1	Justification for new endpoints .....	51
9.7.2	Risk assessment .....	52
9.7.2.1	Risk assessment for in-field exposure.....	52
9.7.2.2	Risk assessment for off-field exposure .....	52
9.7.2.3	Additional higher-tier risk assessment.....	53
9.7.2.4	Risk mitigation measures .....	53
9.7.3	Overall conclusions.....	53
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4) .....	53
9.8.1	Toxicity data .....	54
9.8.1.1	Justification for new endpoints .....	55
9.8.2	Risk assessment .....	55
9.8.2.1	First-tier risk assessment.....	56
9.8.2.2	Higher-tier risk assessment.....	56
9.8.3	Overall conclusions.....	56
9.9	Effects on soil microbial activity (KCP 10.5).....	56
9.9.1	Toxicity data .....	57
9.9.1.1	Justification for new endpoints .....	57
9.9.2	Risk assessment .....	57
9.9.3	Overall conclusions.....	58
9.10	Effects on non-target terrestrial plants (KCP 10.6) .....	58
9.10.1	Toxicity data .....	59
9.10.1.1	Justification for new endpoints .....	59
9.10.2	Risk assessment .....	59
9.10.2.1	Tier-1 risk assessment (based screening data) .....	59
9.10.2.2	Tier-2 risk assessment (based on dose-response data).....	60
9.10.2.3	Higher-tier risk assessment.....	60
9.10.2.4	Risk mitigation measures .....	60
9.10.3	Overall conclusions.....	60
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7) .....	60
9.12	Monitoring data (KCP 10.8) .....	61
9.13	Classification and Labelling .....	62
<b>Appendix 1</b>	<b>Lists of data considered in support of the evaluation .....</b>	<b>63</b>
<b>Appendix 2</b>	<b>Detailed evaluation of the new studies .....</b>	<b>72</b>
A 2.1	KCP 10.1 Effects on birds and other terrestrial vertebrates.....	72
A 2.1.1	KCP 10.1.1 Effects on birds .....	72
A 2.1.2	KCP 10.1.2 Effects on terrestrial vertebrates other than birds .....	72
A 2.1.3	KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians).....	72
A 2.2	KCP 10.2 Effects on aquatic organisms .....	73

---

A 2.2.1	KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes .....	73
A 2.2.2	KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms.....	164
A 2.2.3	KCP 10.2.3 Further testing on aquatic organisms .....	164
A 2.3	KCP 10.3 Effects on arthropods .....	164
A 2.3.1	KCP 10.3.1 Effects on bees .....	164
A 2.4	KCP 10.3.2 Effects on non-target arthropods other than bees.....	198
A 2.4.1	KCP 10.3.2.1 Standard laboratory testing for non-target arthropods .....	198
A 2.5	KCP 10.4 Effects on non-target soil meso- and macrofauna.....	208
A 2.5.1	KCP 10.4.1 Earthworms .....	208
A 2.5.2	KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms) .....	212
A 2.6	KCP 10.5 Effects on soil nitrogen transformation.....	213
A 2.7	KCP 10.6 Effects on terrestrial non-target higher plants.....	213
A 2.7.1	KCP 10.6.1 Summary of screening data .....	213
A 2.7.2	KCP 10.6.2 Testing on non-target plants.....	213
A 2.7.3	KCP 10.6.3 Extended laboratory studies on non-target plants .....	213
A 2.8	KCP 10.7 Effects on other terrestrial organisms (flora and fauna).....	213
A 2.9	KCP 10.8 Monitoring data.....	218

## 9 Ecotoxicology (KCP 10)

### 9.1 Critical GAP and overall conclusions

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

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ 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	Soil organisms	Non-target plants
					Zonal uses (field or outdoor uses, certain types of protected crops)															
1	AT, HU, RO, DE, NL, PL, CZ, SK, SI	Winter and spring oilseed rape	F	<i>Sclerotinia sclerotiorum</i>	Foliar	BBCH 60 – 69	a) 1 b) 1	-	a) 0.5 L/ha b) 0.5 L/ha	a) 200 g a.s./ha b) 200 g a.s./ha	100 – 300	-	The PHI is covered by the time remaining between application and harvest							

\* 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

Comments of zRMS:	The risk is unacceptable if formulation is applied in winter and spring OSR on acidic soils (pH ≤ 5.9). The risk is acceptable if formulation is applied in winter and spring OSR on neutral and basic soils (pH ≥ 7.2) if mitigation measures are applied.
-------------------	--

**Remarks table:**

- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>(1) Numeration necessary to allow references</li> <li>(2) Use official codes/nomenclatures of EU</li> <li>(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (<i>e.g.</i> fumigation of a structure)</li> <li>(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</li> <li>(5) Scientific names <u>and</u> 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</li> <li>(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench<br/>Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated</li> </ul> | <ul style="list-style-type: none"> <li>(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</li> <li>(8) The maximum number of application possible under practical conditions of use must be provided</li> <li>(9) Minimum interval (in days) between applications of the same product.</li> <li>(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</li> <li>(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).</li> <li>(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under "application: method/kind".</li> <li>(13) PHI - minimum pre-harvest interval</li> <li>(14) Remarks may include: Extent of use/economic importance/restrictions</li> </ul> |
|---|---|

## **9.1.1 Overall conclusions**

### **9.1.1.1**



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

An acceptable acute and long-term (reproductive) dietary risk to birds and mammals is concluded based on screening level assessments. Furthermore, an acceptable risk to birds and mammals from exposure via contaminated drinking water is concluded.

An assessment of the risk of secondary poisoning to earthworm-eating birds and mammals was required for the active substance mandestrobin, and an assessment of the risk to fish-eating birds and mammals was required for mandestrobin and metabolites S-2200-OR and S-2200-ORC. An acceptable risk to earthworm- and fish-eating birds and mammals is demonstrated for mandestrobin and both metabolites.

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

For the active substance mandestrobin, an acceptable risk to aquatic organisms is demonstrated for uses in both winter and spring oilseed rape based on FOCUS Step 3 PEC<sub>sw</sub> modelling for the worst-case uses in acidic soils and, in a comprehensive approach, also for basic soils which result in lower exposure levels.

For the metabolites, an acceptable risk for uses in both winter and spring oilseed rape could be demonstrated based on FOCUS Step 1 and FOCUS Step 2 PEC<sub>sw</sub> modelling.

In addition, a risk assessment based on PEC<sub>sw</sub> values calculated for the formulation arising from the drift loading into surface water indicated an acceptable risk.

Therefore, overall an acceptable risk to aquatic organisms is demonstrated without a requirement for risk mitigation.

#### **9.1.1.5 For the active substance mandestrobin, an acceptable risk to aquatic organisms is demonstrated for uses in both winter and spring oilseed rape based on FOCUS Step 3 PEC<sub>sw</sub> modelling for the worst-case uses in acidic soils and, in a comprehensive approach, also for basic soils, which result in lower exposure levels. This conclusion is based on the lowest Tier 1 RAC which is for the acute risk to aquatic invertebrates.**

For the metabolites, an acceptable risk for uses in both winter and spring oilseed rape could be demonstrated based on FOCUS Step 1 and FOCUS Step 2 PEC<sub>sw</sub> modelling.

In addition, a risk assessment based on PEC<sub>sw</sub> values calculated for the formulation arising from the drift loading into surface water indicated an acceptable risk.

Therefore, overall an acceptable risk to aquatic organisms is demonstrated without a requirement for risk mitigation.

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

Overall, an acceptable risk to honeybees is demonstrated based on a SANCO risk assessment (acute risk to adults) and a risk assessment based on the EFSA (2013) scheme (acute and chronic risk to adults and chronic risk to larvae).

In addition, acute oral and contact toxicity data on bumblebees are submitted, which do not indicate that bumblebees are any more sensitive to mandestrobin than honeybees.

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

A first-tier risk assessment demonstrates an acceptable in-field and off-field risk to non-target arthropods for the proposed use of Mandestrobin 40SC in oilseed rape with no requirement for risk mitigation.

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

An acceptable risk to non-target soil meso- and macrofauna and soil microorganisms is demonstrated for the proposed use of Mandestrobin 40SC in oilseed rape.

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

Vegetative vigour and seedling emergence limit tests with the formulation S-2200 25 SC indicated no effects greater than 50% at 200 g a.s./ha. As this rate corresponds to the maximum proposed application rate of 200 g a.s./ha, an acceptable risk to non-target terrestrial plants for the proposed use of Mandestrobin 40SC is concluded.

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

Not required.

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

The following table documents the intended use of Mandestrobin 40SC.

**Table 9.1-2: Critical use pattern of Mandestrobin 40SC**

<b>Crop</b>	<b>Growth stage</b>	<b>Maximum no. of applications</b>	<b>Maximum rate per application</b>
Winter and spring oilseed rape	BBCH 60 – 69	1	0.5 L product/ha (200 g a.s./ha)

### 9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below in Table 9.1-3. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of Mandestrobin 40SC is indicated in the table.

**Table 9.1-3 Metabolites of mandestrobin**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
2-COOH-S-2200	( <i>RS</i> )-2-{2-[1-methoxy-1-( <i>N</i> -methylcarbamoyl)methyl]benzyloxy}-4-methylbenzoic acid	343.38	8.7% soil	Yes (surface water and soil)
5-COOH-S-2200	( <i>RS</i> )-3-{2-[1-methoxy-1-( <i>N</i> -methylcarbamoyl)methyl]benzyloxy}-4-methylbenzoic acid	343.38	18.0% soil, 3.6% water	Yes (surface water and soil, exposure via soil)
S-2200-OR	( <i>RS</i> )-2-[2-(2-hydroxy-3,6-dimethylbenzyl)phenyl]-2-methoxy- <i>N</i> -methylacetamide	313.39	20.7% water	Yes (surface water)
S-2200-ORC	( <i>RS</i> )- <i>N</i> ,1,4-trimethyl-6,11-dihydrodibenzo[ <i>b,e</i> ]oxepine-6-carboxamide	281.35	13.7% water	Yes (surface water)
DX-CA-S-2200	( <i>RS</i> )-2-( <i>N</i> -methylcarbamoyl-methoxymethyl) benzoic acid	223.2	8.3 % soil	Yes (soil and surface water)

## 9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The risk assessment of the acute and long-term risk for birds due to the use of Mandestrobin 40 SC formulation was submitted.					
	The risk assessment was conducted in accordance with Birds and Mammals guidance, 2009.					
	<b>Mandestrobin.</b> The acute risk assessment should be conducted with the lowest endpoint derived from a short-term dietary test (bobwhite quail), a LD <sub>50</sub> > 1136 mg a.s./kg bw/d.					
	<b>Intended use</b>		Oilseed rape			
	<b>Active substance</b>		Mandestrobin			
	<b>Application rate (g a.s./ha)</b>		1 × 200			
	<b>Acute toxicity (mg/kg bw)</b>		> 1136			
	<b>TER criterion</b>		10			
	<b>Crop scenario</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>
	Oilseed rape	Small omnivorous bird	158.8	1.0	31.76	> 36
	TER <sub>A</sub> value for small omnivorous birds is above the trigger value of 10 at screening step indicating an acceptable acute risk for birds.					

The TER <sub>LT</sub> values for long-term risk are above the trigger value of 5 at screening step assessment indicating an acceptable long-term risk for birds.					
No further refinement is required.					
The puddle scenario was used in bird exposure assessment. The submitted assessment was accepted.					
Secondary poisoning. The risk assessment for earthworm-eating birds and fish-eating birds was accepted.					
No relevant metabolite was considered; the justification was accepted.					
The risk to birds following application of Mandestrobin 40 SC formulation in accordance with the proposed pattern use is acceptable.					

## 9.2.1 Toxicity data

Avian toxicity studies have been carried out with mandestrobin. Full details of these studies are provided in the respective EU DAR and related documents. No additional data have been submitted.

Effects of the formulation on birds were not evaluated as part of the EU assessments of the active substance mandestrobin. However, the provision of further data on the formulation Mandestrobin 40SC is not considered essential, because the risk for terrestrial vertebrates other than birds is adequately addressed based on the data for the active substance.

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

Species	Substance	Exposure system	Results	Reference
Bobwhite quail ( <i>Colinus virginianus</i> )	Mandestrobin	Acute, oral (1-day)	<b>LD<sub>50</sub></b> <b>&gt; 2250 mg a.s./kg bw</b>	EFSA Conclusion (2015)
Bobwhite quail ( <i>Colinus virginianus</i> )	Mandestrobin	Short-term, dietary (5-day)	LDD <sub>50</sub> > 1136 mg a.s./kg bw/d	EFSA Conclusion (2015)
Mallard duck ( <i>Anas platyrhynchos</i> )	Mandestrobin	Short-term, dietary (5-day)	LDD <sub>50</sub> > 2460 mg a.s./kg bw/d	EFSA Conclusion (2015)
Bobwhite quail ( <i>Colinus virginianus</i> )	Mandestrobin	Long-term, reproduction (21 weeks)	<b>NOEL</b> <b>= 91.1 mg a.s./kg bw/d</b>	EFSA Conclusion (2015)
Mallard duck ( <i>Anas platyrhynchos</i> )	Mandestrobin	Long-term, reproduction (20 weeks)	NOEL = 129 mg a.s./kg bw/d	EFSA Conclusion (2015)

Note: endpoints shown in **bold** are used in the risk assessment.

### 9.2.1.1 Justification for new endpoints

The EU agreed endpoint for use in the acute risk assessment is the LDD<sub>50</sub> of > 1136 mg a.s./kg bw/day from the short-term dietary study. However, the risk assessment scheme does not routinely use input from short-term dietary studies (i.e. the LDD<sub>50</sub>) and testing is obsolete according to Commission Regulation (EC) 283/2013 unless there are indications that toxicity from dietary exposure is increased. Likewise, the Bird and Mammal Guidance Document (EFSA, 2009) indicates that the endpoint from this study should only be used if the results indicate that the dietary endpoint is lower than the LD<sub>50</sub> based on the acute oral study. For mandestrobin, the dietary endpoint for bobwhite quail (> 1136 mg a.s./kg bw/day) is formally lower than the acute oral endpoint (> 2250 mg a.s./kg bw). However, in both studies the endpoint is unbound (i.e. greater than the highest dose tested). Furthermore, no treatment related mortalities or sub-lethal effects (clinical signs) were noted in either of the studies. Therefore, as there is no indication that the dietary toxicity is greater than the acute oral toxicity, the acute oral LD<sub>50</sub> of > 2250 mg a.s./kg bw has been used in the following acute risk assessment.

Acute oral testing of the formulated product on birds is not considered necessary, as there is no indication of an increased toxicity compared to the active substance, based on data obtained from mammalian testing (LD<sub>50</sub> > 2000 mg product/kg bw) available for the similar formulated product S-2200 25 SC. The acute oral LD<sub>50</sub> derived from the active substance testing has therefore been used in the following acute risk assessment.

## 9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for

Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

### 9.2.2.1 Screening assessment (indicator species)

The results of the acute and reproductive dietary screening assessments are summarised in the following table.

**Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Mandestrobin 40SC in oilseed rape**

<b>Intended use</b>	Oilseed rape				
<b>Active substance</b>	Mandestrobin				
<b>Application rate (g a.s./ha)</b>	1 × 200				
<b>Acute toxicity (mg/kg bw)</b>	≥ 2250				
<b>TER criterion</b>	10				
<b>Crop scenario</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>
Oilseed rape	Small omnivorous bird	158.8	1.0	31.76	≥ 71
<b>Reprod. toxicity (mg/kg bw/d)</b>	91.1				
<b>TER criterion</b>	5				
<b>Crop scenario</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>
Oilseed rape	Small omnivorous bird	64.8	1.0 × 0.53	6.87	13.3

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.

Based on a screening level assessment, an acceptable acute and long-term (reproductive) risk is demonstrated for the proposed use of Mandestrobin 40SC on oilseed rape.

### 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 Mandestrobin 40SC 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 an arithmetic mean  $K(f)_{oc}$  of 449 L/kg, mandestrobin belongs to the group of less sorptive substances. The ratios presented below demonstrate an acceptable acute and long-term (reproductive) risk to birds from the consumption of contaminated drinking water following the proposed use of Mandestrobin 40SC in oilseed rape.

Effective application rate (g/ha) =	200		Trigger = 50
Acute toxicity (mg/kg bw) =	<del>&gt; 2250</del> > 1136	quotient =	<del>&lt; 0.09</del> < 0.18
Reprod. toxicity (mg/kg bw/d) =	91.1	quotient =	2.20

#### 9.2.2.4 Effects of secondary poisoning

The log  $P_{ow}$  of mandestrobin is 3.51 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

The relevant metabolites in soil and water were determined to be 2-COOH-S-2200, 5-COOH-S-2200, DX-CA-S-2200, S-2200-OR (water only) and S-2200-ORC (water only) with an estimated (according to KOWWIN, v.1.68 model) log  $P_{ow}$  of 2.53, 2.88, 0.2, 3.30 and 4.02, respectively. Hence, the potential of the metabolites to bioaccumulate in the food chain is considered to be low for the metabolites 2-COOH-S-2200, 5-COOH-S-2200, and DX-CA-S-2200, with log  $P_{ow}$  values less than 3. However, the potential of the metabolites S-2200-OR and S-2200-ORC to have an effect on fish-eating birds has to be addressed as the estimated log  $P_{ow}$  values are greater than 3. As no avian toxicity studies with the metabolites are available, as a conservative approach they are assumed to be 10 times more toxic than the parent compound.

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

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

**Table 9.2-3: Assessment of the risk for earthworm-eating birds due to exposure to Mandestrobin 40SC via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape**

Parameter	Mandestrobin	Comments
$PEC_{soil}$ (mg/kg soil)	0.069	$PEC_{accumulation}$ ( $PEC_{act} + PEC_{soil\ plateau}$ ) See Part B8 Section 8.7
$P_{ow}$	3236	
$K_{oc}$	449	Arithmetic mean
$f_{oc}$	0.02	Default
$BCF_{worm}$	4.42	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
$PEC_{worm}$	0.30	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.32	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	91.1	Table 9.2-1
$TER_{lt}$	285	Trigger = 5

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

The above risk assessment demonstrates an acceptable risk to earthworm-eating birds for the use of Mandestrobin 40SC in oilseed rape.

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

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

Since no BCF values are available for metabolites S-2200-OR and S-2200-ORC, they are assumed to be 10 times higher than for the parent compound as a conservative approach, as presented in the DAR of mandestrobin (part B.9.1.8.5.2).

**Table 9.2-4: Assessment of the risk for fish-eating birds due to exposure to mandestrobin and its metabolites S-2200-OR and S-2200-ORC via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape**

Parameter	Mandestrobin	S-2200-OR	S-2200-ORC	Comments
PEC <sub>sw</sub> (mg/L)	0.00543	0.00168	0.001	Maximum Step 2 PEC <sub>sw</sub> See Part B8 Section 8.9
BCF <sub>fish</sub>	26	260 <sup>a)</sup>	260 <sup>a)</sup>	Mandestrobin DAR Final (B.9.1.8.5.2)
PEC <sub>fish</sub>	0.141	0.437	0.260	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.022	0.069	0.041	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d)	91.1	9.1 <sup>b)</sup>	9.1 <sup>b)</sup>	Table 9.2-1
TER <sub>it</sub>	4058	131	220	Trigger = 5

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

<sup>a)</sup> BCF for the metabolites is assumed to be 10 times higher than for the parent compound.

<sup>b)</sup> Assumed to be 10 times more toxic than the parent compound.

The above risk assessment demonstrates an acceptable risk to fish-eating birds for the use of Mandestrobin 40SC in oilseed rape.

### 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

Overall, the risk to birds from the proposed use of Mandestrobin 40SC in oilseed rape is concluded to be acceptable.



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

zRMS Comments:	<p>The submitted risk assessment of the acute and long-term risk for birds due to the use of Mandestrobin 40 SC formulation was accepted.</p> <p>The used endpoints were agreed at the EU level.</p> <p>The risk assessment was conducted in accordance with Birds and Mammals guidance, 2009.</p> <p><b>Mandestrobin.</b> The submitted acute and long-term risk assessment for mammals was accepted.</p> <p>TER<sub>A</sub> value for small herbivorous mammals is above the trigger value of 10 at screening step indicating an acceptable acute risk for mammals.</p> <p>The TER<sub>LT</sub> values for long-term risk are above the trigger value of 5 at screening step assessment indicating an acceptable long-term risk for mammals.</p> <p>No further refinement is required.</p> <p>The puddle scenario was used in bird exposure assessment. The submitted assessment was accepted.</p> <p>Secondary poisoning. The risk assessment based on risk envelope approach for earthworm-eating <b>birds mammals</b> and fish-eating <b>birds mammals</b> was accepted.</p> <p>No relevant metabolite was considered; the justification was accepted.</p> <p>The risk to birds following application of <b>PRO-HER-300-EC Mandestrobin 40SC</b> formulation in accordance with the proposed pattern use is acceptable.</p>
-------------------	--

#### 9.3.1 Toxicity data

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

No toxicological studies on the formulation Mandestrobin 40SC are submitted. Effects on mammals of the formulation S-2200 25 SC were assessed as part of the EU evaluation of mandestrobin. In the acute oral study with S-2200 25 SC, there was no indication of greater toxicity compared to the acute oral study performed with the active substance. Based on this data, no mammalian studies with Mandestrobin 40SC are considered necessary.

The selection of studies and endpoints for the risk assessment is therefore 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	Mandestrobin	Acute oral	<b>LD<sub>50</sub></b> <b>&gt; 2000 mg a.s./kg bw</b>	EFSA Conclusion (2015)
Rat	S-2200 25 SC <sup>a)</sup>	Acute oral	LD <sub>50</sub> > 2000 mg product/kg bw (> 499.2 mg a.s./kg bw)	EFSA Conclusion (2015)
Rat	Mandestrobin	Reproduction	<b>NOAEL</b>	EFSA Conclusion

Species	Substance	Exposure system	Results	Reference
		(2-generation)	= 3000 mg/kg feed <b>(166.3 mg a.s./kg bw/d)</b>	(2015)

Note: endpoints shown in **bold** are used in the risk assessment.

<sup>a)</sup> Content of 24.96% active ingredient specified in the study report; the product density is 1.054 g/mL.

### 9.3.1.1 Justification for new endpoints

Not applicable, as there are no new endpoints.

### 9.3.2 Risk assessment for spray applications

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

#### 9.3.2.1 Screening assessment (indicator species)

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

**Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Mandestrobin 40SC in oilseed rape**

Intended use		Oilseed rape				
Active substance		Mandestrobin				
Application rate (g a.s./ha)		1 × 200				
Acute toxicity (mg/kg bw)		> 2000				
TER criterion		10				
Crop scenario	Indicator species		SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Oilseed rape	Small herbivorous mammal		118.4	1.0	23.68	> 85
Reprod. toxicity (mg/kg bw/d)		166.3				
TER criterion		5				
Crop scenario	Indicator species		SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Oilseed rape	Small herbivorous mammal		48.3	1.0 x 0.53	5.12	33

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.

Based on a screening level assessment, an acceptable acute and long-term (reproductive) risk to mammals is demonstrated for the proposed use of Mandestrobin 40SC in oilseed rape.

#### 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 an arithmetic mean  $K(f)_{oc}$  of 449 L/kg, mandestrobin belongs to the group of less sorptive substances. The ratios presented below demonstrate an acceptable acute and long-term (reproductive) risk to mammals from the consumption of contaminated drinking water following the proposed use of Mandestrobin 40SC in oilseed rape.

Effective application rate (g/ha) =	200		Trigger = 50
Acute toxicity (mg/kg bw) =	> 2000	quotient =	< 0.10
Reprod. toxicity (mg/kg bw/d) =	166.3	quotient =	1.20

### 9.3.2.4 Effects of secondary poisoning

The log  $P_{ow}$  of mandestrobin is 3.51 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

The relevant metabolites in soil and water were determined to be 2-COOH-S-2200, 5-COOH-S-2200, DX-CA-S-2200, S-2200-OR (water only) and S-2200-ORC (water only) with an estimated (according to KOWWIN, v.1.68 model) log  $P_{ow}$  of 2.53, 2.88, 0.2, 3.30 and 4.02, respectively. Hence, the potential of the metabolites to bioaccumulate in the food chain is considered to be low for the soil metabolites 2-COOH-S-2200, 5-COOH-S-2200 and DX-CA-S-2200, with log  $P_{ow}$  values less than 3. However, the potential of the metabolites S-2200-OR and S-2200-ORC to have an effect on fish-eating mammals has to be addressed as the log  $P_{ow}$  values are greater than 3. As no mammalian toxicity studies with the metabolites are available, as a conservative approach they are assumed to be 10 times more toxic than the parent compound.

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

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

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

Parameter	Mandestrobin	Comments
PEC <sub>soil</sub> (mg/kg soil)	0.069	PEC <sub>accumulation</sub> (PEC <sub>act</sub> + PEC <sub>soil plateau</sub> ) See Part B8 Section 8.7
P <sub>ow</sub>	3236	
K <sub>oc</sub>	449	Arithmetic mean
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	4.42	$BCF_{worm/soil} = (PEC_{worm,ww} / PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.30	PEC <sub>worm</sub> = PEC <sub>soil</sub> × BCF <sub>worm/soil</sub>
Daily dietary dose (mg/kg bw/d)	0.39	DDD = PEC <sub>worm</sub> × 1.28
NOEL (mg/kg bw/d)	166.3	Table 9.3-1
TER <sub>lt</sub>	426	Trigger = 5

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

The above risk assessment demonstrates an acceptable risk to earthworm-eating mammals for the use of Mandestrobin 40SC in oilseed rape.

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

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

Since no BCF values are available for metabolites S-2200-OR and S-2200-ORC, they are assumed to be 10 times higher than for the parent compound as a conservative approach, as presented in the DAR of mandestrobin (part B.9.1.8.5.2)

**Table 9.3-4: Assessment of the risk for fish-eating mammals due to exposure to mandestrobin and its metabolites S-2200-OR and S-2200-ORC via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape**

Parameter	Mandestrobin	S-2200-OR	S-2200-ORC	Comments
PEC <sub>sw</sub> (mg/L)	0.00543	0.00168	0.001	Maximum Step 2 PEC <sub>sw</sub> See Part B8 Section 8.9
BCF <sub>fish</sub>	26	260 <sup>a)</sup>	260 <sup>a)</sup>	Mandestrobin DAR Final (B.9.1.8.5.2)
PEC <sub>fish</sub>	0.141	0.437	0.260	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.020	0.062	0.037	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d)	166.3	16.63 <sup>b)</sup>	16.63 <sup>b)</sup>	Table 9.3-1
TER <sub>lt</sub>	8295	268	450	Trigger = 5

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

<sup>a)</sup> BCF for the metabolites is assumed to be 10 times higher than for the parent compound.

<sup>b)</sup> Assumed to be 10 times more toxic than the parent compound.

The above risk assessment demonstrates an acceptable risk to fish-eating mammals for the use of Mandestrobin 40SC in oilseed rape.

### 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

Overall, an acceptable acute and long-term risk to mammals is concluded for the proposed use of Mandestrobin 40SC in oilseed rape.

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

No additional data are submitted.

## 9.5 Effects on aquatic organisms (KCP 10.2)

zRMS

Comments:

The risk assessment for aquatic organisms was submitted and accepted.

For risk assessment the PEC<sub>sw</sub> and PEC<sub>sed</sub> values evaluated in Section 8 were taken into consideration.

The D1, D2 and D6 scenarios were not considered as they are not relevant for Central Zone.

**Mandestrobin**, New studies performed with active substance and/or with metabolites were submitted. ~~not considered in the risk assessment for aquatic organisms. All these studies should be submitted and evaluated at the EU level during active substance renewal~~

In accordance with List of Endpoints (EFSA Conclusion, 2015) in the chronic risk assessment for aquatic invertebrates the NOEC endpoint of 0.0056 mg a.s./L should be used. This value was also accepted at zonal assessment.

The risk assessment was corrected and presented below:

**Winter OSR: 20 m VFS + 20 m NSS**  
RAC = 0.56 µg/L

Scenario	PEC <sub>sw</sub> [µg/L]	PEC/RAC	PEC <sub>sw</sub> [µg/L]	PEC/RAC
	Basic condition		Acidic condition	
D3 Ditch	0.095	0.17	0.095	0.17
D4 Pond	0.304	0.54	0.932	<b>1.66</b>
D4 Stream	0.307	0.55	0.913	<b>1.63</b>

D5 Pond	0.092	0.16	0.462	0.83
D5 Stream	0.125	0.22	0.503	0.90
R1 Pond	0.054	0.10	0.055	0.10
R1 Stream	0.427	0.76	0.435	0.78
R3 stream	0.351	0.63	0.385	0.69

**The risk is unacceptable if formulation is applied in winter OSR on acidic soils.**

**Spring OSR: 20 m VFS + 20 m NSS**

RAC = 0.56 µg/L

Scenario	PEC <sub>sw</sub> [µg/L]	PEC/RAC	PEC <sub>sw</sub> [µg/L]	PEC/RAC
	<b>Basic condition</b>		<b>Acidic condition</b>	
D3 Ditch	0.095	0.17	0.095	0.17
D4 Pond	0.366	0.65	1.012	<b>1.81</b>
D4 Stream	0.368	0.66	0.993	<b>1.77</b>
D5 Pond	0.214	0.38	0.609	<b>1.09</b>
D5 Stream	0.257	0.46	0.759	<b>1.36</b>
R1 Pond	0.073	0.13	0.077	0.14
R1 Stream	0.452	0.81	0.458	0.82

**The risk is unacceptable if formulation is applied in spring OSR on acidic soils (pH ≤ 5.9).**

The Applicant has submitted new chronic studies for aquatic invertebrates considering the justification of use of new endpoint for chronic risk for aquatic invertebrates. As all studies/test were carried out on the active substance and were evaluated in this registration report (necessary for safe use in acidic soils).

The new endpoints HC<sub>5</sub> were derived for NOEC and EC<sub>10</sub> values of 0.060 mg a.s/L and 0.056 mg a.s./ha, respectively, Using the AF=3 the SSD-RAC were calculated: 0.020 mg a.s/L and 0.019 mg a.s./ha, respectively. The lower value was used in higher tier risk assessment (Tier 2B).

The Applicant's approach was accepted.

Based on submitted refined risk assessment (Tables 9.5-5 to 9.5-7) it can be concluded that no mitigation measures are required for all soils (neutral, alkaline and acidic).

The risk assessment for the aquatic insect *Chironomus riparius* should be provided as the endpoint of NOEC is available in the List of Endpoints (EFSA Conclusion, 2015). As the Applicant has taken the lower endpoint for freshwater amphipod *Hyaella Azteca*, the risk assessment is accepted as the more conservative approach was used.

Based on PEC/RAC ratio, the risk for aquatic organisms is acceptable if formulation is applied on neutral and basic soils (pH ≥ 7.2) and following mitigation measures are applied:

20 m VFS + 20 m NSS

#### **Metabolites of Mandestrobin.**

The metabolites of active substance were taken into consideration. The submitted risk assessment is based on PEC<sub>sw</sub> i PEC<sub>sd</sub> values reported in Section 8.

The risk assessment for metabolites was accepted.

Metabolites pose an acceptable risk.

**Formulation Mandestrobin 40 SC.**

	<p>The submitted risk considering the drift exposure for aquatic plants and algae was corrected in accordance with PEC<sub>sw</sub> presented in Section 8.</p> <p>The PEC<sub>sw</sub> = 3.48 µg/L as the worst case (0.5 L formulation/ha) was considered in formulation risk assessment.</p> <p>No additional mitigation measure is required.</p>
--	--

### 9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with mandestrobin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. In addition, new studies on the chronic toxicity of mandestrobin to aquatic invertebrates are submitted to support the use of an SSD-RAC for this organism group. These studies are listed in Appendix 1 and summarised in Appendix 2.

The representative formulation for the EU assessment of mandestrobin was S-2200 25 SC. Therefore, the effects of Mandestrobin 40SC on aquatic organisms were not evaluated as part of the EU evaluation. New data on this formulation submitted with this application are listed in Appendix 1 and summarised in Appendix 2. These include an acute study with *Daphnia magna* and a study with the green algae *Pseudokirchneriella subcapitata*. For information purposes, available data on S-2200 25 SC are also presented in the table below.

To avoid unnecessary vertebrate testing, no acute fish studies with Mandestrobin 40SC are submitted. The results of the formulation and active substance studies with *D. magna* and *P. subcapitata* indicate that Mandestrobin 40SC does not appear to be of any greater toxicity than the technical active substance. Therefore, the available fish acute toxicity data with the active substance are considered to cover the risk of the formulation to fish. In addition, an acute fish study with the formulation S-2200 25 SC is available which was assessed as part of the EU evaluation of mandestrobin. This formulation was of no greater toxicity to fish than the technical active substance. Moreover, the available data on *D. magna* and *P. subcapitata* indicate that Mandestrobin 40SC is of comparable (or even lower) toxicity than S-2200 25 SC, which further justifies that an acute fish study with Mandestrobin 40SC is not necessary. A comparison of the available data on the active substance and two formulations is shown in Table 9.5-3.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Further justification for these deviations is provided in Section 9.5.1.1.

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

Species	Substance	Exposure system	Results	Reference
<b>Fish</b>				
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Mandestrobin	96 h acute, s	LC <sub>50</sub> = 0.94 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Fathead minnow ( <i>Pimephales promelas</i> )	Mandestrobin	96 h acute, s	LC <sub>50</sub> = 1.0 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Bluegill sunfish ( <i>Lepomis macrochirus</i> )	Mandestrobin	96 h acute, s	LC <sub>50</sub> = 2.3 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Sheepshead minnow ( <i>Cyprinodon variegatus</i> )	Mandestrobin	96 h acute, f	LC <sub>50</sub> > 2.2 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Fathead minnow ( <i>Pimephales promelas</i> )	Mandestrobin	32 d, ELS, f	NOEC = 0.15 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Sheepshead minnow ( <i>Cyprinodon variegatus</i> )	Mandestrobin	34 d, ELS, f	NOEC = 0.30 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	S-Isomer (S-2354)	96 h acute, s	LC <sub>50</sub> > 12 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	R-Isomer (S-2167)	96 h acute, s	LC <sub>50</sub> = 0.84 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	2-COOH-S-2200	96 h acute, s	LC <sub>50</sub> > 89 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	5-COOH-S-2200	96 h acute, s	LC <sub>50</sub> > 100 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	S-2200-OR	96 h acute, s	LC <sub>50</sub> > 9.0 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	S-2200-ORC	96 h acute, s	LC <sub>50</sub> = 4.0 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
Bluegill sunfish ( <i>Lepomis macrochirus</i> )	Mandestrobin	35 d BCF, f	BCF <sub>steady-state</sub> (whole fish) = 25-26	EFSA Conclusion (2015)
<b>Aquatic invertebrates</b>				
<i>Daphnia magna</i>	Mandestrobin	48 h acute, s	EC <sub>50</sub> = 1.2 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Saltwater mysid ( <i>Americamysis bahia</i> )	Mandestrobin	96 h acute, f	LC <sub>50</sub> = 0.43 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Eastern oyster ( <i>Crassostrea virginica</i> )	Mandestrobin	96 h acute, f	EC <sub>50</sub> = 2.0 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	S-Isomer (S-2354)	48 h acute, s	EC <sub>50</sub> > 14 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	R-Isomer (S-2167)	48 h acute, s	EC <sub>50</sub> = 0.92 mg/L <sub>mm</sub>	EFSA Conclusion (2015)



Species	Substance	Exposure system	Results	Reference
<i>Daphnia magna</i>	2-COOH-S-2200	48 h acute, s	EC <sub>50</sub> > 100 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	5-COOH-S-2200	48 h acute, s	EC <sub>50</sub> > 100 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	S-2200-OR	48 h acute, s	EC <sub>50</sub> > 14 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	S-2200-ORC	48 h acute, s	EC <sub>50</sub> = 9.6 mg/L <sub>mm</sub>	EFSA Conclusion (2015)
<i>Daphnia magna</i>	Mandestrobin	21 d chronic, ss	NOEC = 0.56 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Saltwater mysid ( <i>Americamysis bahia</i> )	Mandestrobin	36 d chronic, f	NOEC = 0.049 mg a.s./L <sub>mm</sub> <sup>b)</sup> NOEC = 0.0056 mg a.s./L <sub>mm</sub>	EFSA Conclusion (2015)
Saltwater mysid ( <i>Americamysis bahia</i> )	Mandestrobin	28 d chronic, f	NOEC = 0.13 mg a.s./L <sub>mm</sub> <sup>a)</sup> EC <sub>10</sub> = 0.15 mg a.s./L <sub>mm</sub>	KCP 10.2.1/01 Urann (2016), ROW-0096
<i>Daphnia pulex</i>	Mandestrobin	28 d chronic, ss	NOEC = 0.92 mg a.s./L <sub>twa</sub> EC <sub>10</sub> = 0.54 mg a.s./L <sub>twa</sub> <sup>a)</sup>	KCP 10.2.1/02 Roessink (2019a) ROW-0103
Daphnid ( <i>Ceriodaphnia dubia</i> )	Mandestrobin	7 d chronic, ss	NOEC = 0.63 mg a.s./L <sub>mm</sub> <sup>a)</sup> EC <sub>10</sub> = 0.69 mg a.s./L	KCP 10.2.1/03 Shaw (2021a) ROW-0126
Freshwater shrimp ( <i>Caridina parvidentata</i> )	Mandestrobin	28 d chronic, ss	NOEC = 2.7 mg a.s./L <sub>twa</sub> EC <sub>10</sub> = 2.6 mg a.s./L <sub>twa</sub> <sup>a)</sup>	KCP 10.2.1/04 Roessink (2019b) ROW-0106
Freshwater isopod ( <i>Gammarus pulex</i> )	Mandestrobin	28 d chronic, ss	NOEC = 0.092 mg a.s./L <sub>twa</sub> <sup>a)</sup> EC <sub>10</sub> = 0.18 mg a.s./L <sub>twa</sub>	KCP 10.2.1/05 Roessink (2019c) ROW-0105
Freshwater isopod ( <i>Asellus aquaticus</i> )	Mandestrobin	28 d chronic, ss	NOEC = 0.029 mg a.s./L <sub>twa</sub> <sup>a)</sup> EC <sub>10</sub> = 0.050 mg a.s./L <sub>twa</sub>	KCP 10.2.1/06 Roessink (2019d) ROW-0104
Freshwater amphipod ( <i>Hyaella azteca</i> )	Mandestrobin	42 d chronic, ss	NOEC = 0.26 mg a.s./L <sub>mm</sub> <sup>a)</sup> EC <sub>10</sub> = 0.66 mg a.s./L <sub>mm</sub>	KCP 10.2.1/07 Shaw (2021b) ROW-0127
Freshwater decapod ( <i>Palaemonetes paludosus</i> )	Mandestrobin	28 d, chronic, ss	NOEC = 1.0 mg a.s./L <sub>mm</sub> <sup>a)</sup> EC <sub>10</sub> = 1.2 mg a.s./L <sub>mm</sub>	KCP 10.2.1/08 Shaw (2022) ROW-0148

Species	Substance	Exposure system	Results	Reference
SSD endpoint (chronic)			$HC_5 = 0.060 \text{ mg a.s./L}$ $AF = 3$ <b>SSD-RAC</b> <b>= 0.020 mg a.s./L</b> $HC_5 = 0.056 \text{ mg a.s./L}$ $AF = 3$ <b>SSD-RAC =</b> <b>0.019 mg a.s./L</b>	KCP 10.2.1/09 White <i>et al.</i> (2024) ROW-0158
<b>Sediment dwelling organisms</b>				
Midge ( <i>Chironomus riparius</i> )	Mandestrobin	28 d, s Spiked water	NOEC = 8.1 mg a.s./L <sub>nom</sub>	EFSA Conclusion (2015)
Freshwater amphipod ( <i>Hyalella azteca</i> )	Mandestrobin	42 d, f Spiked sediment	<b>NOEC (28 d)</b> <b>= 5.0 mg a.s./kg sed.</b> <sub>mm</sub>	EFSA Conclusion (2015)
Marine amphipod ( <i>Leptocheirus plumulosus</i> )	Mandestrobin	28 d, ss Spiked sediment	NOEC = 10.3 mg a.s./kg sed. <sub>mm</sub>	EFSA Conclusion (2015)
<b>Algae</b>				
Green algae ( <i>Pseudokirchneriella subcapitata</i> )	S-Isomer (S-2354)	72 h, s	$E_rC_{50} > 12 \text{ mg/L}$ <sub>mm</sub> $E_bC_{50} = 12 \text{ mg/L}$ <sub>mm</sub> <sup>c)</sup>	EFSA Conclusion (2015)
Green algae ( <i>Pseudokirchneriella subcapitata</i> )	R-Isomer (S-2167)	72 h, s	<b><math>E_rC_{50} = 2.2 \text{ mg/L}</math></b> <sub>mm</sub> $E_bC_{50} = 0.38 \text{ mg/L}$ <sub>mm</sub> <sup>c)</sup>	EFSA Conclusion (2015)
Green algae ( <i>Pseudokirchneriella subcapitata</i> )	2-COOH-S- 2200	72 h, s	<b><math>E_rC_{50} = 62 \text{ mg/L}</math></b> <sub>mm</sub> $E_bC_{50} = 58 \text{ mg/L}$ <sub>mm</sub> <sup>c)</sup>	EFSA Conclusion (2015)
Green algae ( <i>Pseudokirchneriella subcapitata</i> )	5-COOH-S- 2200	72 h, s	<b><math>E_rC_{50} &gt; 54 \text{ mg/L}</math></b> <sub>mm</sub> $E_bC_{50} > 54 \text{ mg/L}$ <sub>mm</sub> <sup>c)</sup>	EFSA Conclusion (2015)
<b>Aquatic plants</b>				
Duckweed ( <i>Lemna gibba</i> )	Mandestrobin	7 d, ss	<b><math>E_rC_{50} &gt; 2.3 \text{ mg a.s./L}</math></b> <sub>mm</sub> $E_bC_{50} > 2.3 \text{ mg a.s./L}$ <sub>mm</sub> <sup>c)</sup>	EFSA Conclusion (2015)
<b>Additional information</b>				
<b>Mandestrobin Renewal: Position Paper on the Relevant Tier 1 Chronic Endpoint for <i>Americamysis bahia</i></b> <b>This position paper defends the original NOEC of 0.049 mg a.s./L concluded in the study report of the chronic test with <i>Americamysis bahia</i> (Urann, 2012) as the relevant Tier 1 chronic endpoint for <i>A. bahia</i>.</b>				<b>KCP 10.2.1/12</b> <b>White &amp; Eck</b> <b>(2024)</b> <b>ROW-0159</b>

Note: endpoints shown in **bold** are used in the risk assessment.

s: static; ss: semi-static; f: flow-through; mm: based on mean measured concentrations; ELS: Early life-stage; BCF: Bioconcentration factor.

<sup>a)</sup> Endpoint used in the chronic SSD for aquatic invertebrates. Where chronic EC<sub>10</sub> values for aquatic invertebrates are available and are lower than the NOEC, these values were used in the SSD.

<sup>b)</sup> Original NOEC in study report (0.049 mg a.s./L). The endpoint of 0.0056 mg a.s./L was noted in the EFSA Conclusion (2015) due to a significant effect at 0.011 mg a.s./L; however, this endpoint is argued to be unduly conservative by the Applicant based on the absence of statistically significant effects at the following two concentrations of 0.024 and 0.049 mg a.s./L in the study. The Applicant argues that 0.049 mg a.s./L is the most reliable NOEC from this study, considering the results of the study and the confirmatory results of the repeat study (ROW-0096).

<sup>c)</sup> Biomass endpoints are reported in addition to growth rate endpoints for consistency with the EFSA Conclusion (2015); however, it is noted that the relevant endpoints for use in the risk assessment are the endpoints for growth rate (ErC<sub>50</sub>).

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Mandestrobin 40SC and S-2200 25SC**

Species	Substance	Exposure system	Results	Reference
<b>Fish</b>				
<i>Oncorhynchus mykiss</i>	S-2200 25 SC	96 h acute, s	<b>LC<sub>50</sub> = 4.4 mg prod/L</b> (1.1 mg a.s./L <sub>mm</sub> )	EFSA Conclusion (2015)
<b>Aquatic invertebrates</b>				
<i>Daphnia magna</i>	Mandestrobin 40SC	48 h acute, s	<b>EC<sub>50</sub> = 2.55 mg prod/L</b> (0.973 mg a.s./L <sub>mm</sub> )	KCP 10.2.1/10 Obert-Rausser (2023a) ROW-0155
<i>Daphnia magna</i>	S-2200 25 SC	48 h acute, s	EC <sub>50</sub> = 2.68 mg prod/L (0.67 mg a.s./L <sub>mm</sub> )	EFSA Conclusion (2015)
<b>Algae</b>				
<i>Pseudokirchneriella subcapitata</i>	Mandestrobin 40SC	72 h, s	<b>ErC<sub>50</sub> = 11.0 mg prod/L</b> (3.94 mg a.s./L <sub>mm</sub> )	KCP 10.2.1/11 Obert-Rausser (2023b) ROW-0154
<i>Pseudokirchneriella subcapitata</i>	S-2200 25 SC	72 h, s	ErC <sub>50</sub> > 11.2 mg prod/L (> 2.8 mg a.s./L <sub>mm</sub> ) EbC <sub>50</sub> = 4.8 mg prod/L <sup>a)</sup> (1.2 mg a.s./L <sub>mm</sub> )	EFSA Conclusion (2015)

s: static; mm: based on mean measured concentrations.

<sup>a)</sup> Biomass endpoints are reported in addition to growth rate endpoints for consistency with the EFSA Conclusion (2015); however, it is noted that the relevant endpoints for use in the risk assessment are the endpoints for growth rate (ErC<sub>50</sub>).

**Table 9.5-3: Comparison of toxicity data with mandestrobin, Mandestrobin 40SC and S-2200 25 SC**

Species/endpoint	Mandestrobin (mg a.s./L)	S-2200 25 SC (mg a.s./L)	Mandestrobin 40SC (mg a.s./L)
<i>Oncorhynchus mykiss</i> 96-hour LD <sub>50</sub> (95% CI)	0.94 (0.57 – 1.3)	1.1 (0.8 – 1.6)	- <sup>a)</sup>
<i>Daphnia magna</i> 48-hour EC <sub>50</sub> (95% CI)	1.2 (0.70 – 2.9)	0.67 (0.36 – 1.5)	0.97 (0.81 – 1.16)
<i>Pseudokirchneriella subcapitata</i> 72-hour ErC <sub>50</sub> (95% CI)	3.4 (3.1 – 3.6) <sup>b)</sup> 2.2 (1.5 – n.a.) <sup>c)</sup>	> 2.8 (n.a.)	3.94 (3.69 – 4.19)

CI: Confidence intervals; n.a.: not applicable.

<sup>a)</sup> No acute studies on fish with Mandestrobin 40SC are submitted in the interest of minimising vertebrate testing. The toxicity of Mandestrobin 40SC to fish is considered to be covered by the acute rainbow trout studies with mandestrobin and the formulation S-2200 25 SC.

<sup>b)</sup> Based on a green algae study with mandestrobin submitted during the EU evaluation but not considered valid/acceptable and therefore not included in the EFSA (2015) List of Endpoints.

<sup>c)</sup> Based on a green algae study with the *R*-isomer, used as a surrogate endpoint for the active substance mandestrobin.

### 9.5.1.1 Justification for new endpoints

The long-term endpoint for *Americamysis bahia* as an additionally tested aquatic invertebrate species in the EFSA conclusion (NOEC = 0.0056 mg a.s./L) is considered unduly conservative by the Notifier, who argues for an endpoint of 0.049 mg a.s./L derived from the same study that led to the EFSA endpoint. This argumentation is based on the fact that a statistically significant effect found for the next higher concentration of 0.011 mg a.s./L tested in the study is not considered to be dose related since there were no statistically significant effects at the following two concentrations of 0.024 and 0.049 mg a.s./L. This was, accordingly, discussed in the study report, concluding on 0.049 mg a.s./L as the appropriate NOEC for this study.

The study was therefore repeated, and the new study (Urann, 2016) is submitted with this dRR. It is listed in Appendix 1 and summarised in Appendix 2. A NOEC of 0.13 mg a.s./L and an EC<sub>10</sub> of 0.15 mg a.s./L were obtained from this study. The new data support the interpretation of the original study results by the Study Director and Notifier, setting the NOEC of the study by Claude *et al.* (2012) to 0.049 mg a.s./L, which is comparable to the NOEC and EC<sub>10</sub> of the new data by Urann (2016).

In agreement with the EFSA Aquatic Guidance (2013) proposing the preferential use of the 10% effect level, the EC<sub>10</sub> of 0.15 mg a.s./L is considered most appropriate for risk assessment. With reference to the lack of an appropriate concentration-response and the supposed outlier, which is now further evidenced by the new data, it is noted that no reliable EC<sub>10</sub> can be derived from the study by Claude *et al.* (2012). However, in a conservative approach and relating to the EU agreed data, the NOEC of 0.049 mg a.s./L is used in the Tier 1 risk assessment.

In further support of the risk assessment, new chronic aquatic invertebrate studies on additional species have been performed and are submitted in support of a Tier 2B RAC (the Species Sensitivity Distribution (SSD)) approach. These studies are listed in Appendix 1 and summarised in Appendix 2. This approach was agreed with the zRMS during the pre-submission exchanges about possible refinements of the aquatic risk assessment (09.08.2023; pre-submission meeting in writing).

Two separate SSDs have been performed and are reported in White *et al.* (2024), shown in KCP 10.2.1/09. The first approach was performed in line with the requirement of the Central Zone Evaluation Manual (v2.0 August 2023) that available estimates (e.g. NOEC and EC<sub>10</sub> values) should not be mixed in the SSD, and the EC<sub>10</sub> is the preferable estimate for use. However, in two of the available studies, no reliable EC<sub>10</sub> values could be calculated. One of these studies is with the standard species *Daphnia magna* (ROW-0020), and the other is the original study with *Americamysis bahia* (ROW-0063) which provides the Tier 1 RAC for the aquatic risk assessment. Nevertheless, in order to comply with the CZ Evaluation Manual (v2.0, 2023), an SSD was performed taking into account the available EC<sub>10</sub> estimates only. This is still feasible even with the omission of the two aforementioned studies, as the required number of eight endpoints from eight different species is available. Moreover, the species *Americamysis bahia* is still accounted for by using the EC<sub>10</sub> derived from the more recent study.

However, the approach outlined above, while in line with the requirements of the CZ Evaluation Manual (2023), leads to the omission of two valuable data points. Therefore, an additional SSD has been performed, also reported in KCP 10.2.1/09, which takes into account all available studies. In contrast to the CZ Evaluation Manual (v2.0, 2023) and in accordance with the EFSA Aquatic GD (2013), for SSDs with chronic aquatic invertebrate data it is acceptable to combine estimate types, e.g. NOECs and EC<sub>10</sub> values. In the second SSD, EC<sub>10</sub> estimates were applied where available. Where no reliable EC<sub>10</sub> estimates could be calculated, the NOECs were applied instead. In order to be inclusive of both available studies for *Americamysis bahia*, and to avoid use of two separate endpoints for the same species, the available NOECs from these studies (both based on the growth parameter) were combined using a geometric mean. This geometric mean NOEC was calculated based on the NOEC of 0.049 mg a.s./L from the original study (argued as the most reliable by the Applicant) and 0.13 mg a.s./L from the repeat study. A resulting geometric mean of 0.08 mg a.s./L was calculated.

With the first approach (EC<sub>10</sub> estimates only), a HC<sub>5</sub> of 0.060 mg a.s./L and RAC of 0.020 mg a.s./L were calculated (using an assessment factor of 3). With the second approach (combined EC<sub>10</sub> and NOEC estimates), a HC<sub>5</sub> value of 0.056 mg a.s./L and RAC of 0.019 mg a.s./L were calculated (using an assessment factor of 3).

For the purpose of the aquatic risk assessment, both SSD-RACs are applied. Regardless of the approach taken, a very similar Tier 2B RAC is derived for the chronic risk to aquatic invertebrates. The available results therefore provide additional reassurance and certainty as to the reliability of the Tier 2B RACs applied to the risk assessment.

For algae, in contrast to the EFSA Conclusion for mandestrobin (2015), in which the biomass endpoints (E<sub>b</sub>C<sub>50</sub>) were used for the risk assessment, the risk assessment in this dossier uses the growth rate endpoint (E<sub>r</sub>C<sub>50</sub>). The use of growth rate endpoints is in line with 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).

#### **Applicant update October 2024**

At the request of the zRMS Poland, the chronic NOEC of 0.0056 mg a.s./L for *Americamysis bahia* listed in the EFSA Conclusion (2015) has now been applied to the risk assessments and presented alternatively in Tables 9.5-4 and 9.5-5 (acidic conditions) and Tables 9.5-6 and 9.5-7 (basic conditions). As already noted above, this endpoint is considered unduly conservative by the Notifier, who argues for an endpoint of 0.049 mg a.s./L derived from the same study that led to the EFSA endpoint. In defence of this stance, a position paper is submitted under data point CP 10.2.1/12, which includes weight of evidence that the NOEC of 0.049 mg a.s./L is sufficiently conservative for use in the aquatic risk assessment. Please refer to Appendix 2 for full details; however, this evidence includes:

- The effects seen on adult first-generation survival at 0.011 mg a.s./L in the 2012 study were slight, not dose responsive and, accordingly, not treatment related. The supposed effects at 0.011 mg a.s./L were concluded by the Study Director in the original study report to be not related to treatment, as the difference from the controls was slight, and not dose responsive (higher rates of survival were actually observed at the next two treatment levels of 0.024 and 0.049 mg a.s./L, with no statistically significant reductions compared to the control in these treatments).
- A second chronic study with *A. bahia* is available (Urann, 2016) which identified no significant effects on survival, growth or reproduction at concentrations up to and including 0.13 mg a.s./L (NOEC). Significant effects were observed at a LOEC of 0.24 mg a.s./L. The lowest of the estimated L/EC<sub>10</sub> values was an LC<sub>10</sub> of 0.15 mg a.s./L. These endpoints are in agreement with the NOEC of 0.049 mg a.s./L from the original study, which was based on mortality and growth. Moreover, the lower 95% confidence interval of this LC<sub>10</sub> (0.071 mg a.s./L) provides further reassurance that a NOEC of 0.049 mg a.s./L is sufficiently protective of *Americamysis bahia*.
- The median time to first brood release in the two main mysid studies suggest that the mysids used in the 2016 study were in better health conditions than those used in the 2012 study. In the 2012 study, median time to first brood release in the combined controls was 27 days, leading to the study duration being extended to 36 days (standard duration is 28 days). Comparatively, in the 2016 study, median time to first brood release in the combined controls was 20.5 days, and the study was terminated on schedule at 28 days. These results indicate that the findings of the 2016 study may be more reliable and that a NOEC/EC<sub>10</sub> of 0.13 and 0.15 mg a.s./L, respectively may even be argued to be more appropriate for the species.
- A well-performed non-GLP range finding study is available, performed prior to the 2016 study. The findings further support the conclusions noted above, with a NOEC of 0.160 mg a.s./L (nominal) based on significant effects observed at the highest test concentration of 0.500 mg a.s./L (nominal).

- Studies on eight species in addition to *Americamysis bahia* are available. The most closely related species to *A. bahia* of these species are *Asellus aquaticus*, *Gammarus pulex* and *Hyalella azteca*. The available endpoints from these studies are all highly comparable and in line with the NOEC of 0.049 mg a.s./L, further demonstrating that this NOEC is sufficiently conservative of the risk to this species.

In line with the conclusions of this position paper, the relevant Tier 1 endpoint for the assessment of the chronic risk to *Americamysis bahia* is considered to be the NOEC of 0.049 mg a.s./L, leading to a Tier 1 RAC of 4.9 µg a.s./L. This endpoint is applied to the aquatic risk assessments. However, in line with the request of zRMS Poland, the conservative NOEC of 0.0056 mg a.s./L (RAC = 0.56 µg a.s./L) is also shown in the risk assessments. It should be noted that the assessments based on this RAC are presented for illustrative purposes only. Conclusions for the chronic risk assessment for aquatic invertebrates should be based on the lowest SSD-RAC of 19 µg/L, as agreed with the zRMS during the pre-submission exchanges about possible refinements and overall conclusions should be based on the lowest Tier 1 acute RAC of 4.3 µg a.s./L for aquatic invertebrates.

### 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 active substance mandestrobin is a racemic mixture (50:50) of two stereoisomers, namely the *R*-isomer (S-2167) and the *S*-isomer (S-2354). The results of the laboratory studies show that the *R*-isomer of mandestrobin and the active substance mandestrobin are of comparable toxicity, whereas the *S*-isomer of mandestrobin is significantly less toxic than the *R*-isomer and the active substance. In line with the EFSA conclusion for mandestrobin (2015), the risk assessment was performed for the *R*-isomer (S-2167) where possible, as the endpoints are formally lower than those for the active substance.

Toxicity data presented above indicate that the metabolites which were considered to potentially be of concern in aquatic systems show clearly less toxicity compared to the parent mandestrobin. Concurrently, relevant PEC<sub>SW</sub> values for these metabolites do not exceed the predicted concentrations calculated for the parent. Thus, either way (from both the toxicity and exposure point of view), it is reasonably concluded that the risk for aquatic organisms arising from these compounds is covered by the parent. Nonetheless, as a comprehensive approach to the risk assessment, separate risk assessments are presented for the metabolites, based on FOCUS Step 1 and 2 PEC<sub>SW</sub> modelling.

The most sensitive endpoints for each test organism group have been divided by the corresponding assessment factors to calculate the RACs. The RACs have been compared with the PEC<sub>SW</sub> values for the active substance and formulated product (see part B8 for details) to determine if an acceptable risk can be demonstrated.

As already noted above under 9.5.1 and 9.5.1.1, the chronic risk to aquatic invertebrates is ultimately based on the SSD-RACs of 19 and 20 µg a.s./L.

The risk assessment is presented separately for winter and spring oilseed rape at FOCUS Step 1 and 2. Additionally, as a comprehensive approach, FOCUS Step 3 and Step 4 modelling (accounting for maximum 20 m vegetated buffers) is presented which, as a conservative approach, is presented for acidic soils which are worst-case compared to estimates for basic soils. However, in a comprehensive approach, risk assessments are now also amended for environmental modelling based on parameterisation as relevant for basic soils. Refer to part B8 for more details about the parameters used to determine PEC<sub>SW</sub> values.

#### **Applicant update October 2024**

At the request of the zRMS Poland, the chronic NOEC of 0.0056 mg a.s./L for *Americamysis bahia* listed in the EFSA Conclusion (2015) has now been alternatively applied to the risk assessments in Tables 9.5-4 and 9.5-5 (acidic conditions) and Tables 9.5-6 and 9.5-7 (basic conditions) as an additional Tier 1 endpoint. FOCUS Step 4 PEC<sub>SW</sub> are also added to the assessments with consideration of risk mitigation measures alongside this RAC. However, it should be noted that these Step 4 PEC<sub>SW</sub> are shown for illustrative purposes only, as the conclusions for the chronic risk to aquatic invertebrates should be based on the lowest SSD-RAC of 19 µg a.s./L, and the overall conclusions for the risk assessment should be based on the acute RAC for aquatic invertebrates of 4.3 µg a.s./L.

#### **Active substance**

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

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mandestrobin for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the use of Mandestrobin 40SC in winter oilseed rape (1 x 200 g a.s./ha, acidic soil - worst case scenario)**

Group		Fish acute	Fish chronic	Inverteb. acute	<i>Inverteb. Chronic (Tier 1)*</i>	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	SSD	SSD	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	Test species	<i>Hyalella azteca</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	<i>NOEC</i>	NOEC	HC <sub>5</sub>	HC <sub>5</sub>	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	Endpoint	NOEC
(µg/L)		840	150	430	<i>5.6</i>	49	56	60	2200	> 2300	(µg a.s./kg)	5000
AF		100	10	100	<i>10</i>	10	3	3	10	10	AF	10
RAC (µg/L)		8.40	15.0	4.30	<i>0.56</i>	4.90	19	20	220	230	RAC (µg/kg)	500
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC									PEC <sub>sed</sub> (µg/kg)	PEC/RAC
Step 1 (winter/spring oilseed rape, 1 x 200 g a.s./ha)												
	43.55	5.18	2.90	10.13	<i>77.77</i>	8.89	2.29	2.18	0.20	0.19	191.90	0.38
Step 2 (winter oilseed rape, 1 x 200 g a.s./ha)												
SEU (Mar – May)	5.43	0.65	0.36	1.26	<i>9.70</i>	1.11	0.29	0.27	0.02	0.02	23.58	0.05
Step 3 (winter oilseed rape, 1 x 200 g a.s./ha, acidic conditions)												
D2 Ditch	3.031	0.36	0.20	0.70	<i>5.41</i>	0.62	0.16	0.15	-	-	-	-
D2 Stream	1.894	0.23	0.13	0.44	<i>3.38</i>	0.39	0.10	0.09	-	-	-	-
D3 Ditch	1.270	0.15	0.08	0.30	<i>2.27</i>	0.26	0.07	0.06	-	-	-	-
D4 Pond	0.938	0.11	0.06	0.22	<i>1.68</i>	0.19	0.05	0.05	-	-	-	-
D4 Stream	1.096	0.13	0.07	0.25	<i>1.96</i>	0.22	0.06	0.05	-	-	-	-
D5 Pond	0.466	0.06	0.03	0.11	<i>0.83</i>	0.10	0.02	0.02	-	-	-	-
D5 Stream	1.182	0.14	0.08	0.27	<i>2.11</i>	0.24	0.06	0.06	-	-	-	-



Group		Fish acute	Fish chronic	Inverteb. acute	<b>Inverteb. Chronic (Tier 1)*</b>	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
R1 Pond	0.231	0.03	0.02	0.05	<b>0.41</b>	0.05	0.01	0.01	-	-	-	-
R1 Stream	1.823	0.22	0.12	0.42	<b>3.26</b>	0.37	0.10	0.09	-	-	-	-
R3 Stream	1.653	0.20	0.11	0.38	<b>2.95</b>	0.34	0.09	0.08	-	-	-	-
<b>Step 4 (winter oilseed rape, 1 x 200 g a.s./ha, acidic conditions) – 20 m drift buffer + vegetated filter strip</b>												
<b>D3 Ditch</b>	<b>0.095</b>	█	█	█	<b>0.17</b>	█	█	█	█	█	█	█
<b>D4 Pond</b>	<b>0.932</b>	█	█	█	<b>1.66</b>	█	█	█	█	█	█	█
<b>D4 Stream</b>	<b>0.913</b>	█	█	█	<b>1.63</b>	█	█	█	█	█	█	█
<b>D5 Stream</b>	<b>0.503</b>	█	█	█	<b>0.90</b>	█	█	█	█	█	█	█
<b>R1 Stream</b>	<b>0.435</b>	█	█	█	<b>0.78</b>	█	█	█	█	█	█	█
<b>R3 Stream</b>	<b>0.385</b>	█	█	█	<b>0.69</b>	█	█	█	█	█	█	█

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

\*RAC of 0.56 µg a.s./L shown for illustrative purposes only. Please refer to CP 10.2.1/12 in Appendix 2 and above under Point 9.5.1.1 for justification.

An acceptable risk to aquatic organisms for the use of Mandestrobin 40SC on winter oilseed rape under acidic conditions is clearly demonstrated at FOCUS Step 3, i.e. without a requirement for risk mitigation, based on the lowest Tier 1 RAC for the acute risk to aquatic invertebrates. For the chronic risk to aquatic invertebrates, an acceptable risk is also demonstrated at FOCUS Step 3 based on the Tier 1 RAC of 4.9 µg a.s./L and the SSD-RACs of 19 and 20 µg a.s./L. The assessment based on the overly conservative RAC of 0.56 µg a.s./L is shown for illustrative purposes only. Please refer to CP 10.2.1/12 for a position paper detailing justification for the irrelevance of this endpoint and the relevance of the chronic Tier 1 RAC of 4.9 µg a.s./L.

**Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mandestrobin for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the use of Mandestrobin 40SC in spring oilseed rape (1 x 200 g a.s./ha, acidic soil - worst case scenario)**

Group		Fish acute	Fish chronic	Inverteb. acute	<i>Inverteb. Chronic (Tier 1)*</i>	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	SSD	SSD	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	Test species	<i>Hyaella azteca</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	<i>NOEC</i>	NOEC	HC <sub>5</sub>	HC <sub>5</sub>	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	Endpoint	NOEC
(µg/L)		840	150	430	<i>5.6</i>	49	56	60	2200	> 2300	(µg a.s./kg)	5000
AF		100	10	100	<i>10</i>	10	3	3	10	10	AF	10
RAC (µg/L)		8.40	15.0	4.30	<i>0.56</i>	4.90	19	20	220	230	RAC (µg/kg)	500
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC									PEC <sub>sed</sub> (µg/kg)	PEC/RAC
Step 1 (winter/spring oilseed rape, 1 x 200 g a.s./ha)												
	43.55	5.18	2.90	10.13	<i>77.77</i>	8.89	2.29	2.18	0.20	0.19	191.90	0.38
Step 2 (spring oilseed rape, 1 x 200 g a.s./ha)												
SEU (Jun – Sep)	4.40	0.52	0.29	1.02	<i>7.86</i>	0.90	0.23	0.22	0.02	0.02	18.97	0.04
Step 3 (spring oilseed rape, 1 x 200 g a.s./ha, acidic conditions)												
D3 Ditch	1.271	0.15	0.08	0.30	<i>2.27</i>	0.26	0.07	0.06	-	-	-	-
D4 Pond	1.018	0.12	0.07	0.24	<i>1.82</i>	0.21	0.05	0.05	-	-	-	-
D4 Stream	1.096	0.13	0.07	0.25	<i>1.96</i>	0.22	0.06	0.05	-	-	-	-
D5 Pond	0.609	0.07	0.04	0.14	<i>1.09</i>	0.12	0.03	0.03	-	-	-	-
D5 Stream	1.182	0.14	0.08	0.27	<i>2.11</i>	0.24	0.06	0.06	-	-	-	-
R1 Pond	0.339	0.04	0.02	0.08	<i>0.61</i>	0.07	0.02	0.02	-	-	-	-
R1 Stream	1.918	0.23	0.13	0.45	<i>3.43</i>	0.39	0.10	0.10	-	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	<i>Inverteb. Chronic (Tier 1)*</i>	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
<b>Step 4 (spring oilseed rape, 1 x 200 g a.s./ha, acidic conditions) – 20 m drift buffer + vegetated filter strip</b>												
D3 Ditch	0.095	█	█	█	0.17	█	█	█	█	█	█	█
D4 Pond	1.012	█	█	█	1.81	█	█	█	█	█	█	█
D4 Stream	0.993	█	█	█	1.77	█	█	█	█	█	█	█
D5 Pond	0.609	█	█	█	1.09	█	█	█	█	█	█	█
D5 Stream	0.759	█	█	█	1.36	█	█	█	█	█	█	█
R1 Stream	0.458	█	█	█	0.82	█	█	█	█	█	█	█

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

\*RAC of 0.56 µg a.s./L shown for illustrative purposes only. Please refer to CP 10.2.1/12 in Appendix 2 and above under Point 9.5.1.1 for justification.

An acceptable risk to aquatic organisms for the use of Mandestrobin 40SC on spring oilseed rape under acidic conditions is clearly demonstrated at FOCUS Step 3, i.e. without a requirement for risk mitigation, based on the lowest Tier 1 RAC for the acute risk to aquatic invertebrates. For the chronic risk to aquatic invertebrates, an acceptable risk is also demonstrated at FOCUS Step 3 based on the Tier 1 RAC of 4.9 µg a.s./L and the SSD-RACs of 19 and 20 µg a.s./L. The assessment based on the overly conservative RAC of 0.56 µg a.s./L is shown for illustrative purposes only. Please refer to CP 10.2.1/12 for a position paper detailing justification for the irrelevance of this endpoint and the relevance of the chronic Tier 1 RAC of 4.9 µg a.s./L.

**Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mandestrobin for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the use of Mandestrobin 40SC in winter oilseed rape (1 x 200 g a.s./ha, basic soil)**

Group		Fish acute	Fish chronic	Inverteb. acute	<i>Inverteb. Chronic (Tier 1)*</i>	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	SSD	SSD	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	Test species	<i>Hyaella azteca</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	NOEC	HC <sub>5</sub>	HC <sub>5</sub>	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	Endpoint	NOEC
(µg/L)		840	150	430	5.6	49	56	60	2200	> 2300	(µg a.s./kg)	5000
AF		100	10	100	10	10	3	3	10	10	AF	10
RAC (µg/L)		8.40	15.0	4.30	0.56	4.90	19	20	220	230	RAC (µg/kg)	500
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC									PEC <sub>sed</sub> (µg/kg)	PEC/RAC
Step 1 (winter/spring oilseed rape, 1 x 200 g a.s./ha)												
	43.55	5.18	2.90	10.13	77.77	8.89	2.29	2.18	0.20	0.19	191.90	0.38
Step 2 (winter oilseed rape, 1 x 200 g a.s./ha)												
SEU (Mar – May)	5.43	0.65	0.36	1.26	9.70	1.11	0.29	0.27	0.02	0.02	23.58	0.05
Step 3 (winter oilseed rape, 1 x 200 g a.s./ha, basic conditions)												
D3 Ditch	1.270	0.15	0.08	0.30	2.27	0.26	0.07	0.06	!	!	!	!
D4 Pond	0.311	0.04	0.02	0.07	0.56	0.06	0.02	0.02	!	!	!	!
D4 Stream	1.096	0.13	0.07	0.25	1.96	0.22	0.06	0.05	!	!	!	!
D5 Pond	0.103	0.01	0.01	0.02	0.18	0.02	0.01	0.01	!	!	!	!
D5 Stream	1.182	0.14	0.08	0.27	2.11	0.24	0.06	0.06	!	!	!	!
R1 Pond	0.228	0.03	0.02	0.05	0.41	0.05	0.01	0.01	!	!	!	!

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. Chronic (Tier 1)*	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
R1 Stream	1.792	0.21	0.12	0.42	3.20	0.37	0.09	0.09				
R3 Stream	1.505	0.18	0.10	0.35	2.69	0.31	0.08	0.08				
<b>Step 4 (winter oilseed rape, 1 x 200 g a.s./ha, basic conditions) – 20 m drift buffer + vegetated filter strip</b>												
D3 Ditch	0.095				0.17							
D4 Stream	0.307				0.55							
D5 Stream	0.125				0.22							
R1 Stream	0.427				0.76							
R3 Stream	0.351				0.63							

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

\*RAC of 0.56 µg a.s./L shown for illustrative purposes only. Please refer to CP 10.2.1/12 in Appendix 2 and above under Point 9.5.1.1 for justification.

An acceptable risk to aquatic organisms for the use of Mandestrobin 40SC on winter oilseed rape under basic conditions is clearly demonstrated at FOCUS Step 3, i.e. without a requirement for risk mitigation, based on the lowest Tier 1 RAC for the acute risk to aquatic invertebrates. For the chronic risk to aquatic invertebrates, an acceptable risk is also demonstrated at FOCUS Step 3 based on the Tier 1 RAC of 4.9 µg a.s./L and the SSD-RACs of 19 and 20 µg a.s./L. The assessment based on the overly conservative RAC of 0.56 µg a.s./L is shown for illustrative purposes only. Please refer to CP 10.2.1/12 for a position paper detailing justification for the irrelevance of this endpoint and the relevance of the chronic Tier 1 RAC of 4.9 µg a.s./L.

**Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mandestrobin for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the use of Mandestrobin 40SC in spring oilseed rape (1 x 200 g a.s./ha, basic soil)**

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. Chronic (Tier 1)*	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	<i>Americamysis bahia</i>	SSD	SSD	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>	Test species	<i>Hyalella azteca</i>
Endpoint (µg/L)		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	NOEC	HC <sub>5</sub>	HC <sub>5</sub>	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	Endpoint (µg a.s./kg)	NOEC
AF		840	150	430	5.6	49	56	60	2200	> 2300	AF	5000
RAC (µg/L)		100	10	100	10	10	3	3	10	10	RAC (µg/kg)	10
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	8.40	15.0	4.30	0.56	4.90	19	20	220	230	PEC <sub>sed</sub> (µg/kg)	500
PEC/RAC												
Step 1 (winter/spring oilseed rape, 1 x 200 g a.s./ha)												
	43.55	5.18	2.90	10.13	77.77	8.89	2.29	2.18	0.20	0.19	191.90	0.38
Step 2 (spring oilseed rape, 1 x 200 g a.s./ha)												
SEU (Jun – Sep)	4.40	0.52	0.29	1.02	7.86	0.90	0.23	0.22	0.02	0.02	18.97	0.04
Step 3 (spring oilseed rape, 1 x 200 g a.s./ha, basic conditions)												
D3 Ditch	1.271	0.15	0.08	0.30	2.27	0.26	0.07	0.06	1	1	1	1
D4 Pond	0.372	0.04	0.02	0.09	0.66	0.08	0.02	0.02	1	1	1	1
D4 Stream	1.096	0.13	0.07	0.25	1.96	0.22	0.06	0.05	1	1	1	1
D5 Pond	0.214	0.03	0.01	0.05	0.38	0.04	0.01	0.01	1	1	1	1
D5 Stream	1.182	0.14	0.08	0.27	2.11	0.24	0.06	0.06	1	1	1	1
R1 Pond	0.322	0.04	0.02	0.07	0.58	0.07	0.02	0.02	1	1	1	1
R1 Stream	1.893	0.23	0.13	0.44	3.38	0.39	0.10	0.09	1	1	1	1

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. Chronic (Tier 1)*	Inverteb. Chronic (Tier 1)	Inverteb. Chronic (Tier 2B)	Inverteb. Chronic (Tier 2B)	Algae	Aquatic plant	Group	Sed. dwell. chronic
<b>Step 4 (spring oilseed rape, 1 x 200 g a.s./ha, basic conditions) – 20 m drift buffer + vegetated filter strip</b>												
D3 Ditch	0.095	█	█	█	0.17	█	█	█	█	█	█	█
D4 Stream	0.368	█	█	█	0.66	█	█	█	█	█	█	█
D5 Stream	0.257	█	█	█	0.46	█	█	█	█	█	█	█
R1 Stream	0.452	█	█	█	0.81	█	█	█	█	█	█	█

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

\*RAC of 0.56 µg a.s./L shown for illustrative purposes only. Please refer to CP 10.2.1/12 in Appendix 2 and above under Point 9.5.1.1 for justification.

An acceptable risk to aquatic organisms for the use of Mandestrobin 40SC on spring oilseed rape under basic conditions is clearly demonstrated at FOCUS Step 3, i.e. without a requirement for risk mitigation, based on the lowest Tier 1 RAC for the acute risk to aquatic invertebrates. For the chronic risk to aquatic invertebrates, an acceptable risk is also demonstrated at FOCUS Step 3 based on the Tier 1 RAC of 4.9 µg a.s./L and the SSD-RACs of 19 and 20 µg a.s./L. The assessment based on the overly conservative RAC of 0.56 µg a.s./L is shown for illustrative purposes only. Please refer to CP 10.2.1/12 for a position paper detailing justification for the irrelevance of this endpoint and the relevance of the chronic Tier 1 RAC of 4.9 µg a.s./L.

### **Metabolites**

Five metabolites of mandestrobin are identified as requiring aquatic risk assessment (see Table 9.1-3). These are S-2200-OR, S-2200-ORC, 2-COOH-S-2200, 5-COOH-S-2200 and DX-CA-S-2200.

Toxicity data presented in Table 9.5-1 indicate that these metabolites show clearly less toxicity compared to the parent mandestrobin. Concurrently, relevant  $PEC_{SW}$  values for these metabolites do not exceed the predicted concentrations calculated for the parent. Thus, either way (from both the toxicity and exposure point of view), it is reasonably concluded that the risk for aquatic organisms arising from these compounds is covered by the parent. Nonetheless, as a comprehensive approach to the risk assessment, separate metabolite risk assessments are presented in **Table 9.5-8 below**, based on FOCUS Step 1 and 2  $PEC_{SW}$  modelling. Where toxicity data are not available for the metabolites, these are assumed to be 10 times more toxic than the parent compound mandestrobin.



**Table 9.5-8:** Aquatic organisms: acceptability of risk for potentially relevant surface water metabolites of mandestrobin for the use of Mandestrobin 40SC in winter and spring oilseed rape (1 x 200 g a.s./ha)

Metabolite	2-COOH-S-2200				5-COOH-S-2200				S-2200-OR			
Organism group		Fish acute	Inverteb. acute	Algae		Fish acute	Inverteb. acute	Algae		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC <sub>50</sub> > 89000	EC <sub>50</sub> > 100000	E <sub>r</sub> C <sub>50</sub> 62000		LC <sub>50</sub> > 100000	EC <sub>50</sub> > 100000	E <sub>r</sub> C <sub>50</sub> > 54000		LC <sub>50</sub> > 9000	EC <sub>50</sub> > 14000	E <sub>r</sub> C <sub>50</sub> 220 <sup>a)</sup>
AF		100	100	10		100	100	10		100	100	10
RAC (µg/L)		> 890	> 1000	6200		> 1000	> 1000	> 5400		> 90	> 140	22
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC			PEC <sub>sw</sub> (µg/L)	PEC/RAC			PEC <sub>sw</sub> (µg/L)	PEC/RAC		
	Step 1				Step 1				Step 1			
Max PEC <sub>sw</sub>	6.97	< 0.01	< 0.01	0.00	14.77	< 0.01	< 0.01	< 0.00	14.00	< 0.16	< 0.10	0.64

AF: Assessment Factor; RAC: Regulatory Acceptable Concentration; PEC<sub>sw</sub>: Predicted Environmental Concentrations in surface water. PEC/RAC ratios above the trigger of 1 are shown in **bold**.

<sup>a)</sup> Based on ten times toxicity of the parent mandestrobin, in the absence of laboratory derived data on this organism group with this metabolite.

**Table 9.5-8 (cont.): Aquatic organisms: acceptability of risk for potentially relevant surface water metabolites of mandestrobin for the use of Mandestrobin 40SC in winter and spring oilseed rape (1 x 200 g a.s./ha)**

Metabolite	S-2200-ORC				DX-CA-S-2200			
Organism group		Fish acute	Inverteb. acute	Algae		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC <sub>50</sub> 4000	EC <sub>50</sub> 9600	E <sub>r</sub> C <sub>50</sub> 220 <sup>a)</sup>		LC <sub>50</sub> 84 <sup>a)</sup>	EC <sub>50</sub> 43 <sup>a)</sup>	E <sub>r</sub> C <sub>50</sub> 220 <sup>a)</sup>
AF		100	100	10		100	100	10
RAC (µg/L)		40	96	22		0.84	0.43	22
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC			PEC <sub>sw</sub> (µg/L)	PEC/RAC		
	Step 1				Step 1			
Max PEC <sub>sw</sub>	8.32	0.21	0.09	0.38	3.91	4.65	9.09	0.18
FOCUS Scenario	Step 2				Step 2			
Winter OSR <sup>b)</sup> SEU (Mar-May)	-	-	-	-	0.23	0.27	0.53	0.01

AF: Assessment Factor; RAC: Regulatory Acceptable Concentration; PEC<sub>sw</sub>: Predicted Environmental Concentrations in surface water. PEC/RAC ratios above the trigger of 1 are shown in **bold**.

<sup>a)</sup> Based on ten times toxicity of the parent mandestrobin, in the absence of laboratory derived data on this organism group with this metabolite.

<sup>b)</sup> Worst-case PEC<sub>sw</sub> value at FOCUS Step 2 for DX-CA-S-2200 was for use in winter oilseed rape in SEU (Mar-May).

The above risk assessment demonstrates an acceptable risk to aquatic organisms from exposure to potentially relevant surface water metabolites of mandestrobin, following the proposed use of Mandestrobin 40SC on oilseed rape at 1 x 200 g a.s./ha, without a requirement for risk mitigation.

## Mandestrobin 40SC

The relevant maximum PEC<sub>sw</sub> values for the formulation Mandestrobin 40SC arising from the drift loading into surface water were calculated from spray drift using the FOCUS drift value of 1.93%. The resulting PEC/RAC ratios are presented in the table below.

**Table 9.5-9:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group for the intended use of Mandestrobin 40SC in winter/spring oilseed rape (541 g product/ha)

Group		Fish acute	Invertebrates acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
Endpoint (µg prod/L)		LC <sub>50</sub> 4400 <sup>a)</sup>	EC <sub>50</sub> 2550	E <sub>r</sub> C <sub>50</sub> 11000
AF		100	100	10
RAC (µg prod./L)		44.0	25.5	1100
FOCUS Scenario	PEC <sub>sw</sub> (µg/L)	PEC/RAC		
Spray drift				
1 meter	3.48	0.08	0.14	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration.

<sup>a)</sup> Endpoint for S-2200 25 SC used in the absence of a study with Mandestrobin 40SC. This is considered to be conservative of Mandestrobin 40SC which is of comparable (or even lower) toxicity than S-2200 25 SC, based on the available data on *D. magna* and *P. subcapitata*. The risk to fish is also addressed in the active substance risk assessment.

An acceptable risk to aquatic organisms is demonstrated in the formulation risk assessment, for the intended use of Mandestrobin 40SC in oilseed rape.

### 9.5.3 Overall conclusions

For the active substance mandestrobin, an acceptable risk to aquatic organisms is demonstrated for uses in both winter and spring oilseed rape based on FOCUS Step 3 PEC<sub>sw</sub> modelling for the worst-case uses in acidic soils and, in a comprehensive approach, also for basic soils, which result in lower exposure levels. This conclusion is based on the lowest Tier 1 RAC which is for the acute risk to aquatic invertebrates.

For the metabolites, an acceptable risk for uses in both winter and spring oilseed rape could be demonstrated based on FOCUS Step 1 and FOCUS Step 2 PEC<sub>sw</sub> modelling.

In addition, a risk assessment based on PEC<sub>sw</sub> values calculated for the formulation arising from the drift loading into surface water indicated an acceptable risk.

Therefore, overall an acceptable risk to aquatic organisms is demonstrated without a requirement for risk mitigation.

## 9.6 Effects on bees (KCP 10.3.1)

<p>zRMS Comments:</p>	<p>The submitted risk assessment is based (SANCO/10329/2002 rev.2 (final), October 17, 2002) guidance.</p> <p>New studies only for acute toxicity were submitted and accepted. No chronic studies (chronic oral and larval toxicity) with formulation were submitted. The</p> <p>The justification considering chronic toxicity of formulation Mandestrobin 40SC was submitted. Mandestrobin 40SC is a fungicide. This formulation contains only one active substance with no significant insecticidal effect. Formulation has much lower acute toxicity (more than 3 times) than active substance. Taking into consideration the acute toxicity of formulation to bees and non-target arthropods other than bees, the negative effect on larvae pupation is not expected. The formulation is applied only once per season, and it should be noted that for NTA the in-field and off-field low risk was concluded.</p> <p><del>The chronic toxicity studies should be submitted to fulfill the regulation requirements (Comm. Reg. 284/2013). These studies should be submitted before the new bee guidance comes into force.</del></p> <p>As the acute toxicity data for mandestrobin and Mandestrobin 40SC demonstrate the formulation is of no greater toxicity than the active substance alone, in accordance with Commission Regulation 284/2013 and considering the recommendations of the EFSA (2013) and (2023) bee guidance documents, no formulation studies are required.</p> <p>The EU agreed endpoints and accepted endpoints from submitted studies were used in acute risk assessment.</p> <p>The hazard quotients are below the trigger values considering SANCO guidance indicating that the active substance and formulation pose an acceptable risk to bees. Therefore, an acceptable acute risk to bees is expected from the application of Mandestrobin 40SC.</p> <p>The risk assessment based on new EFSA, 2013 guidance was added. The submitted risk assessment was accepted.</p>
---------------------------	---

### **9.6.1 Toxicity data**

Studies on the toxicity to bees have been carried out with mandestrobin. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies). Newly submitted studies with mandestrobin include an acute oral/contact toxicity study with the bumblebee, a chronic (adult) honeybee study and a chronic (larvae) honeybee study.

Effects of Mandestrobin 40SC on bees were not assessed as part of the EU evaluation of mandestrobin. A new study on the acute oral and contact toxicity to honeybees is submitted with this application, listed in Appendix 1 and summarised in Appendix 2.

Acute oral/contact toxicity studies with S-2200 25SC were evaluated as part of the EU assessment of mandestrobin. These studies are shown in the table below for information, in addition to two new studies with S-2200 25SC which cover the chronic (adult) and chronic (larvae) risk to honeybees. These two new studies are summarised in Appendix 2.

No chronic adult or larvae studies with Mandestrobin 40SC are submitted. However, the available acute oral and contact toxicity data do not indicate any greater toxicity of Mandestrobin 40SC to honeybees than the technical active substance (see Table 9.6-1). The requirement for chronic testing of bees is considered to be sufficiently addressed by provision of data with the technical active substance.

Chronic adult and larvae studies are also available for the comparable formulation S-2200 25 SC, which reported similar endpoints to the active substance studies. Therefore, no greater chronic toxicity was indicated with this formulation compared to the active substance. Furthermore, based on the acute oral/contact data, Mandestrobin 40SC does not appear to be of any greater toxicity to bees than S-2200 25 SC. Accordingly, these data and comparative toxicities with the active substance provide additional evidence that product toxicity is covered based on data for the technical active substance.

Therefore, based on the data already available, no chronic adult or larvae studies with Mandestrobin 40SC are considered necessary, and the endpoints for S-2200 25 SC should be suitable for use as surrogate endpoints for Mandestrobin 40SC.

The selection of studies and endpoints for the acute risk assessment is in line with the results of the EU review process. In addition, the more recently available chronic data (adults and larvae) are used in the risk assessment.

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

Species	Substance	Exposure system	Results	Reference
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin	Acute oral (adult)	<b>LD<sub>50</sub></b> <b>&gt; 110.71 µg a.s./bee</b>	EFSA Conclusion (2015)
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin	Acute contact (adult)	<b>LD<sub>50</sub></b> <b>&gt; 100 µg a.s./bee</b>	EFSA Conclusion (2015)
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin 40SC	Acute oral (adult)	<b>LD<sub>50</sub></b> 864.31 µg prod/bee <b>(322.04 µg a.s./bee)</b>	KCP 10.3.1.1.1/01 Ansaloni (2023) ROW-0157
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin 40SC	Acute contact (adult)	<b>LD<sub>50</sub></b> > 1358.70 µg prod/bee <b>(&gt; 506.25 µg a.s./bee)</b>	KCP 10.3.1.1.2/01 Ansaloni (2023) ROW-0157
Honeybee ( <i>Apis mellifera</i> )	S-2200 25 SC	Acute oral (adult)	LD <sub>50</sub> > 109 µg a.s./bee	EFSA Conclusion (2015)
Honeybee ( <i>Apis mellifera</i> )	S-2200 25 SC	Acute contact (adult)	LD <sub>50</sub> > 100 µg a.s./bee	EFSA Conclusion (2015)
Bumblebee ( <i>Bombus terrestris</i> )	Mandestrobin	Acute oral (adult)	<b>LD<sub>50</sub></b> <b>&gt; 193.03 µg a.s./bee</b>	KCP 10.3.1.1.1/02 Aguilar-Alberola (2022) ROW-0112
Bumblebee ( <i>Bombus terrestris</i> )	Mandestrobin	Acute contact (adult)	<b>LD<sub>50</sub></b> <b>&gt; 200 µg a.s./bee</b>	KCP 10.3.1.1.2/02 Aguilar-Alberola (2022) ROW-0112
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin	Chronic oral (adult)	<b>LDD<sub>50</sub></b> <b>&gt; 45 µg a.s./bee/day</b> NOEDD = 45 µg a.s./bee/day	KCP 10.3.1.2/01 Picard (2018a) ROW-0102
Honeybee ( <i>Apis mellifera</i> )	S-2200 25 SC	Chronic oral (adult)	LDD <sub>50</sub> > 65.8 µg prod/bee/day (> 16.2 µg a.s./bee/day) NOEDD = 65.8 µg prod/bee/day (16.2 µg a.s./bee/day)	KCP 10.3.1.2/02 Noël (2016) ROW-0099
Honeybee ( <i>Apis mellifera</i> )	Mandestrobin	Chronic oral (larvae)	<b>NOED</b> <b>= 100 µg a.s./larva/dev. period</b>	KCP 10.3.1.3/01 Picard (2018b) ROW-0100
Honeybee ( <i>Apis mellifera</i> )	S-2200 25 SC	Chronic oral (larvae)	NOED = 409.73 µg prod/larva/dev. period (100 µg a.s./larva/dev. period)	KCP 10.3.1.3/02 Aguilar-Alberola (2019) ROW-0101

Note: endpoints shown in **bold** are used in the risk assessment.

<sup>a)</sup> Although there was a statistically significant difference in body weight of bees at 100 µg a.s./larva/dev period when compared to the negative control, the percentage difference from the control was relatively low (8.5%), suggesting that the difference may be within the range of biological variability and not ecologically relevant. Therefore, the most relevant endpoint recommended for use in the risk assessment is 100 µg a.s./larva/dev. period, the NOED for all other measured parameters.

### 9.6.1.1 Justification for new endpoints

A new acute oral and contact toxicity study with Mandestrobin 40SC is submitted. In addition, in order to address the chronic effects on adult bees and bees larvae, these studies are submitted with the active substance mandestrobin and with a comparable formulation S-2200 25 SC. Due to the availability of these studies and considering that Mandestrobin 40SC appears to be of no greater toxicity to bees than S-2200 25 SC or the technical active substance, no chronic studies with Mandestrobin 40SC are submitted (see Section 9.6.1 above for further explanation).

Additionally, in order to address the risk to bumblebees, an acute oral and contact toxicity study with the bumblebee and the active substance is submitted, which does not indicate greater sensitivity of bumble bees as for honeybees.

### 9.6.2 Risk assessment

The evaluation of the acute risk to bees is 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). There is currently no agreed chronic risk assessment scheme for adult and larval honeybees, therefore only an acute risk assessment in accordance with SANCO (2002) is presented.

#### 9.6.2.1 Hazard quotients for bees

##### **SANCO (2002) Risk Assessment**

**Table 9.6-2: First-tier assessment of the risk for bees due to the use of Mandestrobin 40SC in oilseed rape**

Intended use	Oilseed rape		
Active substance	Mandestrobin		
Application rate (g a.s./ha)	1 × 200		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g a.s./ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 110.71	200	< 1.8
Contact toxicity	> 100		< 2.0
Product	Mandestrobin 40SC		
Application rate (g a.s./ha)	1 × 200		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g a.s./ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	322.04	200	< 0.6
Contact toxicity	> 506.25		< 0.4

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 resulting hazard quotients are clearly below the trigger of 50, indicating an acceptable acute risk to honeybees following the proposed use of Mandestrobin 40SC in oilseed rape.

In addition, chronic (adult) and chronic (larvae) studies are available with the active substance mandestrobin and with comparable formulation S-2200 25 SC. Since, based on the available acute data, Mandestrobin 40SC appears to be of no greater toxicity to bees than S-2200 25 SC or the technical active substance, these studies are considered suitable for use to cover the chronic risk of Mandestrobin 40SC to adult honeybees and honeybee larvae.

In response to the request of some cMS, an assessment of the acute risks to adult honeybees and bumblebees, and the chronic risk to honeybee adults and larvae is presented below, in accordance with the EFSA (2013) Bee Guidance. The chronic risk assessments are performed based on available testing with the active substance, as available formulation data (on S-2200 25 SC) do not indicate any increase in toxicity of the formulation compared to the technical active substance alone, with less than 50% mortality observed in both studies. In the study with S-2200 25 SC, 0% mortality was observed at the highest dose of 16.2 µg a.s./bee/day, while 17% mortality was observed at the highest dose of 45 µg a.s./bee in the study with the active substance mandestrobin. Therefore, unbound LDD<sub>50</sub> values were derived in each case, and the endpoint of > 45 µg a.s./bee/day from testing with the technical active substance is applied to the risk assessment.

### EFSA (2013) Bee Risk Assessment

#### Screening assessment

**Table 9.6-3: Screening assessment of the acute risk to adult honeybees and bumblebees resulting from contact exposure**

Crop	Single application rate (g a.s./ha)	Species (life stage)	Endpoint	HQ <sub>contact</sub>	Trigger
Oilseed rape	200	Honeybee (adult)	LD <sub>50</sub> > 100 µg a.s./bee	< 2.0	> 42
		Bumblebee (adult)	LD <sub>50</sub> > 200 µg a.s./bee	< 1	> 7

HQ: Hazard quotient. Values shown in **bold** exceed the relevant trigger value, indicating a potential concern.

An acceptable acute contact risks to adult honeybees and bumblebees are demonstrated at the screening tier.

**Table 9.6-4: Screening assessment of the acute risk to adult honeybees and bumblebees resulting from oral exposure**

Crop	Single application rate (g a.s./ha)	Species (life stage)	Endpoint	Ef x SV	ETR <sub>oral</sub>	Trigger
Oilseed rape (DW)	200	Honeybee (adult)	LD <sub>50</sub> > 110.71 µg a.s./bee	7.6	< 0.01	> 0.2
		Bumblebee (adult)	LD <sub>50</sub> > 193.03 µg a.s./bee	11.2	< 0.01	> 0.036

DW: Downward spray; Ef: Exposure factor; SV: Shortcut value; ETR: Exposure Toxicity Ratio. Values shown in **bold** exceed the relevant trigger value, indicating a potential concern.

An acceptable acute oral risks to adult honeybees and bumblebees are demonstrated at the screening tier.



**Table 9.6-5: Screening assessment of the chronic risk to adult honeybees and honeybee larvae resulting from oral exposure**

Crop	Single application rate (g a.s./ha)	Species (life stage)	Endpoint	Ef x SV	ETR <sub>oral</sub>	Trigger
Oilseed rape (DW)	200	Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	7.6	< 0.034	> 0.03
		Honeybee (larvae)	NOED = 100 µg a.s./larva/dev. period	4.4	0.01	> 0.2

DW: Downward spray; Ef: Exposure factor; SV: Shortcut value; ETR: Exposure Toxicity Ratio. Values shown in **bold** exceed the relevant trigger value, indicating a potential concern.

An acceptable risk to honeybee larvae is demonstrated at the screening tier. For the chronic risk to adult honeybees, an acceptable risk is not formally demonstrated, as the ETR is slightly greater than the trigger value of 0.03 (< 0.034). Since the LDD<sub>50</sub> is unbound, with less than 50% effects on mortality (actual: 17%) observed at the highest dose of 45 µg a.s./bee/day, the ETR<sub>oral</sub> would formally be lower than the required trigger value of 0.03 based on a realistic, calculated LDD<sub>50</sub>. Nevertheless, a first-tier assessment of the chronic risk to adult honeybees is provided below for completeness.

#### ***First-tier assessment (chronic oral risk to adult honeybees)***

There are a number of different scenarios to be assessed in a first-tier risk assessment, in accordance with the EFSA Guidance Document (2013). The routes of exposure considered in the first-tier assessment are as follows:

- Risk from foraging on the treated crop
- Risk from foraging on an adjacent crop
- Risk from foraging on weeds in the treated field
- Risk from foraging in the field margin
- Risk from foraging following year on permanent crop or succeeding crop for annual crops

**Table 9.6-6: First-tier assessment of the chronic risk to adult honeybees from oral exposure**

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR <sub>oral</sub>	Trigger
<b>Scenario: Foraging on the Treated Crop</b>							
Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	40 – 69	1	5.8	0.72	< 0.019	> 0.03
<b>Scenario: Foraging on Weeds in the Treated Field</b>							
Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	40 – 69	0.25	2.9	0.72	< 0.002	> 0.03
<b>Scenario: Foraging in the Field Margin</b>							
Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	40 – 69	0.0092	2.9	0.72	< 0.000	> 0.03
<b>Scenario: Foraging on an Adjacent Crop</b>							
Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	40 – 69	0.0033	5.8	0.72	< 0.000	> 0.03
<b>Scenario: Foraging on the Next Crop</b>							
Honeybee (adult)	LDD <sub>50</sub> > 45 µg a.s./bee/day	40 – 69	1	0.54	0.72	< 0.002	> 0.03

DW: Downward spray; Ef: Exposure factor; SV: Shortcut value; ETR: Exposure Toxicity Ratio. Values shown in **bold** exceed the relevant trigger value, indicating a potential concern.

In the above first-tier assessment, an acceptable chronic oral risk to adult honeybees is demonstrated for all exposure scenarios for the intended use of Mandestrobin 40SC in oilseed rape.

Therefore, overall, an acceptable risk to bees is demonstrated.

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

Not relevant.

### 9.6.3 Effects on bumble bees

An acute oral/contact toxicity study with bumblebees is submitted (refer to Appendix 1 and 2 for details). No risk assessment scheme for these studies is available under the current Guidance Document (SANCO/10329/2002). However, for illustration purposes, an assessment of the acute risk to adult bumble bees is presented in 9.6.2.1 in accordance with the EFSA (2013) Bee Guidance. An acceptable acute contact and oral risks to adult bumblebees are demonstrated at the screening tier. No treatment related effects were observed in the study, with LD<sub>50</sub> values greater than the highest dose tested in both the oral and contact exposures. The available data do not indicate any greater sensitivity of bumblebees to mandestrobin compared to honeybees. Since the testing with honeybees does not indicate any greater toxicity of the formulation Mandestrobin 40SC compared to the active substance, no acute bumblebee studies with the formulation are considered necessary.

### 9.6.4 Effects on solitary bees

Not required.

## 9.6.5 Overall conclusions

Overall, an acceptable risk to bees from the proposed use of Mandestrobin 40SC in oilseed rape is concluded.

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

zRMS Comments:	<p>The submitted risk assessment based on the “Guidance Document on Terrestrial Ecotoxicology”, 2002, was accepted.  New studies for formulation at Tier 1 were submitted and accepted.</p> <p><b>Risk assessment.</b> <del>The vdf value was corrected to 10 (in accordance with zonal harmonization).</del> As the vdf = 5 is recommended in the central zone risk assessment, the risk assessment was accepted.</p> <p>The hazard quotient for active substance is below the trigger value (<math>HQ \leq 2</math>) at Tier 1, the refinement at higher tier is not required.</p> <p><b>Formulation.</b> The following endpoints at Tier 1 were used in risk assessment:</p> <ul style="list-style-type: none"> <li>• <i>Typhlodromus pyri</i>: <math>LR_{50} &gt; 2480</math> mL/ha, equivalent to <math>&gt; 1000</math> g a.s./ha;</li> <li>• <i>Aphidius rhopalosiphi</i>: <math>LR_{50} &gt; 2480</math> mL/ha, equivalent to <math>&gt; 1000</math> g a.s./ha.</li> </ul> <p>As the hazard quotients are lower than trigger value (<math>HQ \leq 2</math>) at Tier 1, indicating that the formulation poses an acceptable risk to arthropods other than bees.</p> <p>Therefore, an acceptable risk to arthropods other than bees is expected if the application of Mandestrobin 40SC is in accordance with intended uses.</p>
-------------------	--

## 9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative formulated product S-2200 25 SC during the EU review of the active substance mandestrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects of Mandestrobin 40SC on non-target arthropods were not evaluated as part of the EU assessment of mandestrobin. New data on Mandestrobin 40SC submitted with this application are listed in Appendix 1 and summarised in Appendix 2. The studies with S-2200 25 SC are also shown in the table below for information.

The selection of studies for the risk assessment therefore deviates from the results of the EU review process; however, the endpoints in terms of active substance content remain the same as those reported in the EFSA Conclusion (2015) due to limit rate endpoints resulting from these new studies (see Table 9.7-1).

**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>	Mandestrobin 40SC	Laboratory test glass plates (2D)	<b>LR<sub>50</sub> / ER<sub>50</sub></b> > 2480 mL prod/ha (> <b>1000 g a.s./ha</b> )	KCP 10.3.2.1/01 Leopold (2023a) ROW-0152
<i>Typhlodromus pyri</i>	Mandestrobin 40SC	Laboratory test glass plates (2D)	<b>LR<sub>50</sub> / ER<sub>50</sub></b> > 2480 mL prod/ha (> <b>1000 g a.s./ha</b> )	KCP 10.3.2.1/02 Leopold (2023b) ROW-0153
<i>Aphidius rhopalosiphi</i>	S-2200 25 SC	Laboratory test glass plates (2D)	LR <sub>50</sub> > 1000 g a.s./ha	EFSA Conclusion (2015)
<i>Typhlodromus pyri</i>	S-2200 25 SC	Laboratory test glass plates (2D)	LR <sub>50</sub> > 1000 g a.s./ha	EFSA Conclusion (2015)

Note: endpoints shown in **bold** are used in the risk assessment.

### 9.7.1.1 Justification for new endpoints

New studies on the toxicity of Mandestrobin 40SC to *Aphidius rhopalosiphi* and *Typhlodromus pyri* are submitted. These new studies provide the relevant endpoints for the risk assessment; however, the endpoints obtained from the new studies (> 1000 g a.s./ha) are identical to those obtained for S-2200 25 SC reported in the EFSA Conclusion (2015) and therefore there is no change to the endpoints used.

## 9.7.2 Risk assessment

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

### 9.7.2.1 Risk assessment for in-field exposure

**Table 9.7-2: First tier assessment of the in-field risk for non-target arthropods due to the use of Mandestrobin 40SC in oilseed rape**

<b>Intended use</b>	Oilseed rape		
<b>Active substance/product</b>	Mandestrobin / Mandestrobin 40SC		
<b>Application rate (g a.s./ha)</b>	1 × 200		
<b>MAF</b>	1.0		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>PER<sub>in-field</sub></b>	<b>HQ<sub>in-field</sub></b>
<b>First tier</b>	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 1000	200	< 0.2
<i>Aphidius rhopalosiphi</i>	> 1000		< 0.2

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

The above risk assessment demonstrates an acceptable in-field risk to non-target arthropods following the proposed use of Mandestrobin 40SC in oilseed rape.

### 9.7.2.2 Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas will be mainly due to spray drift from field applications. Off-field foliar PER values were calculated from the in-field foliar PERs in conjunction with the drift values of ESCORT 2. A vegetation distribution factor (VDF) of 5 is used, in line with the recommendations of the EFSA Technical Report (2019<sup>1</sup>).

<sup>1</sup> EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.

**Table 9.7-3: First-tier assessment of the off-field risk for non-target arthropods due to the use of Mandestrobin 40SC in oilseed rape**

<b>Intended use</b>	Oilseed rape				
<b>Active substance/product</b>	Mandestrobin / Mandestrobin 40SC				
<b>Application rate (g a.s./ha)</b>	1 × 200				
<b>MAF</b>	1.0				
<b>vdf</b>	5				
<b>Test species</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (g/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 1000	2.77%	1.11	10	< 0.011
<i>Aphidius rhopalosiphi</i>	> 1000		1.11		< 0.011

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

<sup>a)</sup> In line with the EFSA Technical Report on Recurring Issues in Ecotoxicology (2019).

The above risk assessment demonstrates an acceptable off-field risk to non-target arthropods following the proposed use of Mandestrobin 40SC in oilseed rape.

### 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

Overall, an acceptable in-field and off-field risk to non-target arthropods is demonstrated for the proposed use of Mandestrobin 40SC in oilseed rape.

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

<b>zRMS</b>	The submitted risk assessment was accepted.
<b>Comments:</b>	<p>The risk assessment for formulation is based on accepted endpoint earthworm reproduction study.</p> <p>The justification considering non-submission studies for <i>Folsomia candida</i> and <i>Hypoaspis aculeifer</i> was accepted.</p> <p>The max PECs values for active substances, their metabolites and formulation (see Section 8. Fate and behavior) were used for acute risk assessment</p> <p>Since risk assessment for non-target soil meso- and macrofauna (earthworm) is acceptable at Tier 1, the higher-tier risk assessment is not required.</p>

	An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the Mandestrobin 40SC is used in accordance with proposed uses.
--	---

### 9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mandestrobin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Mandestrobin 40SC were not evaluated as part of the EU assessment of mandestrobin. A new earthworm reproduction study with Mandestrobin 40SC submitted with this application is listed in Appendix 1 and summarised in Appendix 2.

No studies on *Folsomia candida* or *Hypoaspis aculeifer* are submitted or considered necessary, in accordance with Commission Regulation (EC) 283/2013 and 284/2013 (“*For plant protection products applied as a foliar spray, data on the relevant two non-target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on Folsomia candida and Hypoaspis aculeifer shall be required.*”). The first-tier risk assessment for non-target arthropods shows an acceptable risk, with no effects seen in the two NTA species at 1000 g a.s./ha, neither on mortality nor on reproduction. Furthermore, Mandestrobin 40SC is intended for post-emergence foliar applications at BBCH  $\geq 60$ , i.e. there is no direct soil treatment. Therefore, studies on *Folsomia candida* and *Hypoaspis aculeifer* are not considered necessary. Finally, since the available acute earthworm data indicate that the relevant soil metabolites are less toxic than the parent compound, studies with metabolites are also not considered necessary.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process except for the additional inclusion of the endpoint derived from chronic earthworm testing with Mandestrobin 40SC.

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

Species	Substance	Exposure system	Results	Reference
<i>Eisenia fetida</i>	Mandestrobin	Mixed into substrate 14 d, acute 10% peat content	LC <sub>50corr</sub> <sup>a)</sup> = 84 mg a.s./kg dw	EFSA Conclusion (2015)
<i>Eisenia fetida</i>	Metabolite 2-COOH-S-2200	Mixed into substrate 14 d, acute 10% peat content	LC <sub>50corr</sub> <sup>a)</sup> > 500 mg/kg soil dw	EFSA Conclusion (2015)
<i>Eisenia fetida</i>	Metabolite 5-COOH-S-2200	Mixed into substrate 14 d, acute 10% peat content	LC <sub>50corr</sub> <sup>a)</sup> > 500 mg/kg soil dw	EFSA Conclusion (2015)
<i>Eisenia fetida</i>	Mandestrobin	Mixed into substrate 56 d, chronic 10% peat content	NOEC <sub>corr</sub> <sup>a)</sup> = <b>3.75 mg a.s./kg soil dw</b>	EFSA Conclusion (2015)
<i>Eisenia andrei</i>	Mandestrobin 40SC	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 30.9 mg prod/kg soil dw (11.5 mg a.s./kg soil dw) NOEC <sub>corr</sub> <sup>a)</sup> = <b>15.45 mg prod/kg soil dw</b> <b>(5.75 mg a.s./kg soil dw)</b>	KCP 10.4.1.1/01 Straube (2023) ROW-0156

Note: endpoints shown in **bold** are used in the risk assessment.

<sup>a)</sup> Endpoint corrected by a factor of 2 in line with the EFSA Technical Report (2015), due to log P<sub>ow</sub> value greater than 2.

### 9.8.1.1 Justification for new endpoints

A new earthworm reproduction study with Mandestrobin 40SC is submitted and included in the risk assessment.

### 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). It is noted that acute assessments are no longer required.

The risk assessment includes mandestrobin and potentially relevant metabolites in soil 5-COOH-S-2200, 2-COOH-S-2200 and DX-CA-S-2200. As no chronic toxicity endpoints are available for metabolites 5-COOH-S-2200, 2-COOH-S-2200 and DX-CA-S-2200, they are assumed to be 10 times more toxic than the parent compound mandestrobin.

The relevant PEC<sub>soil</sub> for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). For the purpose of the soil organism risk assessments, as applicable the PEC<sub>accumulation</sub> (PEC<sub>actual</sub> + PEC<sub>soil plateau</sub>) is used for mandestrobin, 5-COOH-S-2200 and 2-COOH-S-2200, whereas for DX-CA-S-2200, the initial PEC<sub>soil</sub> is relevant.



### 9.8.2.1 First-tier risk assessment

**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 Mandestrobin 40SC in oilseed rape**

Intended use	Oilseed rape		
Chronic effects on earthworms			
Product/active substance	NOEC <sub>corr</sub> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>lt</sub> (criterion TER ≥ 5)
Mandestrobin	3.75	0.069 <sup>b)</sup>	54
Mandestrobin 40SC	5.75 (a.s.)	0.069 <sup>b)</sup>	83
Mandestrobin 40SC	15.45 (product)	0.144 <sup>c)</sup>	107
2-COOH-S-2200	0.375 <sup>a)</sup>	0.005 <sup>b)</sup>	75
5-COOH-S-2200	0.375 <sup>a)</sup>	0.011 <sup>b)</sup>	34
DX-CA-S-2200	0.375 <sup>a)</sup>	0.003 <sup>d)</sup>	125

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

<sup>a)</sup> Assumed to be ten times more toxic than the parent compound mandestrobin, in the absence of laboratory derived toxicity endpoints for these metabolites.

<sup>b)</sup> PEC<sub>accumulation</sub> (PEC<sub>act</sub> + PEC<sub>soil plateau</sub>).

<sup>c)</sup> PEC<sub>act</sub>.

<sup>d)</sup> Initial PEC<sub>soil</sub>.

The above risk assessment demonstrates an acceptable risk to earthworms following the proposed use of Mandestrobin 40SC in oilseed rape.

### 9.8.2.2 Higher-tier risk assessment

Not relevant.

### 9.8.3 Overall conclusions

Overall, an acceptable risk to non-target soil meso- and macro-organisms is demonstrated for the proposed use of Mandestrobin 40SC in oilseed rape.

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

zRMS Comments:	<p>The submitted information and data were accepted.</p> <p>New study was submitted and accepted.</p> <p>The formulation Mandestrobin 40 SC poses no adverse effect on nitrate formation in soil.</p> <p>An acceptable risk to soil microorganisms is expected if the application of the Mandestrobin 40 SC is in accordance with proposed pattern use.</p>
-------------------	---

### 9.9.1 Toxicity data

Studies on soil microorganisms have been carried out with mandestrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects of Mandestrobin 40SC on soil microorganisms were not evaluated as part of the EU assessment of mandestrobin and no data are available on the representative formulation S-2200 25 SC. The available studies with mandestrobin are considered sufficient to address the risk of the formulation, as was the case during the EU evaluation of mandestrobin.

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

Endpoint	Substance	Exposure system	Results	Reference
N-transformation	Mandestrobin	28 d, aerobic soil type	<b>NOAEC</b> <b>= 1.5 mg a.s./kg soil dw</b> ( <b>&lt; 25% effect at 0.3 and .5 mg a.s./kg soil dw</b> )	EFSA Conclusion (2015)
C-mineralisation	Mandestrobin	28 d, aerobic soil type	<b>NOAEC</b> <b>= 1.5 mg a.s./kg soil dw</b> ( <b>&lt; 25% effects at 0.3 and 1.5 mg a.s./kg soil dw</b> )	EFSA Conclusion (2015)

NOAEC: < 25% effect at ≤ 100 days. Note: endpoints shown in **bold** are used in the risk assessment.

#### 9.9.1.1 Justification for new endpoints

Not applicable, as there are no new endpoints.

### 9.9.2 Risk assessment

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

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

The risk assessment includes mandestrobin and potentially relevant metabolites in soil 5-COOH-S-2200, 2-COOH-S-2200 and DX-CA-S-2200. As no toxicity values are available for metabolites 5-COOH-S-2200, 2-COOH-S-2200 and DX-CA-S-2200, they are assumed to be ten times more toxic than the parent compound mandestrobin (NOAEC as defined by less than 25% effects at ≤ 100 days divided by 10).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Mandestrobin 40SC in oilseed rape**

Intended use	Oilseed rape		
N-transformation / C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Mandestrobin	1.5 (at 28 d)	0.069 <sup>b)</sup>	Yes
2-COOH-S-2200	0.15 <sup>a)</sup>	0.005 <sup>b)</sup>	Yes
5-COOH-S-2200	0.15 <sup>a)</sup>	0.011 <sup>b)</sup>	Yes
DX-CA-S-2200	0.15 <sup>a)</sup>	0.003 <sup>c)</sup>	Yes

<sup>a)</sup> Assumed to be ten times more toxic than the parent compound mandestrobin, in the absence of laboratory derived toxicity endpoints for these metabolites.

<sup>b)</sup> PEC<sub>accumulation</sub> (PEC<sub>act</sub> + PEC<sub>soil plateau</sub>).

<sup>c)</sup> Initial PEC<sub>soil</sub>.

The above risk assessment demonstrates an acceptable risk to soil micro-organisms from the proposed use of Mandestrobin 40SC in oilseed rape.

### 9.9.3 Overall conclusions

Overall, an acceptable risk to soil micro-organisms is demonstrated for the proposed use of Mandestrobin 40SC in oilseed rape.

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

zRMS Comments:	No new toxicity effects of fungicide Mandestrobin 40 SC on the vegetative vigour and seedling emergence were presented. The additional studies, not GLP, considering herbicidal activity of metabolite DX-CA-S-2200 were submitted, but they were not evaluated. The Applicant submitted a statement considering toxicity comparability of Mandestrobin 40 SC and representative formulation S-2200 25 SC. The statement is based on the same active substance content. The submitted justification was accepted.				
	The following endpoints for representative formulation were accepted at the EU level: ER <sub>50</sub> > 200 g a.s./ha (seedling emergence, cabbage); ER <sub>50</sub> > 200 g a.s./ha (vegetative vigour, lettuce); and were used in risk assessment. and the risk assessment for NTTP was added by evaluator:				
	Intended use		Applications to cereals		
	Active substance/product		Mandestrobin / Mandestrobin 40 SC		
	Application rate (g a.s./ha)		200		
	MAF		1		
	Test species	ER <sub>50</sub> (g/ha)	Drift rate %	PER <sub>off-field</sub> (g/ha)	TER criterion: TER ≥ 5
	Tested species (most sensitive, EFSA, 2015)	> 200	2.77	5.54	36.1

	<p>Based on EFSA Conclusion, 2015, no effect on vegetative vigour and seedling emergence were noted at 200 g a.s./ha with large margin of safety, so it can be concluded that Mandestrobin 40 SC has no herbicidal activity.</p> <p>An acceptable risk to non-target terrestrial plants is expected if the formulation Mandestrobin 40 SC is applied in accordance with intended use.</p>
--	---

### 9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants are available with S-2200 25 SC, the representative formulated product during EU review of the active substance mandestrobin. Full details of these studies are provided in the respective EU DAR and related documents.

No studies on the toxicity of Mandestrobin 40SC to non-target terrestrial plants are available; however, the available studies with S-2200 25 SC are considered suitable to address the risk of Mandestrobin 40SC.

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

Species	Substance	Exposure system	Results	Reference
<i>Triticum aestivum</i> (m) <i>Zea mays</i> (m) <i>Cucumis sativa</i> (d) <i>Glycine max</i> (d) <i>Solanum lycopersicum</i> (d) <i>Lactuca sativa</i> (d)	S-2200 25 SC	21 d Seedling emergence	<b>ER<sub>50</sub></b> <b>&gt; 200 g a.s./ha</b>	EFSA Conclusion (2015)
<i>Triticum aestivum</i> (m) <i>Zea mays</i> (m) <i>Cucumis sativa</i> (d) <i>Glycine max</i> (d) <i>Solanum lycopersicum</i> (d) <i>Lactuca sativa</i> (d)	S-2200 25 SC	21 d Vegetative vigour	<b>ER<sub>50</sub></b> <b>&gt; 200 g a.s./ha</b>	EFSA Conclusion (2015)

m: monocotyledonous; d: dicotyledonous.

Note: endpoints shown in **bold** are used in the risk assessment.

#### 9.10.1.1 Justification for new endpoints

Not applicable, as there are no new endpoints.

### 9.10.2 Risk assessment

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

The evaluation of the risk to non-target plants was performed in accordance with the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). S-2200 25 SC is a fungicide and is therefore not expected to have significant herbicidal activity. Hence, a preliminary assessment is conducted, using the available screening data.

According to the Terrestrial Guidance Document, the risk to non-target terrestrial plants should be considered acceptable if less than 50% effect on at least six species is seen at the highest nominal application rate (1x). For mandestrobin, studies on seedling emergence and vegetative vigour of terrestrial higher plants with limit test rates of up to 200 g a.s./ha were conducted based on the solo formulation S-2200 25 SC as part of the EU assessment. The available data demonstrate no adverse effects on six species at the tested rate of 200 g a.s./ha. The ER<sub>50</sub> was therefore considered to be > 200 g a.s./ha for all plant species tested and as stated in the respective EU DAR 2014, can also be considered to be the NOER.

The tested rate (and NOER) of 200 g a.s./ha is equal to the application rate proposed for use in oilseed rape. Therefore, in line with the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002), as no effects were observed at the maximum proposed in-field application rate, an acceptable risk can be concluded (screening level assessment).

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

Not required.

#### **9.10.2.3 Higher-tier risk assessment**

Not required.

#### **9.10.2.4 Risk mitigation measures**

No risk mitigation needed.

### **9.10.3 Overall conclusions**

Overall, an acceptable risk to non-target terrestrial plants is demonstrated for the proposed use of Mandestrobin 40SC based on a screening level assessment.

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

Screening studies on the herbicidal, insecticidal and fungicidal activity of mandestrobin and metabolites De-Xy-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 are available which were evaluated in the DAR (2014). The details of these studies are shown in Table 9.11-1 below. Additionally, the herbicidal, insecticidal and fungicidal activity of mandestrobin (S-2200) and its metabolite DX-CA-S-2200 were also investigated. These studies are submitted with this dossier, listed in Appendix 1 and summarised in Appendix 2.

**Table 9.11-1: Studies on other terrestrial organisms (flora and fauna)**

Species	Test substance	Exposure System	Results	Reference
Blackgrass (M) Italian ryegrass (M) Barnyardgrass (M) Giant foxtail (M) Cleavers/catchweed bedstraw (D) Velvetleaf (D) Wheat (M)	Mandestrobin DX-CA-S-2200	Herbicidal activity	No herbicidal activity at 400 g a.s./ha	KCP 10.7/01 Soma (2017) ROG-0007
Diamondback moth Cotton aphid Tobacco whitefly Brown planthopper Two-spotted spider mite	Mandestrobin DX-CA-S-2200	Insecticidal activity	No insecticidal activity at 200 ppm	KCP 10.7/02 Kamezaki (2017) ROG-0008
White mould ( <i>Sclerotinia sclerotiorum</i> )	DX-CA-S-2200	Fungicidal activity	No fungicidal activity of the metabolite (EC <sub>50</sub> > 5 ppm)	KCP 10.7/03 Suemoto (2017) ROG-0006
	Mandestrobin		EC <sub>50</sub> = 0.020 ppm	
7 species: Blackgrass (M) Italian ryegrass (M) Barnyardgrass (M) Giant foxtail (M) Wheat (M) Cleavers/catchweed bedstraw (D) Velvetleaf (D)	Mandestrobin De-Xy-S-2200 2-COOH-S-2200 5-COOH-S-2200	Herbicidal activity	No herbicidal activity at 400 g/ha	DAR (2014)
Diamondback moth Cotton aphid Tobacco whitefly Brown planthopper Two-spotted spider mite	Mandestrobin De-Xy-S-2200 2-COOH-S-2200 5-COOH-S-2200	Insecticidal activity	No insecticidal activity (effects on mortality or sublethal effects) at 200 ppm	DAR (2014)
White mould ( <i>Sclerotinia sclerotiorum</i> )	2-COOH-S-2200 5-COOH-S-2200	Fungicidal activity	No fungicidal activity of metabolites (EC <sub>50</sub> > 5 ppm)	DAR (2014)
	Mandestrobin		EC <sub>50</sub> = 0.032 ppm	

M: Monocot; D: Dicot.

## 9.12 Monitoring data (KCP 10.8)

No data are submitted.

### 9.13 Classification and Labelling

The following is proposed in accordance with Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures:

**Category:** Acute category 1 ( $EC_{50}$  *Americamysis bahia* = 0.43 mg a.s./L)  
Chronic category 1 (NOEC *Americamysis bahia* = 0.049 mg a.s./L)

**Pictogram:**



**Signal word:** Warning

**Hazard statements:** H410: Very toxic to aquatic life with long lasting effects

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

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Urann, K.	2016	Mandestrobin (S-2200) –Life-Cycle Toxicity Test with RATS ( <i>Americamysis bahia</i> ) XXXX. Report No. ROW-0096, Study No.: 13048.6921 Smithers Viscient, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.2.1/02	Roessink, I.	2019a	Chronic effects of the fungicide Mandestrobin to <i>Daphnia pulex</i> XXXX. Report No.: ROW-0103, Lab Report No.: ALT.IR.2018.1 Wageningen Environmental Research, The Netherlands GLP Unpublished	N	XXXX
KCP 10.2.1/03	Shaw, A.C.	2021a	Mandestrobin - Full Life-Cycle Toxicity Test with Daphnids ( <i>Ceriodaphnia dubia</i> ) Under Static-Renewal Conditions XXXX. Report No.: ROW-0126, Lab Report No.: 13048.7201 Smithers, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.2.1/04	Roessink, I.	2019b	Chronic effects of the fungicide Mandestrobin to <i>Caridina parvidentata</i> XXXX. Report No.: ROW-0106, Lab Report No.: ALT.IR.2018.7 Wageningen Environmental Research, The Netherlands GLP Unpublished	N	XXXX



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 10.2.1/05	Roessink, I.	2019c	Chronic effects of the fungicide Mandestrobin to <i>Gammarus pulex</i> XXXX. Report No.: ROW-0105, Lab Report No.: ALT.IR.2018.4 Wageningen Environmental Research, The Netherlands GLP Unpublished	N	XXXX
KCP 10.2.1/06	Roessink, I.	2019d	Chronic effects of the fungicide Mandestrobin to <i>Asellus aquaticus</i> XXXX. Report No.: ROW-0104, Lab Report No.: ALT.IR.2018.2 Wageningen Environmental Research, The Netherlands GLP Unpublished	N	XXXX
KCP 10.2.1/07	Shaw, A.C.	2021b	Mandestrobin – 42-Day Toxicity Test Exposing Freshwater Amphipods ( <i>Hyaella azteca</i> ) Under Static-Renewal Conditions XXXX. Report No.: ROW-0127, Lab Report No.: 13048.7202 Smithers, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.2.1/08	Shaw, A.C.	2022	Mandestrobin – 28-Day Toxicity Test Exposing Freshwater Decapods ( <i>Palaemonetes paludosus</i> ) Under Static-Renewal Conditions XXXX. Report No.: ROW-0148, Lab Report No.: 13048.7203 Smithers, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.2.1/09	White, K., Lopez Mangas, A. and Eck, G.	2024	Mandestrobin: Species Sensitivity Distribution for the Refinement of the Chronic Risk to Aquatic Invertebrates Based on Two Approaches XXXX. Report No.: ROW-0158, Report No.: 2202311.UK0-6874 Non-GLP Unpublished	N	XXXX

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 10.2.1/10	Obert-Rauser, P.	2023a	Mandestrobin 400 g/L: Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static) XXXX. Report No.: ROW-0155 Eurofins Aquatic Ecotoxicology GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	XXXX
KCP 10.2.1/11	Obert-Rauser, P.	2023b	Mandestrobin 400 g/L: Toxicity to the Single Cell Green Alga <i>Pseudokirchneriella subcapitata</i> Hindák under Laboratory Conditions XXXX. Report No.: ROW-0154 Eurofins Aquatic Ecotoxicology GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	XXXX
KCP 10.2.1/12	White, K. and Eck, G.	2024	Mandestrobin Renewal: Position Paper on the Relevant Tier 1 Chronic Endpoint for <i>Americamysis bahia</i> Report No.: ROW-0159, Report No.: 1810776.UK0 - 5829 Non-GLP Unpublished	N	XXXX
KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01	Ansaloni, T.	2023	Mandestrobin 40SC: Acute Oral and Contact Toxicity to the Honey bees ( <i>Apis mellifera</i> L.), under Laboratory Conditions XXXX. Report No.: ROW-0157 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain GLP Unpublished	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1.1/02 & KCP 10.3.1.1.2/02	Aguilar-Alberola, J.A.	2022	S-2200 (Mandestrobin) Technical Grade: Acute Oral and Contact Toxicity Test to the Bumblebee ( <i>Bombus terrestris</i> L.), under Laboratory Conditions XXXX. Report No.: ROW-0112 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain GLP Unpublished	N	XXXX
KCP 10.3.1.2/01	Picard, C.	2018a	S-2200 (Mandestrobin): 10-Day Oral Toxicity Test with the Adult Honey Bee ( <i>Apis Mellifera</i> ) Valent U.S.A. LLC. Report No.: ROW-0102 Smithers Viscient, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.3.1.2/02	Noël, E.	2016	S-2200 25 SC - A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae). XXXX. Report No.: ROW-0099 SynTech Research France S.A.S., La Chapelle de Guinchay, France GLP Unpublished	N	XXXX
KCP 10.3.1.3/01	Picard, C.	2018b	S-2200 (Mandestrobin): Honey Bee ( <i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure Valent U.S.A. LLC. Report No.: ROW-0100 Smithers Viscient, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.3.1.3/02	Aguilar-Alberola, J.A.	2019	S-2200 (Mandestrobin) 25SC: Honey Bee ( <i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions XXXX. Report No.: ROW-0101 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain GLP Unpublished	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.1/01	Leopold, J.	2023a	Mandestrobin 40 SC: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates XXXX. Report No.: ROW-0152 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	XXXX
KCP 10.3.2.1/02	Leopold, J.	2023b	Mandestrobin 40 SC: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates XXXX. Report No.: ROW-0153 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	XXXX
KCP 10.4.1.1/01	Straube, D.	2023	Mandestrobin 40 SC: Effects on Reproduction and Growth of Earthworms <i>Eisenia andrei</i> in Artificial Soil XXXX. Report No.: ROW-0156 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	XXXX
KCP 10.7/01	Soma, M.	2017	Evaluation of Herbicidal Activity of DX-CA-S-2200 and S-2200 ROG-0007 XXXX. Non-GLP Unpublished	N	XXXX
KCP 10.7/02	Kamezaki, M.	2017	Evaluation of Insecticidal Activity of DX-CA-S-2200 and S-2200 ROG-0008 XXXX. Non-GLP Unpublished	N	XXXX

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 10.7/03	Suemoto, H.	2017	Comparison of Fungicidal Activity of DX-CA-S-2200 with S-2200 ROG-0006 XXXX. Non-GLP Unpublished	N	XXXX

\* XXXX.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1	XXXX	2011a	S-2200 25% SC – Acute Toxicity to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1, OPPTS Draft Guideline 850.1075, JMAFF 12 NohSan, No. 8147 Fish Acute Toxicity Test (2-7-1-1) and JMAFF 13 SeiSan No. 3986 ROW-0024 XXXX GLP Unpublished	N	XXXX
KCP 10.2.1	Fournier, A. E.	2011b	S-2200 25% SC – Acute Toxicity to Water Fleas ( <i>Daphnia magna</i> ) Under Static Conditions, Following OECD Guideline #202, OPPTS Draft Guideline 850.1010, The Official Journal of the European Communities L383A, Method C.2 and JMAFF 12 NohSan, No. 8147 <i>Daphnia</i> Acute Immobilization Test (2-7-2-1) and JMAFFD 13 Sei San No. 3986. ROW-0025 Smithers Viscient, Massachusetts, USA GLP Unpublished	N	XXXX

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	Softcheck, K.A.	2011	S-2200 25% SC – 72-Hour Toxicity Test with the Freshwater Green Alga, <i>Pseudokirchneriella subcapitata</i> , Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 NohSan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 ROW-0026 Smithers Viscient, Massachusetts, USA GLP Unpublished	N	XXXX
KCP 10.3.1.1.1 and KCP 10.3.1.1.2	Vergé, E.	2010	S-2200 25 SC – Acute Oral and Contact Toxicity to the Honeybee <i>Apis mellifera</i> L. in the Laboratory ROW-0023 Eurofins Agrosiences Services GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	XXXX
KCP 10.3.2	Klug, T.	2010a	S-2200 25 SC: Toxicity to the Aphid Parasitoid, <i>Aphidius rhopalosiphi</i> De Stefanie Perez (Hymenoptera, Braconidae) in the Laboratory (Rate Response Test). ROW-0021 Eurofins Agrosiences Services GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	XXXX
KCP 10.3.2	Klug, T.	2010b	S-2200 25 SC: Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) in the Laboratory (Rate Response Test) ROW-0022 Eurofins Agrosiences Services GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	XXXX
KCP 10.6	Sindermann, A.B., Porch, J.R., Krueger, H.O., Martin, K.H.	2012a	S-2200 25 SC: Toxicity Effects on the Seedling Emergence of Six Species of Plants ROW-0045 Wildlife International, Ltd., Easton, Maryland, USA GLP Unpublished	N	XXXX

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study</b> <b>Y/N</b>	<b>Owner</b>
KCP 10.6	Sindermann, A.B., Porch, J.R., Krueger, H.O., Martin, K.H.	2012b	S-2200 25 SC: Toxicity Effects on the Vegetative Vigour of Six Species of Plants ROW-0046 Wildlife International, Ltd., Easton, Maryland, USA GLP Unpublished	N	XXXX

\*XXXX

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</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study</b> <b>Y/N</b>	<b>Owner</b>

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

<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>



## **Appendix 2 Detailed evaluation of the new studies**

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

#### **A 2.1.1 KCP 10.1.1 Effects on birds**

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

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

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

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

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

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

## A 2.2 KCP 10.2 Effects on aquatic organisms

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

#### A 2.2.1.1 Study 1

<b>Data point:</b>	KCP 10.2.1/01
<b>Report author:</b>	Urann, K.
<b>Report year:</b>	2016
<b>Report title:</b>	Mandestrobin (S-2200) – Life-Cycle Toxicity Test with Mysids ( <i>Americamysis bahia</i> )
<b>Report No.:</b>	13048.6921
<b>Document No.:</b>	ROW-0096
<b>Guidelines followed in study:</b>	U.S. EPA OPPTS 850.1350 (1996)
<b>Deviations from current test guideline:</b>	<p>Compared to U.S. EPA OPPTS 850.1350 (1996):</p> <ul style="list-style-type: none"> <li>- Dissolved oxygen saturation was 32 – 97% during the study, which was at times less than the required range of 60 – 105%. Since the mysids were only exposed to dissolved oxygen levels that were less than 60% of saturation for less than 48 hours and the control met acceptable criteria for survival and offspring per female, this deviation did not have a negative impact on the results or interpretation of the study.</li> <li>- A 16-hour photoperiod with a 15-30 minute transition was used, which deviates from the guideline requirement of 14 hours with a 15-30 minute transition. This minor deviation did not impact on the validity or reliability of the study.</li> </ul>
<b>Previous evaluation:</b>	Evaluated and accepted in the 2019 Southern Zone Registration Report for S-2200 25 SC, for which France was the zRMS.
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to saltwater mysids (*Americamysis bahia*) was evaluated under flow-through conditions in the laboratory over 28 days, at five nominal concentrations between 0.031 and 0.50 mg a.s./L. An untreated control and solvent control (DMF) were tested in parallel. Two generations of saltwater mysids (F<sub>0</sub> and F<sub>1</sub>) were continuously exposed to the test substance. The test was initiated with four replicates per treatment group, each containing 20 F<sub>0</sub> mysids (80 mysids per treatment level). When sexual maturity was reached (day 13), male and female mysids were paired for the reproductive assessment, with a maximum of five pairs per replicate. Reproduction was monitored until termination on day 28. Observations for mortality and behaviour were conducted daily throughout the test and counts of offspring were performed daily after pairing of mysids on day 13. At the time an F<sub>1</sub> generation pairing chamber was established and daily thereafter for 96 hours, observations of stress, abnormal behaviour and survival were made. At test termination, the total body lengths and dry weights of all surviving F<sub>0</sub> mysids were measured.

There was a statistically significant reduction in F<sub>0</sub> survival at 28 days at the highest mean measured concentration of 0.48 mg a.s./L. Due to this significant reduction, this treatment group was excluded from all other statistical analysis (i.e. reproduction and growth parameters).

There was a significant reduction in number of offspring per female, average body length of male and female F<sub>0</sub> mysids and mean dry body weight of F<sub>0</sub> females at 0.24 mg a.s./L.

There were no significant effects on F<sub>1</sub> survival at 96 hours post-release at any treatment level.

Based on male and female length, female dry weight, and offspring per female (the most sensitive indicators of toxicity), the NOEC was determined to be 0.13 mg a.s./L.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.5%
2. **Controls:** Negative control: dilution water  
Solvent control: dilution water + dimethylformamide (DMF)
3. **Reference item:** Not applicable.

### B. STUDY DESIGN AND METHODS

1. **Test animals:** Saltwater mysid (*Americamysis bahia*)  
**Age:** Juveniles, < 24 hours post-release  
**Source:** In-house culture  
**Diet:** Throughout the exposure, mysids were fed live brine shrimp (*Artemia salina*) nauplii, ≤ 48 hours old (post-hydration), twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco®, a substance high in saturated fatty acids.
2. **Dilution water:** Diluted, filtered natural seawater  
**pH:** 7.5 – 7.8  
**Salinity:** 20 – 21‰
3. **Test vessels:** Glass aquaria measuring 30 × 15 × 20 cm with a 10 cm high side drain maintaining a constant exposure solution volume of approximately 4.5 L. For the first 12 days of exposure, each exposure aquarium contained one retention chamber, which were used to retain sexually immature mysids and were constructed of glass petri dishes, 10 cm in diameter, 2 cm deep, to which a 14 cm high Nitex screen collar (350 µm mesh size opening) was attached with silicone sealant. The solution volume within the retention chambers was approximately 785 mL.  
  
Once sexual maturity was reached (test day 13), male and female pairs were transferred to separate pairing chambers. Following this distribution, each exposure aquarium contained one retention chamber and five pairing chambers. Pairing chambers, used to retain sexually mature male and female organisms, were constructed of 6-cm diameter petri dishes, to which a 14 cm high Nitex screen collar (350 µm mesh size opening) was attached with silicone sealant. Solution volume within the pairing chambers was approximately 250 mL.

#### 4. Environmental conditions:

<b>Temperature:</b>	25 – 27°C
<b>pH:</b>	7.4 – 8.0
<b>Salinity:</b>	19 – 22‰
<b>Dissolved oxygen:</b>	2.31 – 6.94 mg/L (32 – 97% saturation)
<b>Photoperiod:</b>	16 hours light : 8 hours dark (260 – 370 lux)

#### 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to saltwater mysids (*Americamysis bahia*) was evaluated under flow-through conditions in the laboratory over 28 days, at five nominal concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 mg a.s./L. An untreated control and solvent control (DMF) were tested in parallel. Two generations of saltwater mysids (F<sub>0</sub> and F<sub>1</sub>) were continuously exposed to the test substance.

To initiate the test, F<sub>0</sub> mysids (juveniles, < 24 hours old) were impartially selected and distributed to beakers until all vessels contained 20 mysids. Each group of 20 mysids was then randomly distributed to one of 28 retention chambers. The exposure was initiated when the retention chambers were replaced in their respective exposure aquaria. Each exposure aquarium contained one retention chamber, yielding 20 mysids per replicate vessel and 80 for each treatment level and control (four replicates per treatment).

When sexual maturity was reached (day 13), male and female mysids were paired for the reproductive assessment, with a maximum of five pairs per replicate to initiate the reproductive phase.

During the reproductive phase, groups of 10 offspring per replicate, 40 per treatment were collected and evaluated. Offspring were removed from adult mysid chambers in each replicate vessel and placed in separate pairing chambers in that replicate. Chambers were established based on the number of available juvenile F<sub>1</sub> mysids; therefore, each chamber was not necessarily initiated on the same day. If ten offspring could not be collected, a reduced number of F<sub>1</sub> mysids were collected and evaluated. One F<sub>1</sub> group was established and monitored for each replicate vessel, with the exception of two replicates in the 0.25 mg/L treatment level and all replicates in the 0.50 mg/L treatment level. The chambers with F<sub>1</sub> mysids were established to monitor survival 96 hours post-release. This observation period ensured an equal observation period across all treatment levels and the control prior to exposure termination.

#### 6. Dose preparation:

A 50 mg/mL diluter stock solution was prepared prior to exposure initiation and as needed throughout the exposure thereafter by placing, for example, 5.3816 g of mandestrobin (5.0318 g as active ingredient) in a 100 mL volumetric flask and bringing it to volume with DMF. The resulting stock solution was observed to be clear and yellow in colour with no visible undissolved test substance following mixing by inversions of the flask and approximately ten minutes of sonication.

An 80 µL/mL solvent control stock solution was prepared prior to exposure initiation and as needed throughout the exposure thereafter by diluting 80 mL of DMF to 1000 mL with reagent grade water. The resultant stock solutions were observed to be clear and colourless following preparation.

Prior to exposure initiation, the system was calibrated to deliver 0.0194 mL/cycle of the 50 mg/mL diluter stock solution to the diluter system's mixing chamber which also received 1.938 L of dilution water each cycle. The mixing chamber was positioned over a magnetic stir plate which aided in the solubilization of the test substance into the dilution water. The solution contained in the mixing chamber constituted the highest nominal test concentration (0.50 mg/L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (0.25, 0.13, 0.063 and 0.031 mg/L).

The concentration of DMF in the solution in the mixing chamber, also the highest test concentration, constituted the highest DMF concentration (0.010 mL/L). The DMF concentration in the solvent control and the treatment levels was 0.010 mL/L, which was equal to that of the highest test concentration.

## **7. Measurements and observations:**

The number of dead and living  $F_0$  mysids were counted daily and any abnormal appearance or behaviour was recorded. Due to the rapid movement of mysids in a single chamber containing up to 20 mysids, survival of the test organisms was estimated for the first 12 days of the test, i.e. prior to pairing. After pairing (day 13), definitive counts of survival were made and the number of dead males and females, the number of offspring produced by each individual female and any abnormal appearance or behaviour was recorded. Observations were performed daily throughout the study. Reproductive success was calculated for each replicate aquarium (treatments and the controls) as

the total number of offspring produced per female. In addition, the percentage of actively reproducing females in each replicate of each treatment and the control was determined. At the time an  $F_1$  generation pairing chamber was established and daily thereafter for 96 hours, observations of stress, abnormal behaviour (including discoloration, immobilization and inability to maintain position in the water column) and survival were made.

At test termination on day 28, all mysids were euthanised and separated into male and female groups for each replicate exposure level. A digital photograph was taken of each mysid using a binocular microscope to measure individual body length. Mysids were then dried in an oven at 98 to 100°C for approximately 24 hours and placed in a desiccator. Individual dry body weight was determined.

Prior to the start of the definitive exposure, samples were removed from one replicate of each treatment level, the solvent control and the control and analysed for mandestrobin concentration. Results of the pre-test analysis were used to determine if the appropriate concentrations of test substance were being maintained in the exposure aquaria to initiate the definitive exposure.

During the in-life phase of the definitive study, samples were removed from alternating replicate solutions of each treatment level, the solvent control and the control on days 0, 7, 14, 21 and 28 for analysis of mandestrobin concentrations. Analysis was performed using high performance liquid chromatography with ultraviolet detection (HPLC/UV)

Temperature, dissolved oxygen concentrations, pH and salinity were measured in each replicate on day 0 and alternated between replicates daily thereafter throughout the exposure period, for each treatment level and the control. In addition, exposure solution temperature was continuously monitored in one control vessel.

## **8. Statistics:**

The performance of the negative control was compared to the solvent control using the Equal Variance Two-Sample t-Test to determine any statistically significant differences. No significant difference was detected for all measured parameters, with the exception of total female body length. Therefore, comparison of treatment data for all parameters, except total female body length, was performed using the pooled control data. Total female body length data was compared to the solvent control data.

Data were assessed for normal distribution using the Shapiro-Wilk's test, and the Bartlett's test or Variance Ratio F Test were used to assess homogeneity of variance. All data met the assumptions of normal distribution, homogeneity and were non-monotonic; therefore, Dunnett's Multiple Comparison Test was used to evaluate the data.

Data for the survival endpoints (e.g. 28-day survival, male and female survival and  $F_1$  survival) were analysed using Fisher's Exact Test with Bonferroni-Holm's Adjustment.

If possible,  $EC_x$  values were calculated using a regression model.

CETIS (Ives, 2013) was used to perform the statistical computations.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

#### Survival and behaviour of F<sub>0</sub> mysids

A summary of the first generation (F<sub>0</sub>) survival and reproductive success data is presented in Table 10.2.1/01-1. Statistical analysis of survival of F<sub>0</sub> mysids included survival data post-pairing only.

During the reproductive phase (from pairing to day 28), there were no significant effects on male or female survival compared to the pooled control. LC<sub>x</sub> values could not be determined for male or female survival, since the test concentrations did not bracket the predicted LC<sub>x</sub> values and since no confidence limits could be calculated.

Following 28 days of exposure, there was a significant difference in survival among mysids exposed to the 0.48 mg a.s./L treatment compared to the pooled control. Due to the significantly reduced survival, this group was excluded from further statistical analysis (i.e. of reproduction and growth parameters).

No behavioural abnormalities were observed during the exposure period.

A summary of endpoints for F<sub>0</sub> survival are shown in Table 10.2.1/01-4.

#### Reproduction of F<sub>0</sub> mysids

Due to the significantly reduced survival at the highest treatment level of 0.48 mg a.s./L, this group was excluded from statistical analysis of reproduction.

There was a statistically significant reduction in the mean number of offspring per female at 0.24 mg a.s./L compared to the pooled control. The EC<sub>10</sub> value could not be determined, since the lower confidence limit could not be calculated. The EC<sub>20/50</sub> values for reproduction, as well as the NOEC and LOEC, are presented in Table 10.2.1/01-4.

**Table 10.2.1/01-1: Summary of first generation (F<sub>0</sub>) survival and reproduction**

Mean measured concentration (mg a.s./L)	Male survival (%) <sup>a)</sup>	Female survival (%) <sup>a)</sup>	Post-pairing survival (%) <sup>b)</sup>	28-day survival (%) <sup>b)</sup>	% of females producing young	Number of offspring/female
Negative control	80	94	89	86	95	11.2
Solvent control	90	82	86	81	100	9.8
Pooled control	85	88	88	83	98	10.5
0.035	73	95	84	78	100	8.5
0.058	86	97	89	77	90	9.4
0.13	84	89	86	82	95	8.0
0.24	73	80	74	72	88	5.5*
0.48 <sup>c)</sup>	86	87	86	56*	31	2.0

<sup>a)</sup> Calculations of male and female survival began after pairing.

<sup>b)</sup> Calculations of survival are of both male and female mysids combined.

<sup>c)</sup> Due to significantly reduced survival, this treatment level was excluded from statistical analyses (other than survival).

\* Statistically reduced compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment for 28-day survival, Dunnett's Multiple Comparison Test for number of offspring per female).

#### Growth of F<sub>0</sub> mysids

A summary of the F<sub>0</sub> growth data (length and dry weight) is shown in Table 10.2.1/01-2. Due to the significantly reduced survival at the highest treatment level of 0.48 mg a.s./L, this group was excluded from statistical analysis of length and dry weight. For the statistical analyses, male length and dry weight data were compared to the pooled control, while female data were compared to the solvent control.

There was a statistically significant reduction in average total body length of male mysids at 0.24 mg a.s./L compared to the pooled control. At the same treatment level, there was also a significant reduction in average total body length of female mysids, compared to the solvent control. EC<sub>x</sub> values could not be

determined for the average total body length of male or female mysids, since there was a significant lack of fit in both regression models used.

There was no statistically significant difference in male dry body weight at any treatment level compared to the pooled control; however, there was a significant reduction in dry body weight of female mysids exposed to the 0.24 mg a.s./L treatment level.

EC<sub>x</sub> values could not be determined for average dry body weight of male mysids, since the test concentrations did not bracket the predicted EC<sub>x</sub> values and since one or both of the confidence limits could not be calculated. The calculated endpoints for dry weight of female mysids are shown in Table 10.2.1/01-4.

**Table 10.2.1/01-2: Summary of first generation (F<sub>0</sub>) mean body length and dry weight after 28 days exposure**

Mean measured concentration (mg a.s./L)	Mean body length (mm)		Mean dry body weight (mg)	
	Males	Females	Males	Females
Negative control	7.09	7.28	0.79	1.06
Solvent control	7.03	7.14	0.82	1.02
Pooled control	7.06	n.a. <sup>a)</sup>	0.80	1.04
0.035	7.05	7.19	0.79	1.03
0.058	6.92	6.99	0.82	1.06
0.13	6.99	7.09	0.79	1.06
0.24	6.81*	6.90*	0.80	0.93*
0.48 <sup>b)</sup>	7.18	7.21	0.75	0.87

n.a.: not applicable.

<sup>a)</sup> Female length data was not pooled.

<sup>b)</sup> Due to significantly reduced survival, this treatment level was excluded from statistical analyses.

\* Statistically significant difference compared to the pooled control (male length and female dry weight) and solvent control (female length), based on Dunnett's Multiple Comparison Test.

### Survival of F<sub>1</sub> mysids (96 hours post-release)

There were no statistically significant differences in F<sub>1</sub> mysid survival among organisms exposed at any treatment level compared to the pooled control. EC<sub>x</sub> values could not be determined for the F<sub>1</sub> generation 96-hour post-release survival, since no models could be fit to the data.

**Table 10.2.1/01-3: F<sub>1</sub> survival at 96 hours post-release**

Mean measured concentration (mg a.s./L)	Mean F <sub>1</sub> survival at 96 hours post-release (%)
Negative control	95
Solvent control	98
Pooled control	96
0.035	88
0.058	90
0.13	98
0.24	94
0.48 <sup>a)</sup>	n.a.

n.a.: not applicable.

<sup>a)</sup> No F<sub>1</sub> populations were established at this treatment level.

### Overall endpoints

The endpoints for each parameter are shown in Table 10.2.1/01-4 below.

**Table 10.2.1/01-4: Summary of endpoints**

Endpoint	Based on mean measured concentrations (mg a.s./L)				
	LC <sub>10</sub> /EC <sub>10</sub> (95% CL)	LC <sub>20</sub> /EC <sub>20</sub> (95% CL)	LC <sub>50</sub> /EC <sub>50</sub> (95% CL)	NOEC	LOEC
Male survival (pairing to D28)	n.d.	n.d.	n.d.	0.48	> 0.48
Female survival (pairing to D28)	n.d.	n.d.	n.d.	0.48	> 0.48
28-day survival	0.15 (0.071 – 0.23)	0.31 (0.18 – 0.45)	> 0.48 (n.d.)	0.24	0.48
Offspring per female	n.d.	0.110 (0.049 – 0.17)	0.260 (0.19 – 0.32)	0.13	0.24
Male length	n.d.	n.d.	n.d.	0.13	0.24
Female length	n.d.	n.d.	n.d.	0.13	0.24
Male body weight	n.d.	n.d.	n.d.	0.24	> 0.24
Female body weight	0.26 (0.18 – 0.35)	n.d.	n.d.	0.13	0.24
F <sub>1</sub> survival at 96 hours post-release	n.d.	n.d.	n.d.	0.24	> 0.24

n.d.: not determined.

## B. ANALYSIS

Measured concentrations of mandestrobin were generally consistent between sampling intervals and the expected concentration gradient (50% dilution series) was maintained throughout the exposure. Mean measured mandestrobin concentrations ranged from 92 to 110% of nominal concentrations (see Table 10.2.1/01-5).

**Table 10.2.1/01-5: Summary of analytical results**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)					Mean measured concentration (mg a.s./L)	% of nominal
	Day 0	Day 7	Day 14	Day 21	Day 28		
Negative control	< MDL	< MDL	< MDL	< MDL	< MDL	n.a.	n.a.
Solvent control	< MDL	< MDL	< MDL	< MDL	< MDL	n.a.	n.a.
0.031	0.038	0.037	0.042	0.028	0.030	0.035	110
0.063	0.065	0.064	0.068	0.045	0.049	0.058	92
0.13	0.15	0.14	0.15	0.11	0.12	0.13	100
0.25	0.26	0.26	0.27	0.19	0.22	0.24	95
0.50	0.55	0.54	0.60	0.33	0.40	0.48	97

MDL: Minimum detectable limit (0.0040 mg a.s./L); n.a.: not applicable.

## C. VALIDITY CRITERIA

The study was compared to the acceptability criteria outlined in the most recent test guideline (U.S. EPA OPPTS 850.1350, 1996), as detailed below:



- Less than 25% of first generation females in the control groups failed to produce young (actual: 5% and 0% in the negative and solvent controls, respectively).
- The average number of young produced per female in the control groups should be greater than 3. In this study, the number of offspring produced per female was 11.2 in the control and 9.8 in the solvent control.

### III. CONCLUSION

The chronic toxicity of mandestrobin to saltwater mysids (*Americamysis bahia*) was evaluated under flow-through conditions in the laboratory over 28 days. The results of the study are reported based on the mean measured concentrations.

There was a statistically significant reduction in F<sub>0</sub> survival at 28 days at the highest mean measured concentration of 0.48 mg a.s./L. Due to this significant reduction, this treatment group was excluded from all other statistical analysis (i.e. reproduction and growth parameters).

There was a significant reduction in number of offspring per female, average body length of male and female F<sub>0</sub> mysids and mean dry body weight of F<sub>0</sub> females at 0.24 mg a.s./L.

There were no significant effects on F<sub>1</sub> survival at 96 hours post-release at any treatment level.

Based on male and female length, female dry weight, and offspring per female (the most sensitive indicators of toxicity), the NOEC was determined to be 0.13 mg a.s./L.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>Based on the mean measured concentrations, the relevant endpoint derived from the original study is:</p> <p>NOEC: 0.13 mg a.s./L (growth and reproduction)</p>
Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met. Some deviations from study protocol were noted, they are not ecotoxicologically relevant.</p> <p>The following endpoints were derived: NOEC: 0.13 mg a.s./L (growth and reproduction) EC<sub>10</sub> = 0.094 mg a.s./L</p>

### A 2.2.1.2 Study 2

<b>Data point:</b>	KCP 10.2.1/02
<b>Report author:</b>	Roessink, I.
<b>Report year:</b>	2019a
<b>Report title:</b>	Chronic Effects of the Fungicide Mandestrobin to <i>Daphnia pulex</i>
<b>Report No.:</b>	ALT.IR.2018.1
<b>Document No.:</b>	ROW-0103
<b>Guidelines followed in study:</b>	OECD 211 (2012)
<b>Deviations from current test guideline:</b>	<p>Compared to OECD 211 (2012):</p> <ul style="list-style-type: none"> <li>- The study was performed with <i>Daphnia pulex</i> rather than <i>Daphnia magna</i>. The two species have different biological characteristics which affected the validity criteria of OECD 211, which is specific to <i>Daphnia magna</i> (see Section C, Validity Criteria, for full explanation). As the validity criteria are specific to <i>D. magna</i>, this is not considered to invalidate the study.</li> <li>- Immediately after initiation of the study, several replicates per treatment level showed non-treatment related mortality. As a result, these replicates were re-started 4 days later. The results were appropriately integrated for statistical analysis and therefore this is not considered to have impacted on the reliability of the study.</li> </ul>
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The chronic toxicity of mandestrobin to *Daphnia pulex* was determined over 28 days under semi-static conditions. Initially the test was planned to last 21 days; however, on day 21, less than 60 offspring per surviving female were present, therefore the test was prolonged to 28 days.

Daphnids were exposed to five nominal concentrations between 0.10 and 10 mg a.s./L. An untreated control and solvent control (acetone) were tested in parallel. Fifteen replicates were set up per test item treatment, control and solvent control, each containing one individual daphnid. Medium refreshment and spiking with mandestrobin was conducted three times per week with the same dosage as on day 0. Measured parameters included parental mortality, reproduction and parental growth based on length and weight at the end of the test. Length and dry weight biomass were measured for 30 representative test individuals at the start of the test. The results of the study were reported based on the time-weighted average (TWA) concentrations.

The most sensitive parameters were dry weight of survivors and reproduction. The 28-day EC<sub>10/20/50</sub> values for dry weight were 0.54, 0.99 and 2.8 mg a.s./L, respectively, and the NOEC was 0.92 mg a.s./L. The 28-day EC<sub>10/20/50</sub> values for reproduction were 0.82, 0.93 and 1.2 mg a.s./L and the NOEC was 0.92 mg a.s./L. The 28-day LC<sub>10/20/50</sub> values were determined to be 2.5, 2.6 and 2.9 mg a.s./L, respectively, and the NOEC for adult survival was 2.9 mg a.s./L.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not stated  
**Lot/Batch:** ST-0811G  
**Purity:** 93.1%
2. **Controls:** Negative control: dilution water  
Solvent control: dilution water + acetone

### B. STUDY DESIGN AND METHODS

1. **Test organism:** *Daphnia pulex*  
**Age:** ≤ 24 hours old  
**Source:** In-house culture  
**Diet:** Daphnids were fed with *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*) algae, which was administered at every medium refreshment (three times per week).
2. **Dilution water:** RT medium
3. **Test vessels:** 100 mL glass tubes, each containing 80 mL test solution
4. **Environmental conditions:**  
**Temperature:** 17.6 – 20.7°C  
17.6 – 20.8°C (re-started replicates)  
**pH:** 7.5 – 9.2  
7.5 – 9.1 (re-started replicates)  
**Dissolved oxygen:** 5.9 – 12.1 mg/L (62.9 – 129.1% saturation)  
5.90 – 12.15 (62.7 – 129.4% saturation) (re-started replicates)  
**Electrical conductivity:** 226.0 – 316.8 µS/cm  
226.0 – 317.5 (re-started replicates)  
**Photoperiod:** 16 hours light : 8 hours dark ( $15.6 \pm 1.0 \mu\text{mol s}^{-1} \text{m}^{-2}$ )

### 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to *Daphnia pulex* was determined over 28 days under semi-static conditions. Initially the planned study duration was 21 days; however, after this time, less than 60 offspring per surviving female were present, therefore the test was prolonged to 28 days.

Daphnids were exposed to five nominal concentrations of 0.10, 0.30, 1.0, 3.0 and 10 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Fifteen replicates were set up per test item treatment, control and solvent control, each containing one individual daphnid. To keep the exposure sufficiently worst-case, medium refreshment and spiking with mandestrobin was conducted three times per week with the same dosage as on day 0.

Immediately after initiation of the study, several replicates per treatment level showed non-treatment related mortality. As a result, these replicates were re-started 4 days later. Consequently, the individual results from the original and restarted replicates were integrated based on nominal days post start of the test in the statistical analysis (calculation of effect concentrations and NOEC values).

### 6. Dose preparation:

To dose the different treatment levels, different stock solutions were prepared. The preparation and concentrations of the stock solutions used to dose the test vessels is shown in Table 10.2.1/02-1 below.

**Table 10.2.1/02-1: Preparation of stock solutions**

Code stock		Concentration (mg a.s./L)	Mass of mandestrobin TG added (g)	Mass acetone added (g)	Final volume stock (mL)
MDB2-6	Intended	100,000	3.222	23.73	30
	Actual	99,993.81	3.2224	23.7319	30.0024
MDB2-5	Intended	30,000	1.611	39.55	50
	Actual	30,010.43	1.6120	39.5566	50.008
MDB2-4	Intended	10,000	0.537	39.55	50
	Actual	9949.62	0.5344	39.5536	50.005
MDB2-3	Intended	3000	0.161	39.55	50
	Actual	3083.59	0.1660	39.6440	50.119
MDB2-2	Intended	1000	0.054	39.55	50
	Actual	1041.47	0.0562	39.7390	50.239
Code		Intended concentration (mg a.s./L)	Intended mass stock MDB-4 added (g)	Mass of final volume (after addition with acetone) (g)	Final volume stock
MDB2-1	Intended	300	3.955	39.55	50
	Actual	308.34	3.9916	39.5797	50.038

The dose solutions were made once and all were stored in the freezer for later use. After manual shaking of the stock solution, a subsample of approximately 4.0 mL was transferred into a 4 mL vial. Per treatment level, one vial was used for dosing the test systems.

On days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28 new test medium was created. During application, the freshly made dosage solution was added to a 2 L volume of RT medium using a Multipettor, after which the medium was stirred with a magnetic stirrer. The volume of 2 L was created per treatment level after which the individual 100 mL tubes were filled from the dosed medium.

## 7. Measurements and observations:

Before the start of the experiment, 30 individuals were used as a representative sample for length and weight measurements. The daphnids were individually measured with a stereo microscope. Since the animals were too small to be weighed individually, their weight was determined per five (twice) or ten individuals (twice) using a microbalance.

Parental mortality and number of offspring were observed on day 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28 in test tubes of all treatments. Daphnids were classed as dead when an animal was immobile, i.e. when it was not able to swim, or if there was no observed movement of appendages or post abdomen, within 15 seconds after gentle agitation of the test container.

At the end of the experimental period, length and dry weight were determined in all surviving parental daphnids. To measure length, daphnids were photographed using a stereomicroscope and measurements made using ImageJ. For dry weight estimation, the surviving individuals from each test vessel were dried at 105°C for 2 hours. The dry weight of these individuals per vessel was weighed on the micro-balance.

Temperature, electrical conductivity, pH and dissolved oxygen were measured in the test tubes of the controls and highest treatment level, directly before medium refreshment and at the end of the test. At the start of the experiment and prior to each medium refreshment, conductivity, pH and dissolved oxygen were measured in the vessel with the fresh stock of exposure medium used to refresh the medium of all test systems.

For analysis of test substance in the test system, in both the control and treated systems, 3 mL samples were taken immediately after mandestrobin application and before refreshment of the exposure medium on days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28. The analytical method was carried out by means of triple quadrupole LC-MS (LC-QQQ).

## 8. Statistics:

NOEC and LC<sub>x</sub> values were calculated for the endpoints parental survival (mortality) and number of offspring on sampling days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28. It should be noted that on day 0, 2 and 4, no offspring were present yet.

E(L)C<sub>10/20/50</sub> values and 95% confidence limits were calculated by a log concentration-logit effect regression method. Within the regression, calculated E(L)C<sub>x</sub> values were corrected for mortality in the controls. The regression method was programmed in GENSTAT (version 19).

NOEC estimations ( $p \leq 0.05$ ) were carried out using ToxRat Professional (v3.2). It should be noted that this program automatically selects the most appropriate test for the data (either quantal or metric responses) from an array available in the software. At the end of the prolonged test period, mortality of *Daphnia pulex* in the controls (without solvent) was 40% and consequently this treatment level was not used and further statistical analysis and discussion of the results was performed with the solvent control only.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

On day 21, mortality in the control and solvent controls comprised 20.0 and 13.3%, respectively, and on day 28, mortality in the control and solvent controls comprised 40.0 and 20.0%, respectively. Due to the high mortality in the control (without solvent), this control was not used and test item treatments were statistically compared to the solvent control.

100% mortality occurred in the highest TWA treatment level of 9.7 mg a.s./L by the 21-day assessment, which was statistically significant compared to the solvent control. No significant effects on mortality were observed at any other treatment level at either the 21- or 28-day assessments. Effects on mortality at 21 and 28 days are shown in Table 10.2.1/02-2 and relevant endpoints are shown in Table 10.2.1/02-4.

After 21 days, less than 60 offspring per surviving female were present, therefore the test was prolonged to 28 days.

As 100% mortality was observed at the highest concentration, reproduction was not assessed for this treatment level. A statistically significant reduction in reproduction was observed at a TWA concentration of 2.8 mg a.s./L at both the 21- and 28-day assessments. No significant effects on reproduction were observed at any other treatment level. Effects on reproduction are shown in Table 10.2.1/02-2 and relevant endpoints are shown in Table 10.2.1/02-4.

**Table 10.2.1/02-2: Summary of effects on adult survival and reproduction**

TWA concentration (mg a.s./L)	% Mortality (D21)	% Mortality (D28)	Mean no. of offspring (D21)	Mean no. of offspring (D28)
Control	20.0	40.0	n.r.	n.r.
Solvent control	13.3	20.0	35.23	57.91
0.095	33.3	40	36.2	62
0.29	40	40	41.78	66.33
0.92	20	20	27.92	48.17
2.8	46.7	60	0.13*	0.17*
9.7	100*	100*	n.a.	n.a.

n.a.: not applicable; n.r.: not reported.

\* Significantly reduced compared to the solvent control.

Statistically significant reductions in mean body length, mean dry weight and mean growth rate (based on both length and weight) were observed at 2.8 mg a.s./L. No significant reductions were observed at any other treatment level.

As 100% mortality was observed at the highest treatment level of 9.7 mg a.s./L, no analysis of body length or dry weight was performed at this concentration. Effects on body length and dry weight are shown in Table 10.2.1/02-3 and relevant endpoints are shown in Table 10.2.1/02-4.

**Table 10.2.1/02-3: Summary of effects on growth on day 28**

<b>TWA concentration (mg a.s./L)</b>	<b>Mean body length (mm)</b>	<b>Mean growth rate (mm/day)</b>	<b>Mean dry weight (mg)</b>	<b>Mean growth rate (mg/day)</b>
Control	2.36	0.05	0.11	0.03
Solvent control	2.36	0.05	0.11	0.02
0.095	2.41	0.05	0.11	0.02
0.29	2.28	0.05	0.12	0.03
0.92	2.2	0.04	0.09	0.02
2.8	1.64*	0.03*	0.06*	0*
9.7	n.a.	n.a.	n.a.	n.a.

n.a.: not applicable.

\* Significantly reduced compared to the solvent control.

**Table 10.2.1/02-4: Summary of endpoints based on nominal and TWA concentrations**

Parameter	Endpoint	Based on nominal concentrations (mg a.s./L)	Based on TWA concentrations (mg a.s./L)
21-day adult survival	LC <sub>10</sub> (95% CI)	2.8 <sup>a)</sup>	2.6 (2.2 – 3.1)
	LC <sub>20</sub> (95% CI)	2.9 <sup>a)</sup>	2.8 (2.3 – 3.3)
	LC <sub>50</sub> (95% CI)	3.2 <sup>a)</sup>	3.1 (2.6 – 3.7)
	NOEC	3.0	2.9
28-day adult survival	LC <sub>10</sub> (95% CI)	2.6 (2.3 – 3.0)	2.5 (2.2 – 2.8)
	LC <sub>20</sub> (95% CI)	2.8 (2.4 – 3.2)	2.6 (2.3 – 3.0)
	LC <sub>50</sub> (95% CI)	3.1 (2.7 – 3.5)	2.9 (2.6 – 3.3)
	NOEC	3.0	2.9
Mean individual dry weight of survivors (mg)	EC <sub>10</sub> (95% CI)	0.58 (0.090 - 3.9)	0.54 (0.080 - 3.7)
	EC <sub>20</sub> (95% CI)	1.1 (0.30 - 3.7)	0.99 (0.28 - 3.6)
	EC <sub>50</sub> (95% CI)	2.9 (1.4 - 5.9)	2.8 (1.4 - 5.7)
	NOEC	1.0	0.92
Mean individual length of survivors (mm)	EC <sub>10</sub> (95% CI)	1.3 (0.41 - 3.8)	1.1 (0.35 - 3.6)
	EC <sub>20</sub> (95% CI)	2.1 (1.2 - 3.8)	1.9 (1.0 - 3.6)
	EC <sub>50</sub> (95% CI)	5.1 (2.3 - 11)	4.9 (2.2 - 11)
	NOEC	1.0	0.92
Mean individual daily growth in dry weight of survivors (mg/day) <sup>b)</sup>	NOEC	1.0	0.92
Mean individual daily growth in length of survivors (mm/day)	EC <sub>10</sub> (95% CI)	1.3 (0.36 - 4.5)	1.1 (0.31 - 4.2)
	EC <sub>20</sub> (95% CI)	2.1 (1.1 - 4.2)	2.0 (0.98 - 4.0)
	EC <sub>50</sub> (95% CI)	5.2 (2.1 - 13)	5.1 (1.9 - 13)
	NOEC	1.0	0.92
21-day no. of offspring per surviving female	EC <sub>10</sub> (95% CI)	0.85 (0.59 – 1.2)	0.77 (0.52 – 1.1)
	EC <sub>20</sub> (95% CI)	0.96 (0.72 – 1.3)	0.88 (0.66 – 1.2)
	EC <sub>50</sub> (95% CI)	1.2 (0.89 – 1.6)	1.1 (0.81 – 1.5)
	NOEC	1.0	0.92
28-day no. of offspring per surviving female	EC <sub>10</sub> (95% CI)	0.90 (0.62 – 1.3)	0.82 (0.57 – 1.2)
	EC <sub>20</sub> (95% CI)	1.0 (0.75 – 1.4)	0.93 (0.68 – 1.3)
	EC <sub>50</sub> (95% CI)	1.2 (0.87 – 1.8)	1.2 (0.79 – 1.7)
	NOEC	1.0	0.92

<sup>a)</sup> No convergence of the model, therefore no confidence limits are reported.

<sup>b)</sup> EC<sub>10/20/50</sub> values for this parameter could not be calculated.

CI: Confidence intervals.

## B. ANALYSIS

Measured mandestrobin concentrations in the dosing solutions ranged between 91 to 121% of nominal concentrations.

In the test systems, mandestrobin concentrations remained relatively stable throughout the experiment, and overall measured concentrations comprised 95.2% (± 2.0%) of nominal concentrations when expressed as TWA concentrations.

On a few occasions, mandestrobin could be detected in the control and solvent control. However, these concentrations, comprised on average 0.9 and 0.5% of the lowest treatment level (0.10 mg a.s./L) or 0.06 to 0.07% when expressed as TWA concentrations over the whole experimental period. As this would translate into respectively 0.006 and 0.007 mg a.s./L, these levels were considered to be very low. Because

the lowest effect concentration established (28-day EC<sub>10</sub> for dry weight of survivors) was almost two orders of magnitude greater, the levels observed in the controls were considered ecotoxicologically not relevant and were not considered to have impacted on the study. A summary of the TWA concentrations are shown in Table 10.2.1/02-5.

**Table 10.2.1/02-5: Calculated time weighted average concentrations of mandestrobin over the whole experimental period**

Nominal concentration (mg a.s./L)	Mean TWA concentration (mg a.s./L)	Mean TWA concentration (% of nominal)
Control	0.000057	0.06 <sup>a)</sup>
Solvent control	0.000072	0.07 <sup>a)</sup>
0.1	0.095	94.9
0.3	0.29	97.6
1.0	0.92	91.8
3.0	2.8	94.7
10.0	9.7	96.7

<sup>a)</sup> Expressed as a percentage of the lowest treatment level (0.1 mg a.s./L).

### C. VALIDITY CRITERIA

The study was compared to the validity criteria outlined in the most recent version of the EU test guideline (OECD 211, 2012) detailed below:

- Mortality of the parent animals (female *Daphnia*) in the control should not exceed 20% at the end of the test.
- The mean number of living offspring produced per parent animal surviving in the control at the end of the test should be  $\geq 60$ .

On day 21, the observed mortality in the control and solvent control never exceeded 20% (20.0 and 13.3%, respectively). Therefore, the test was considered to fulfil this validity criterion at this date. However, on this date, the number of offspring in both the control and solvent control comprised 35 individuals. Since this did not meet the criterion for  $\geq 60$  offspring per female, the test was prolonged to 28 days. After 28 days, however, the number of offspring per surviving female comprised 55 and 57 in the control and solvent control, respectively.

Wu *et al.* (2007)<sup>2</sup> reports that the lifespan of *D. pulex* approximates 22.2 ( $\pm 1.93$ ) days and that number of offspring per female approximates 34.4 ( $\pm 11.94$ ). Based on this published data, the validity criterion of 60 offspring per female is considered to be specific to *Daphnia magna* and not suitable for *D. pulex*. As a result, in the present study, the criterion for reproduction was replaced by the value reported by Wu *et al.* (2007). Based on the revised values, the test meets the validity criteria on both day 21 and day 28 (35.23 and 57.91 on day 21 and 28 respectively in the solvent control).

Consequently, the assessment endpoints parental mortality and number of offspring per female on day 21 in both the control and solvent control are considered suitable for use. However, on day 28, mortality in the control and solvent control had increased to 40 and 20%, respectively. Since the 28-day mortality in the solvent control was considered to be in accordance with the validity criteria, the solvent control data were used for statistical comparison with the test item treatments, and the study is considered suitable for use.

### III. CONCLUSION

The chronic toxicity of mandestrobin to *Daphnia pulex* was determined over 28 days under semi-static conditions. Initially the test was planned to last 21 days; however, on day 21, less than 60 offspring per surviving female were present, and the test was prolonged to 28 days. The results of the study were reported based on the time-weighted average (TWA) concentrations.

<sup>2</sup> Y., Lin, C. and Yuan, L. (2007). "Characteristics of six cladocerans in relation to ecotoxicity testing." *Ecological Indicators* 7(4): 768-775.



The most sensitive parameters were dry weight of survivors and reproduction. The 28-day EC<sub>10/20/50</sub> values for dry weight of survivors were 0.54, 0.99 and 2.8 mg a.s./L, respectively, and the NOEC was 0.92 mg a.s./L. The 28-day EC<sub>10/20/50</sub> values for reproduction were 0.82, 0.93 and 1.2 mg a.s./L and the NOEC was 0.92 mg a.s./L.

The 28-day LC<sub>10/20/50</sub> values were determined to be 2.5, 2.6 and 2.9 mg a.s./L, respectively, and the NOEC for adult survival was 2.9 mg a.s./L.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>Based on the time weighted average concentrations, the relevant 28-day endpoint derived from the study is:</p> <p>NOEC: 0.92 mg a.s./L (reproduction, dry weight and length)</p>
---	---

Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met.</p> <p>The minor deviations were noted, not ecotoxicologically relevant.</p> <p>The following endpoints were derived:</p> <p>NOEC: 1.00 mg a.s./L (based on nominal concentration)</p> <p>NOEC: 0.92 mg a.s./L (based on TWA concentration)</p> <p>EC<sub>10</sub> = 0.54 mg a.s./L (based on TWA concentration)</p>
-------------------	--

### A 2.2.1.3 Study 3

<b>Data point:</b>	KCP 10.2.1/03
<b>Report author:</b>	Shaw, A.C.
<b>Report year:</b>	2021a
<b>Report title:</b>	Mandestrobin - Full Life-Cycle Toxicity Test with Daphnids ( <i>Ceriodaphnia dubia</i> ) Under Static-Renewal Conditions
<b>Report No.:</b>	13048.7201
<b>Document No.:</b>	ROW-0126
<b>Guidelines followed in study:</b>	U.S. EPA Test Method 1002.0
<b>Deviations from current test guideline:</b>	None/not applicable.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to *Ceriodaphnia dubia* was determined over 7 days under semi-static conditions. Daphnids were exposed to five nominal concentrations between 0.16, and 2.5 mg a.s./L (mean measured concentrations between 0.17 and 2.7 mg a.s./L). An untreated control and solvent (acetone) control were tested in parallel. Ten replicates were set up per treatment group and control each containing a single daphnid. Test medium was renewed daily. Observations for immobility and abnormal behaviour were performed daily. Offspring production was measured upon the first brood release and daily until three broods had been counted. Total body length of each surviving daphnid was determined on day 7. The results of the study were reported based on the mean measured concentrations.

Based on the results for parental survival, reproduction, and growth (measured as mean total length), the overall NOEC was determined to be 0.63 mg a.s./L (reproduction and body length). The EC<sub>50</sub> value for survival was calculated to be 2.0 mg a.s./L, the EC<sub>50</sub> for reproduction was 1.0 mg a.s./L and the EC<sub>50</sub> for body length was >1.4 mg a.s./L.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.7%
- Controls:** Negative control: dilution water  
 Solvent control: dilution water + acetone
- Reference item:** Not applicable.

### B. STUDY DESIGN AND METHODS

- Test animals:** *Ceriodaphnia dubia*  
**Age:** < 24 hours old, within 8 hours of the same age  
**Source:** In-house culture

- Diet:** Unicellular green algae, *Rhaphidocelis subcapitata* ( $3 \times 10^7$  cells/mL) and a suspension of YTC (yeast, trout chow, and wheat grass). During testing a rate of 200  $\mu$ L of a 50:50 ratio was provided per test vessel.
2. **Dilution water:** Fortified well water  
**Total hardness:** 96 – 100 mg/L as  $\text{CaCO}_3$   
**Total alkalinity:** 50 – 60 mg/L as  $\text{CaCO}_3$   
**pH:** 7.4 – 7.9  
**Conductivity:** 430 – 480 mg/L as  $\mu\text{S/cm}$
3. **Test vessels:** 30 mL plastic disposable vessels each containing 15 mL of test solution
4. **Environmental conditions:**  
**Temperature:** 24 – 26°C  
**pH:** 7.3 – 7.9  
**Dissolved oxygen:** 7.3 – 8.1 mg/L (new solutions), 6.5 – 7.2 mg/L (old solutions)  
**Total hardness:** 96 – 110 mg/L as  $\text{CaCO}_3$   
**Total alkalinity:** 50 – 60 mg/L as  $\text{CaCO}_3$   
**Conductivity:** 430 – 500  $\mu\text{S/cm}$   
**Photoperiod:** 16 hours light : 8 hours dark (810 – 990 lux)

## 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to *C. dubia* was determined over 7 days under semi-static conditions. Daphnids were exposed to five nominal concentrations of 0.16, 0.31, 0.63, 1.3 and 2.5 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Ten replicates were set up per treatment group and control each containing a single daphnid.

The test was initiated when the daphnids were impartially distributed to each of the ten replicates for each nominal concentration and the controls. At exposure initiation and daily thereafter, fresh exposure solutions were prepared and added to a new set of test vessels containing approximately 15 mL of solution. During each renewal, daphnids were carefully transferred from the aged solution into the freshly prepared test solutions and the food solutions were then added.

## 6. Dose preparation:

Prior to exposure initiation, a 25 mg/mL primary stock solution was prepared by placing 0.6627 g (0.6209 g as active ingredient) of mandestrobin in a volumetric flask and bringing it to a volume of 25 mL with acetone. After mixing by inversions, the resulting stock solution was observed to be clear and yellow with no visible undissolved test substance. Secondary stock solutions were prepared as dilutions at nominal concentrations of 13, 6.3, 3.1 and 1.6 mg/mL. All resulting stock solutions were observed to be clear and yellow following preparation with decreasing yellow intensity with decreasing concentration. These stock solutions were used to prepare exposure solutions at test initiation and at each renewal interval. A volume of 0.10 mL of the appropriate stock solution was added to 1.0 L of dilution water to achieve the desired test concentrations.

A solvent control was established by adding 0.10 mL of acetone to 1.0 L of dilution water. Each test solution was mixed for approximately one minute using a glass rod. All test solutions were observed to be clear and colourless with no visible undissolved test substance present following preparation.

## 7. Measurements and observations:

The number of immobilised adult daphnids and observations of abnormal behaviour were recorded daily. Immobilisation was defined as the inability of daphnids to swim within 15 seconds of gentle agitation of the test vessel. Numbers of offspring were determined upon the first brood release in any vessel and daily until three broods had been counted. Offspring were counted and discarded at each observation interval.

Offspring from the fourth or higher broods were not counted or included in the total number of offspring produced during the exposure. The day of appearance of first brood release was recorded for each treatment level and the controls. Observations of physical characteristics of test solutions (e.g. precipitate, cloudy solution) were recorded, if applicable, whenever the test organisms were observed.

At test termination (day 7), the total body length of each surviving adult daphnid was measured. Daphnids were measured (to the nearest 0.05 mm) from the apex of the helmet to the base of the shell spine using a dissecting microscope.

Dissolved oxygen, temperature and pH were measured in each test and control solution at the beginning (new solutions) and end (aged solutions) of each renewal period, where appropriate. In addition, hardness, alkalinity and conductivity were measured in new solutions of the controls and high treatment level daily and in aged solutions of the controls and high treatment level at test termination. The temperature of the water bath was continuously monitored throughout the study in a satellite vessel.

During the definitive exposure, one sample was removed from each treatment level, negative control, and solvent control solution for analysis of mandestrobin concentration at day 0 (new), 1 (aged), 3 (new), 4 (aged), 6 (new) and 7 (aged). New solutions were collected from intermediate mixing vessels before addition to individual tests vessels and old solutions were collected from composited (pooled) replicates. All samples were analysed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

## **8. Statistics:**

At the termination of the study, data obtained on organism survival, reproduction and growth (as total body length) were statistically analysed to identify significant treatment-related effects. Analyses were performed using the individual response values. Reproduction was evaluated using total living offspring and total living offspring/surviving female.

All statistical analyses were conducted at the 95% level of certainty except in the case of the qualifying tests, in which the 99% level of certainty was applied. CETIS Version 1.9 was used to perform the statistical computations. Both the NOEC and LOEC were determined.

The negative and solvent controls were assessed for differences. Survival was assessed using Fisher's Exact Test. Reproduction and growth were assessed for normality using Shapiro-Wilk's Test and homogeneity using Variance Ratio F Test before analysis with the Two-sample t-Test. Survival and growth for the treatment data were compared to the pooled control whereas reproduction was compared to the solvent control. For survival treatment analysis, Fisher's Exact/Bonferroni-Holm Test were used. For reproduction treatment analysis, Shapiro-Wilk's Test was used for normality and Bartlett's Equality of Variance Test for homogeneity before analysis with Dunn/Bonferroni-Holm Test. For growth treatment analysis, Shapiro-Wilk's Test was used for normality and Bartlett's Equality of Variance Test for homogeneity before analysis with Jonckheere-Terpstra Step-Down Test.

The data for survival (as immobilization), reproduction (as the total living offspring per surviving female), and growth (total body length) were used to estimate the 7-day  $EC_{10/20/50}$  values and the corresponding 95% confidence intervals. If at least one test concentration caused a  $\geq 10$ , 20, or 50% reduction in survival, reproduction or growth of the test population, then CETIS Version 1.9 was used to calculate the  $EC_x$  values and 95% confidence intervals.

## **II. RESULTS AND DISCUSSION**

### **A. BIOLOGICAL EFFECTS**

Results for adult survival, reproduction and growth (total body length) are shown in Table 10.2.1/03-1. A summary of endpoints is presented in Table 10.2.1/03-2.

There was a statistically significant reduction in survival among daphnids at 2.7 mg a.s./L compared to the pooled control, as all daphnids died at this treatment level. There was a statistically significant reduction in reproduction among daphnids at 1.4 mg a.s./L compared to the solvent control. There was a statistically significant reduction in total body length among daphnids at 1.4 mg a.s./L compared to the pooled control. First brood release occurred on test day 3 in the negative control, solvent control, 0.17, 0.32, and 0.63 mg/L treatment levels, and on test day 4 in the 1.4 mg/L treatment level. No offspring were produced in the 2.7 mg/L treatment level as 100% mortality was observed in this concentration by test day 1.

**Table 10.2.1/03-1: Summary of effects on adult survival, reproduction and growth**

Mean measured concentration (mg a.s./L)	Mean % adult survival	Mean no. of offspring per surviving daphnid $\pm$ SD	Mean body length (mm) $\pm$ SD
Control	100	34 $\pm$ 1.6	1.04 $\pm$ 0.03
Solvent control	100	32 $\pm$ 2.5	1.03 $\pm$ 0.03
Pooled control	100	33 $\pm$ 2.3	1.03 $\pm$ 0.03
0.17	90	31 $\pm$ 2.1	1.04 $\pm$ 0.03
0.32	100	32 $\pm$ 1.9	1.04 $\pm$ 0.03
0.63	100	31 $\pm$ 1.0	1.02 $\pm$ 0.04
1.4	100	4 $\pm$ 5.2**	0.93 $\pm$ 0.05***
2.7	0*	n.a.	n.a.

SD: Standard deviation; n.a.: not applicable.

\* Significantly reduced compared to the pooled control (Fisher's Exact Test/Bonferroni-Holm Test)

\*\* Significantly reduced compared to the solvent control (Dunn/Bonferroni-Holm Test)

\*\*\* Significantly reduced compared to the pooled control (Jonckheere-Terpstra Step-Down Test)

**Table 10.2.1/03-2: Summary of endpoints**

Endpoint (mg a.s./L)	Survival	Reproduction	Body length
EC <sub>10</sub> (95% CI)	1.5 (1.4 – 1.5)	0.69 (0.69 – 0.71)	n.c.
EC <sub>20</sub> (95% CI)	1.6 (1.5 – 1.6)	0.77 (0.73 – 0.79)	>1.4 (n.c.)
EC <sub>50</sub> (95% CI)	2.0 (1.9 – 2.0)	1.0 (0.98 – 1.1)	>1.4 (n.c.)
NOEC	1.4	0.63	0.63
LOEC	2.7	1.4	1.4
<b>Overall NOEC = 0.63 mg a.s./L</b>			
<b>Overall LOEC = 1.4 mg a.s./L</b>			

CI: Confidence intervals; n.c.: not calculable.

## B. ANALYSIS

Measured concentrations were generally consistent between sampling intervals for both the newly prepared and aged solutions. Mean measured concentrations ranged from 100 to 110% of nominal concentrations (see Table 10.2.1/03-3).

**Table 10.2.1/03-3: Measured concentrations of mandestrobin in the exposure solutions**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)								% of nominal
	Day 0 (new)	Day 1 (aged)	Day 3 (new)	Day 4 (aged)	Day 6 (new)	Day 7 (aged)	Mean	% CV	
Control	< 0.027	< 0.027	< 0.027	< 0.027	< 0.027	< 0.027	n.a.	n.a.	n.a.
Solvent control	< 0.027	< 0.027	< 0.027	< 0.027	< 0.027	< 0.027	n.a.	n.a.	n.a.
0.16	0.17	0.16	0.17	0.18	0.17	0.16	0.17	3.6	110
0.31	0.32	0.31	0.32	0.33	0.32	0.32	0.32	2.2	100
0.63	0.66	0.61	0.64	0.64	0.62	0.60	0.63	3.4	100
1.3	1.4	1.4	1.4	1.5	1.4	1.5	1.4	4.1	110
2.5	2.7	2.7	- <sup>a)</sup>	-	-	-	2.7	0.61	110

CV : Coefficient of variation; n.a.: not applicable.

<sup>a)</sup> Samples were not analysed due to 100% immobilisation in this treatment level.

### C. VALIDITY CRITERIA

The study was performed with *C. dubia*, which is not a standard test species in the EU. As such, there is no adopted guideline for this species. The study was conducted to U.S. EPA Test Method 1002.0 (2002) and OCSPP 850.1000 (2016). The study fulfilled the validity criteria outlined in the study protocol, as detailed below:

- The test organism, *C. dubia*, came from the same source.
- The test was initiated with organisms that were < 24 hours old and these organisms were within 8 hours of the same age at the initiation of the test.
- ≥ 80% survival of organisms in the control(s) at test termination (100% survival).
- An average of ≥ 15 young per surviving female was produced in the control(s) by test termination (actual average of 34 and 32 young per surviving female in the negative control and solvent control, respectively).
- ≥ 60% of the surviving females in the control(s) produced their third brood, (actual 100% of the surviving females in the negative control and solvent control produced their third brood).

### III. CONCLUSION

The chronic toxicity of mandestrobin to *C. dubia* was determined over 7 days under semi-static conditions. Results are reported based on the mean measured concentrations.

Based on the results for parental survival, reproduction and growth (measured as mean total length), the overall NOEC was determined to be 0.63 mg a.s./L (reproduction and body length). The EC<sub>50</sub> value for survival was calculated to be 2.0 mg a.s./L, the EC<sub>50</sub> for reproduction was 1.0 mg a.s./L and the EC<sub>50</sub> for body length was >1.4 mg a.s./L.

Assessment and Conclusion by Applicant:	Although not a standard species, the study has been performed to current standards and is considered to be fully valid.  Based on the mean measured concentrations, the relevant endpoints derived from the study is: NOEC: 0.63 mg a.s./L
---	---

Comments of zRMS:	The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.  In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted. The study was conducted in accordance with EPA test method. The validation criteria were met.
-------------------	---

	No deviations from study protocol were noted.
	The following endpoints were derived: NOEC: 0.63 mg a.s./L EC <sub>10</sub> = 0.69 mg a.s./L

#### A 2.2.1.4 Study 4

<b>Data point:</b>	KCP 10.2.1/04
<b>Report author:</b>	Roessink, I.
<b>Report year:</b>	2019b
<b>Report title:</b>	Chronic Effects of the Fungicide Mandestrobin to <i>Caridina parvidentata</i>
<b>Report No.:</b>	ALT.IR.2018.7
<b>Document No.:</b>	ROW-0106
<b>Guidelines followed in study:</b>	OECD 211 (2012); OECD 219 (2004); OECD 233 (2010).
<b>Deviations from current test guideline:</b>	None/not applicable.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to the aquatic shrimp *Cardina parvidentata* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations of between 0.10 and 10 mg a.s./L, (time weighted average concentrations between 0.096 and 8.9 mg a.s./L). An untreated control and solvent (acetone) control were tested in parallel. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. Test medium was renewed once per week. Immobility, mortality and possible number of offspring were recorded on day 7, 14, 21 and 28. Length and dry weight biomass of surviving test animals was assessed on day 28. Mandestrobin concentrations were measured before and after mandestrobin application at renewal where appropriate, at day 0, 7, 14, 21 and 28. The results of the study were reported based on the time weighted average concentrations (TWA).

For survival clear mortality was observed at the highest concentration of 8.9 mg a.s./L, a 28 day NOEC, LC<sub>10</sub> and LC<sub>50</sub> of 2.7, 2.7 and 3.1 mg a.s./L, respectively were observed.

For mean individual dry weight of survivors and mean individual daily growth in dry weight of survivors, a 28 day NOEC of 2.7 mg a.s./L was observed and no EC<sub>10</sub> or EC<sub>50</sub> values could be determined.

For mean individual length of survivors, a 28 day NOEC, EC<sub>10</sub> and EC<sub>50</sub> of 2.7, 4.0 and 4.5 mg a.s./L, respectively was observed. No NOEC, EC<sub>10</sub> or EC<sub>50</sub> values could be determined for mean individual daily growth in length of survivors.

### I. MATERIALS AND METHODS

#### A. MATERIALS

1. **Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported

- |                           |   |
|---------------------------|---|
| <b>Lot/Batch:</b>         | ST-0811G  |
| <b>Purity:</b>            | 93.1%   |
| <b>2. Controls:</b>       | Negative control: dilution water<br>Solvent control: dilution water + acetone |
| <b>3. Reference item:</b> | Not applicable.   |

## B. STUDY DESIGN AND METHODS

- 1. Test animals:** Aquatic shrimp, Decapoda Crustacea (*Cardina parvidentata*)  
**Age:** Not determined, mean length of subadults/adults  $11.2 \pm 1.8$  mm/ $20.5 \pm 0.6$  mm, mean weight of subadults/adults  $6.2 \pm 0.5$  mg/ $21.0 \pm 2.7$  mg (subadults distinguished as not containing eggs)  
**Source:** In-house culture  
**Acclimation:** Not needed as culture kept under similar conditions to those used in the test exposure  
**Diet:** Preconditioned *Populus* leaves, biofilm and a pellet of fish food (Trouvit)
- 2. Dilution water:** Aerated groundwater (Sinderhoeve complex, Netherlands)
- 3. Test vessels:** 1.5 L glass beakers filled with 1000 mL of aerated groundwater and a shoot of *Elodea* sp. to act as a substrate and provide oxygen
- 4. Environmental conditions:**

<b>Temperature:</b>	18.7 – 19.8°C
<b>pH:</b>	7.6 – 8.2
<b>Dissolved oxygen:</b>	7.1 – 9.3 mg/L (77.7 – 102.4% saturation)
<b>Conductivity:</b>	148.3 – 189.2 µS/cm
<b>Photoperiod:</b>	16 hours light : 8 hours dark (approx. $100 \mu\text{mol s}^{-1} \text{m}^{-2}$ )

### 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to the aquatic shrimp *C. parvidentata* was evaluated under semi-static conditions in the laboratory over 28 days, at nominal concentrations of 0, 0 (acetone control), 0.10, 0.30, 1.0, 3.0 and 10 mg a.s./L. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. Mandestrobin was applied once per week from a prepared stock/dosing solution, immediately after the renewal of the test medium.

### 6. Dose preparation:

To dose the different treatment levels, different stock solutions were prepared. The preparation and concentrations of the stock solutions used to dose the test vessels is shown in Table 10.2.1/04-1 below.



**Table 10.2.1/04-1: Preparation of stock solutions**

Code stock	Intended concentration (mg a.s./L)	Mass of mandestrobin TG added (g)	Mass acetone added (g)	Final volume stock (mL)	
MDB2-6	100,000	3.222	23.73	30	Intended
	99,993.81	3.2224	23.7319	30.0024	Actual
MDB2-5	30,000	1.611	39.55	50	Intended
	30,010.43	1.6120	39.5566	50.008	Actual
MDB2-4	10,000	0.537	39.55	50	Intended
	9949.2	0.5344	39.5536	50.005	Actual
MDB2-3	3000	0.161	39.55	50	Intended
	3083.59	0.1660	39.6440	50.119	Actual
MDB2-2	1000	0.054	39.55	50	Intended
	1041.47	0.0562	39.7390	50.239	Actual
Code	Intended concentration (mg a.s./L)	Intended mass stock MDB-4 added (g)	Mass of final volume (after addition with acetone) (g)	Final volume stock	
MDB2-1	300	3.955	39.55	50	Intended
	308.34	3.9916	39.5797	50.038	Actual

The dose solutions were made once and stored in the freezer for later use. After manual shaking of the stock solution, a subsample of approximately 4.0 mL was transferred into a 4 mL wisp vial. Per treatment level, one vial was used for dosing the test systems.

## 7. Measurements and observations:

Observations for immobility, mortality and possible number of offspring were performed on day 7, 14, 21 and 28 in test vessels of all treatments. Effects were scored under mortality when no response of any kind was observed over a time period of 15 seconds after tactile stimulation. Effects were scored under immobility when after tactile stimulation only limited movement was visible. Living animals were not extracted from the test water during observation but were counted in a shallow white tray.

At test termination, length and dry weight were determined for all surviving test organisms. For the length measurement, animals were photographed using a stereomicroscope, after which their length was measured using computer software ImageJ. For dry weight estimation, the surviving individuals from each test vessel were dried at 105°C for 24 hours. The average dry weight of these individuals was established by dividing the total weight of the number of individuals per vessel.

Mandestrobin concentrations were determined in all treatments before and after mandestrobin application where appropriate, at day 0, 7, 14, 21 and 28 using Liquid Chromatography with triple quadrupole mass detection (LC/QQQ). Concentrations of mandestrobin were also determined in the stock/dosing solutions.

Temperature, electrical conductivity, pH and dissolved oxygen were measured in the controls and highest surviving treatment level, directly before media refreshment and at the end of the test and at the start of the experiment and prior to each media refreshment (from the fresh stock of exposure media used to refresh the media of all test systems). Light intensity was measured in the test room at the start of the experiment.

## 8. Statistics:

NOEC and LC<sub>x</sub> values were calculated for survival (mortality) on days 7, 14, 21 and 28. Clear immobility effects could not be scored during the test, therefore NOEC and EC<sub>x</sub> values for immobility were not calculated.

28-day NOEC and EC<sub>x</sub> values were calculated for endpoints total dry weight of survivors, mean individual dry weight of survivors, mean individual length of survivors, mean individual daily growth in dry weight of survivors and mean individual daily growth in length of survivors. The growth endpoints for biomass and length were calculated by subtracting mean initial dry weight biomass and length of *C. parvidentata* individuals at the start of the experiment from the mean length and dry weight of surviving individuals at the end of the experiment.

E(L)C<sub>x</sub> values and 95% confidence limits were calculated by a log concentration-logit effect regression method. Within the regression, calculated E(L)C<sub>x</sub> values were corrected for mortality in the controls. The regression method was programmed in GENSTAT (version 19).

NOEC estimations for each endpoint ( $p \leq 0.05$ ) were carried out using the Williams test. The analyses were performed with the Community Analysis (CA) computer program.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

#### Survival

For survival clear mortality was observed at the highest concentration of 8.9 mg a.s./L (TWA) compared to the pooled control. No significant effects on survival were observed at any other treatment level. A summary of the mortality data is shown in Table 10.2.1/04-2 below.

**Table 10.2.1/04-2: Summary of effects on survival**

TWA concentration (mg a.s./L)	% Mortality			
	Day 7	Day 14	Day 21	Day 28
Control	8	16	20	20
Solvent control	4	8	18	20
0.096	20	27	33	40
0.29	20	20	23	23
0.90	7	7	7	7
2.7	13	20	30	30
8.9	87*	100*	100*	100*

\* Significantly reduced compared to the pooled controls (Williams test).

NOEC and LC<sub>x</sub> values for survival are presented in Table 10.2.1/04-4 below.

#### Reproduction

No offspring were observed during the experimental period.

#### Growth

At the end of the experiment (day 28) surviving *C. parvidentata* individuals were collected, weighed, and measured for their length. For both dry weight biomass and length clear treatment-related responses could be observed, while this was not the case for their respective growth rates. This latter phenomenon is very likely caused by the fact that not much growth had occurred in the experimental period and the responses observed are consequently a result of mortality in the highest surviving treatment level of 2.7 mg a.s./L.

For unknown reasons, however, the selection of the twenty individuals to characterize the start situation was biased towards slightly larger individuals than used in the test, resulting in slightly negative values for growth. The fact that growth did not occur in the test can be explained by the fact that crustaceans, like insects, only grow in 'bursts'. In order to grow, the old carapax needs to be shed (e.g. moulted) so the

individual can increase in size when the new carapax is still soft. Once the new carapax is hard, growth only occurs at the next moulting event again.

Since no newly-bourne individuals were available, subadult animals were selected whose growth (i.e. moulting frequency) is lower than in very young individuals. These subadult individuals did not appear to moult/grow within the 28-day experimental period, thus resulting in growth rates in controls and treatment groups that were approximately zero. However, it is clear that the weight and length endpoints were unaffected in the 0.096 to 2.7 mg a.s./L treatment levels.

For the endpoints mean individual dry weight of survivor, mean individual daily growth in dry weight of survivors and mean individual length of survivors a NOEC value of 2.7 mg a.s./L could be calculated but EC<sub>x</sub> values could only be established for the endpoint mean individual length of survivors. It should be noted that the NOEC value calculated for the endpoint mean individual daily growth in dry weight of survivors is considered to be ecotoxicologically less relevant, because of the limited growth observed in the experimental period. As a result, the lowest EC<sub>10</sub> value was 4.0 mg a.s./L. The lowest 28-day EC<sub>50</sub> estimate was observed for the same endpoint at 4.5 mg a.s./L.

A summary of the mean growth data (pooled replicates) is presented in Table 10.2.1/04-3 and NOEC and EC<sub>x</sub> values for growth are presented in Table 10.2.1/04-4 below.

**Table 10.2.1/04-3: Summary of effects on growth on day 28 <sup>a)</sup>**

<b>TWA concentration (mg a.s./L)</b>	<b>Mean individual length (mm)</b>	<b>Mean growth rate (mm/day)</b>	<b>Mean individual dry weight (mg)</b>	<b>Mean growth rate (mg/day)</b>
Control	10.685	-0.002	4.792	-0.009
Solvent control	10.766	-0.002	4.276	-0.014
0.096	10.825	-0.001	4.702	-0.010
0.29	10.469	-0.003	4.600	-0.011
0.90	9.135	-0.008	4.483	-0.012
2.7	12.966	0.005*	4.239	-0.014
8.9	n.a. <sup>b)</sup> *	n.a. <sup>b)</sup>	n.a. <sup>b)</sup> *	n.a. <sup>b)</sup> *

n.a.: not applicable.

\* Significantly reduced compared to the pooled controls (Williams test).

<sup>a)</sup> Values were calculated by the Applicant as the mean values were not clearly presented in the report.

<sup>b)</sup> All individuals died.

**Table 10.2.1/04-4: Summary of endpoints based on nominal and TWA concentrations**

Parameter	Endpoint	Based on nominal concentrations (mg a.s./L)	Based on TWA concentrations (mg a.s./L)
28-day adult survival	LC <sub>10</sub> (95% CI)	3.0 (2.6 – 3.5)	2.7*
	LC <sub>20</sub> (95% CI)	3.2 (2.7 – 3.7)	2.9*
	LC <sub>50</sub> (95% CI)	3.5 (3.0 – 4.1)	3.1*
	NOEC	3.0	2.7
Total dry weight of survivors (mg)	EC <sub>10</sub> (95% CI)	2.9 (2.1 – 4.0)	2.6 (1.9 – 3.6)
	EC <sub>20</sub> (95% CI)	3.0 (2.2 – 4.2)	2.8 (2.0 – 3.8)
	EC <sub>50</sub> (95% CI)	3.3 (2.4 – 4.6)	3.0 (2.2 – 4.2)
	NOEC	3.0	2.7
Mean individual dry weight of survivors (mg)	EC <sub>10</sub> (95% CI)	-	-
	EC <sub>20</sub> (95% CI)	-	-
	EC <sub>50</sub> (95% CI)	-	-
	NOEC	3.0**	2.7**
Mean individual length of survivors (mm)	EC <sub>10</sub> (95% CI)	4.6 (n.c.)	4.0 (n.c.)
	EC <sub>20</sub> (95% CI)	4.7 (n.c.)	4.2 (n.c.)
	EC <sub>50</sub> (95% CI)	5.1 (n.c.)	4.5 (n.c.)
	NOEC	3.0	2.7
Mean individual daily growth in dry weight of survivors (mg/day)	EC <sub>10</sub> (95% CI)	-	-
	EC <sub>20</sub> (95% CI)	-	-
	EC <sub>50</sub> (95% CI)	-	-
	NOEC	3.0	2.7
Mean individual daily growth in length of survivors (mm/day)	EC <sub>10</sub> (95% CI)	-	-
	EC <sub>20</sub> (95% CI)	-	-
	EC <sub>50</sub> (95% CI)	-	-
	NOEC	-	-

CI: Confidence intervals.

\* No conversion of the model.

\*\* Value considered less reliable due to too low growth values in the test. Not taken into account in further.

n.c.: not calculable.

## B. ANALYSIS

Measured mandestrobin concentrations in the dosing solutions ranged between 96 to 119% of nominal concentrations. mandestrobin concentrations measured in the test system remained relatively stable throughout the experiment between 82 and 108% of nominal. No mandestrobin was detected in the control and solvent control. A summary of the analytical measurements is presented in Table 10.2.1/04-5 below.

**Table 10.2.1/04-5: Summary of analytical measurements**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)					TWA concentration (mg a.s./L) <sup>a)</sup>
	Day 0	Day 7 (before/after dosing)	Day 14 (before/after dosing)	Day 21 (before/after dosing)	Day 28	
Control	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	-
Solvent control	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	-
0.10	0.095	0.089/0.105	0.098/0.099	0.092/0.098	0.089	0.096 (96)
0.30	0.304	0.259/0.304	0.287/0.298	0.280/0.298	0.278	0.29 (96)
1.0	0.933	0.847/0.960	0.863/0.932	0.879/0.946	0.862	0.90 (90)
3.0	2.849	2.527/2.690	2.711/2.920	2.755/2.791	2.675	2.7 (91)
10	8.553	8.604/9.143	9.168/9.339	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>	8.9 (89)

n.a.: not applicable.

<sup>a)</sup> Percent of nominal value shown in brackets.

<sup>b)</sup> All individuals died.

LOQ = 0.270 µg a.s./L.

### C. VALIDITY CRITERIA

The study was performed with *C. parvidentata*, which is not a standard test species in the EU. As such, there is no adopted guideline for this species. The study was designed based on a combination of OECD 211 (2012), OECD 219 (2004) and OECD 233 (2010), and appropriate validity criteria were designed in the study protocol. The study fulfilled the validity criteria outlined in the study protocol, as detailed below:

- The mortality in the controls (i.e. control and solvent control) should not exceed 20% at the end of the test (actual: 20% in both controls on day 28).
- The oxygen concentration should be at least 60% of the air saturation at the temperature used (actual: ≥ 77.7%).

### III. CONCLUSION

The chronic toxicity of mandestrobin to the aquatic shrimp *C. parvidentata* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations of 0.10, 0.30, 1.0, 3.0 and 10 mg a.s./L, (time weighted average concentrations 0.096, 0.29, 0.90, 2.7, and 8.9 mg a.s./L).

For survival clear mortality was observed at the highest concentration of 8.9 mg a.s./L, a 28-day NOEC, LC<sub>10</sub> and LC<sub>50</sub> of 2.7, 2.7 and 3.1 mg a.s./L, respectively were observed.

For mean individual dry weight of survivors and mean individual daily growth in dry weight of survivors, a 28 day NOEC of 2.7 mg a.s./L was observed and no EC<sub>10</sub> or EC<sub>50</sub> values could be determined.

For mean individual length of survivors, a 28 day NOEC, EC<sub>10</sub> and EC<sub>50</sub> of 2.7, 4.0 and 4.5 mg a.s./L, respectively was observed. No NOEC, EC<sub>10</sub> or EC<sub>50</sub> values could be determined for mean individual daily growth in length of survivors.

Assessment and Conclusion by Applicant:	<p>Although not a standard test species, the study has been performed according to current standards and is considered to be fully valid.</p> <p>Based on time weighted average mean measured concentrations, the relevant endpoint derived from the study is:</p> <p>NOEC: 2.7 mg a.s./L, based on 28-day survival, mean individual dry weight of survivors, mean individual length of survivors and mean individual daily growth in dry weight of survivors.</p>
---	--

Comments of zRMS:	<p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met.</p>
-------------------	--

	The minor deviations were noted, not ecotoxicologically relevant.
	The following endpoints were derived: NOEC: 3.0 mg a.s./L (based on nominal concentration) NOEC: 2.7 mg a.s./L (based on TWA concentration) EC <sub>10</sub> = 2.9 mg a.s./L (based on nominal concentration) EC <sub>10</sub> = 2.6 mg a.s./L (based on TWA concentration)

#### A 2.2.1.5 Study 5

<b>Data point:</b>	KCP 10.2.1/05
<b>Report author:</b>	Roessink, I.
<b>Report year:</b>	2019c
<b>Report title:</b>	Chronic Effects of the Fungicide Mandestrobin to <i>Gammarus pulex</i>
<b>Report No.:</b>	ALT.IR.2018.4
<b>Document No.:</b>	ROW-0105
<b>Guidelines followed in study:</b>	OECD 211 (2012); OECD 219 (2004); OECD 233 (2010).
<b>Deviations from current test guideline:</b>	None/not applicable.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to the aquatic isopod *Gammarus pulex* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations between 0.030 and 3.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. Test medium was renewed once per week. Immobility, mortality and possible number of offspring were recorded on day 7, 14, 21 and 28. Length and dry weight biomass of survivors was assessed on day 28. The results of the study were reported based on the time weighted average concentrations.

100% mortality (statistically significant) occurred in the highest treatment level of 2.8 mg a.s./L. A significant effect on mortality was also observed at 0.91 mg a.s./L. A significant reduction in individual dry weight was observed at 0.91 mg a.s./L and a significant reduction in individual length was observed at 0.25 and 0.91 mg a.s./L. As all isopods had died by day 28 in the highest treatment level, no analysis of dry weight and length was performed for this treatment group.

The 28-day NOEC for survival was 0.25 mg a.s./L and the lowest NOEC for growth was 0.092 mg a.s./L, based on effects on individual length and growth in terms of mm/day.

### I. MATERIALS AND METHODS

#### A. MATERIALS

- Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.1%

2. **Controls:** Negative control: dilution water  
Solvent control: dilution water + acetone
3. **Reference item:** Not applicable.

## B. STUDY DESIGN AND METHODS

1. **Test animals:** Aquatic isopod (*Gammarus pulex*)  
**Age:** Not reported  
**Source:** Uncontaminated brook near Heelsum, The Netherlands.  
**Acclimation:** 5 days  
**Diet:** Preconditioned *Populus* leaves
2. **Dilution water:** Aerated groundwater
3. **Test vessels:** 1.5 L glass beakers filled with aerated groundwater and equipped with a small stainless steel mesh to provide additional substrate for the organisms. Test medium volume in each test vessel was 1000 mL.
4. **Environmental conditions:**  
**Temperature:** 18.4 – 19.9°C  
**pH:** 7.8 – 8.5  
**Dissolved oxygen:** 8.0 – 10.1 mg/L (88 – 111% saturation)  
**Conductivity:** 151.0 – 181.9 µS/cm  
**Photoperiod:** 16 hours light : 8 hours dark (100 µmol s<sup>-1</sup> m<sup>-2</sup>)
5. **Animal assignment and treatment:**

The chronic toxicity of mandestrobin to the aquatic isopod *Gammarus pulex* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations of 0.030, 0.10, 0.30, 1.0 and 3.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. mandestrobin was applied once per week, immediately after the renewal of the test medium.

### 6. Dose preparation:

To dose the different treatment levels, different stock solutions were prepared. The preparation and concentrations of the stock solutions used to dose the test vessels is shown in Table 10.2.1/05-1 below.

The dose solutions were made once and stored in the freezer for later use. After manual shaking of the stock solution, a subsample of approximately 4.0 mL was transferred into a 4 mL vial. Per treatment level, one vial was used for dosing the test systems. Each test system was dosed with 0.1 mL of the respective stock solution. The control systems received water only, while the solvent control systems were dosed with 0.1 mL acetone. All test systems, except the negative control, received an equal amount of acetone.

**Table 10.2.1/05-1: Preparation of stock solutions**

Code stock		Concentration (mg a.s./L)	Mass of mandestrobin TG added (g)	Mass acetone added (g)	Final volume stock (mL)
MDB-7 <sup>a)</sup>	Intended	100,000	3.222	23.73	30
	Actual	99,909.66	3.2227	23.7541	30.030
MDB-6	Intended	30,000	1.611	39.55	50
	Actual	29,986.64	1.6106	39.5536	50.005
MDB-5	Intended	10,000	0.537	39.55	50
	Actual	9985.95	0.5364	39.5572	50.009
MDB-4	Intended	3000	0.161	39.55	50
	Actual	3001.40	0.1612	39.5519	50.002
MDB-3	Intended	1000	0.054	39.55	50
	Actual	1021.87	0.0549	39.5641	50.018
		<b>Intended concentration (mg a.s./L)</b>	<b>Intended mass stock MDB-4 added (g)</b>	<b>Mass of final volume (after addition with acetone) (g)</b>	<b>Final volume stock</b>
MDB-2	Intended	300	3.955	39.55	50
	Actual	300.21	3.9577	39.5675	50.022

<sup>a)</sup> This solution was not used in this particular experiment since it was intended for a treatment level of 10 mg a.s./L.

## 7. Measurements and observations:

Observations for immobility, mortality and possible number of offspring were performed on day 7, 14, 21 and 28 in test vessels of all treatments. Effects were scored under mortality when no response of any kind was observed over a time period of 15 seconds after tactile stimulation. Effects were scored under immobility when after tactile stimulation only limited movement was visible.

At test termination, length and dry weight were determined for all surviving test organisms. For the length measurement, animals were photographed using a stereomicroscope, after which their length was measured using computer software ImageJ. For dry weight estimation, the surviving individuals from each test vessel were dried at 105°C for 24 hours. The average dry weight of these individuals per vessel was weighed on a micro-balance. On day 0, length and dry weight biomass was measured for 20 representative test individuals to provide these measurements for the start of the experiment.

Mandestrobin concentrations were monitored in the dosing solutions and in each test vessel immediately after each treatment and before refreshment of the medium. Analysis was performed using LC-QQQ.

Temperature, electrical conductivity (EC), pH and dissolved oxygen (DO) were measured in the controls and highest treatment level, directly before medium refreshment and at the end of the test. At the start of the experiment and prior to each medium refreshment EC, pH and DO were measured in the vessel with the fresh stock of exposure medium used to refresh the medium of all test systems.

## 8. Statistics:

NOEC and LC<sub>x</sub> values were calculated for mortality on days 7, 14, 21 and 28. Clear immobility effects could not be scored during the test, therefore NOEC and EC<sub>x</sub> values for immobility were not calculated.

28-day NOEC and EC<sub>x</sub> values were calculated for dry weight of survivors, length of survivors, daily growth in dry weight of survivors and daily growth in length of survivors.

E(L)C<sub>x</sub> values and 95% confidence limits were calculated by a log concentration-logit effect regression method. Within the regression, calculated E(L)C<sub>x</sub> values were corrected for mortality in the controls. The regression method was programmed in GENSTAT (version 19).



NOEC estimations for each endpoint ( $p \leq 0.05$ ) were carried out using the Williams test. The analyses were performed with the Community Analysis (CA) computer program.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

#### Survival

On day 28, there was a statistically significant increase in mortality the two highest treatment levels compared to the pooled control. No significant effects on mortality were observed at any other treatment level. A summary of the mortality data on day 28 is shown in Table 10.2.1/05-2 below.

**Table 10.2.1/05-2: Summary of effects on adult survival**

TWA concentration (mg a.s./L)	% Mortality (D28) <sup>a)</sup>
Control	6
Solvent control	12
0.028	3.33
0.092	6.67
0.25	0.00
0.91	36.67*
2.8	100.00*

\* Significantly reduced compared to the pooled controls.

<sup>a)</sup> Mortality values for the treatment levels calculated by the Applicant as the values were not presented in the report.

#### Reproduction

No offspring were observed during the experimental period.

#### Growth

At test initiation, 20 isopods were separated into two groups of 10 to determine average dry weight per group. Reported dry weights per group of 10 individuals were 11.766 mg and 9.401 mg, corresponding to 1.177 mg and 0.940 mg per individual, respectively.

At test termination (day 28), the dry weight of surviving isopods was determined in groups (per replicate). A statistically significant reduction in individual dry weight was observed at 0.91 mg a.s./L compared to the pooled control. All isopods in the highest treatment level of 2.8 mg a.s./L had died, therefore data are not available for this treatment group. The mean individual dry weights per control and treatment group are reported in Table 10.2.1/05-3.

At test initiation, lengths of 20 isopods were measured individually. The mean individual length at test initiation was 6.24 mm.

At test termination (day 28) the length of surviving isopods was again determined individually. A statistically significant reduction in individual length was observed at 0.25 and 0.91 mg a.s./L compared to the pooled control. All isopods in the highest treatment level of 2.8 mg a.s./L had died, therefore data are

not available for this treatment group. The mean individual lengths of survivors per control and treatment group are reported in Table 10.2.1/05-3.

**Table 10.2.1/05-3: Summary of effects on growth on day 28 <sup>a)</sup>**

TWA concentration (mg a.s./L)	Mean body length per specimen (mm)	Mean growth rate (mm/day)	Mean dry weight per organism (mg)	Mean growth rate (mg/day)
Control	8.944	0.013	2.922	0.036
Solvent control	9.288	0.014	3.152	0.039
0.028	9.080	0.013	3.137	0.038
0.092	8.963	0.013	2.789	0.035
0.25	8.457*	0.011*	2.668	0.033
0.91	6.643*	0.002*	1.072*	0.000*
2.8	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>

\* Significantly reduced compared to the pooled controls.

<sup>a)</sup> Values were calculated by the Applicant as the mean values were not clearly presented in the report.

<sup>b)</sup> All individuals died.

A summary of endpoints based on both the nominal and TWA concentrations is shown in Table 10.2.1/05-4 below.

**Table 10.2.1/05-4: Summary of endpoints based on nominal and TWA concentrations**

Parameter	Endpoint	Based on nominal concentrations (mg a.s./L)	Based on TWA concentrations (mg a.s./L)
28-day adult survival	LC <sub>10</sub> (95% CI)	0.92 (0.78 - 1.1)	0.84 (0.68 - 1.0)
	LC <sub>20</sub> (95% CI)	0.97 (0.85 - 1.1)	0.88 (0.76 - 1.0)
	LC <sub>50</sub> (95% CI)	1.0 (0.93 - 1.2)	0.94 (0.82 - 1.1)
	NOEC	0.30	0.25
Mean individual dry weight of survivors (mg)	EC <sub>10</sub> (95% CI)	0.86 (0.78 - 0.94)	0.78 (0.72 - 0.85)
	EC <sub>20</sub> (95% CI)	0.90 (0.82 - 0.98)	0.81 (0.75 - 0.89)
	EC <sub>50</sub> (95% CI)	0.97 (0.88 - 1.1)	0.88 (0.81 - 0.96)
	NOEC	0.30	0.25
Mean individual length of survivors (mm)	EC <sub>10</sub> (95% CI)	0.94 (0.88 - 1.0)	0.86 (0.79 - 0.93)
	EC <sub>20</sub> (95% CI)	0.98 (0.92 - 1.1)	0.89 (0.82 - 0.97)
	EC <sub>50</sub> (95% CI)	1.1 (0.98 - 1.1)	0.96 (0.88 - 1.0)
	NOEC	0.10	0.092
Mean individual daily growth in dry weight of survivors (mg/day)	EC <sub>10</sub> (95% CI)	0.28 (0.12 - 0.67)	0.23 (0.09 - 0.58)
	EC <sub>20</sub> (95% CI)	0.35 (0.17 - 0.72)	0.29 (0.13 - 0.64)
	EC <sub>50</sub> (95% CI)	0.50 (0.30 - 0.85)	0.43 (0.24 - 0.76)
	NOEC	0.30	0.25
Mean individual daily growth in length of survivors (mm/day)	EC <sub>10</sub> (95% CI)	0.23 (0.10 - 0.50)	0.18 (0.08 - 0.43)
	EC <sub>20</sub> (95% CI)	0.30 (0.15 - 0.57)	0.24 (0.12 - 0.50)
	EC <sub>50</sub> (95% CI)	0.48 (0.30 - 0.75)	0.41 (0.25 - 0.67)
	NOEC	0.10	0.092

CI: Confidence intervals.

## B. ANALYSIS

Measured mandestrobin concentrations in the dosing solutions ranged between 96 to 107% of nominal concentrations.

In the test systems, mandestrobin concentrations remained relatively stable throughout the experiment. A mean concentration below 80% (range 10 -21%) of the intended initial level was measured on day 21, directly after application, in the 0.3 mg a.s./L treatment only. This most likely is an artefact since on day 28 mean measured concentrations in the 0.3 mg a.s./L treatment level were higher than 80% of the initial intended concentration.

In addition, the time weighted average concentrations over the complete 28-day test period were calculated. Note that the TWA concentration of the highest treatment level was calculated over 7 days, since 100% mortality was observed after this period. The calculated TWA concentrations are shown in Table 10.2.1/05-5 below.

**Table 10.2.1/05-5: Summary of analytical measurements**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)					TWA concentration (mg a.s./L) <sup>a)</sup>
	Day 0	Day 7 (before/after dosing)	Day 14 (before/after dosing)	Day 21 (before/after dosing)	Day 28	
Control	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	-
Solvent control	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	-
0.03	0.030	0.025/0.032	0.025/0.030	0.026/0.030	0.024	0.028 (92)
0.1	0.096	0.085/0.112	0.085/0.097	0.088/0.097	0.081	0.092 (92)
0.3	0.304	0.251/0.367	0.260/0.295	0.268/0.042	0.250	0.25 (82)
1.0	0.912	0.891/1.061	0.848/0.941	0.890/0.916	0.818	0.91 (91)
3.0	2.747	2.841/2.824	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>	n.a. <sup>b)</sup>	2.8 (93)

n.a.: not applicable.

<sup>a)</sup> Percent of nominal value shown in brackets.

<sup>b)</sup> All individuals died.

LOQ = 0.114 µg a.s./L.

## C. VALIDITY CRITERIA

The study was performed with *Gammarus pulex*, which is not a standard test species in the EU. As such, there is no adopted guideline for this species. The study was designed based on a combination of OECD 211 (2012), OECD 219 (2004) and OECD 233 (2010), and appropriate validity criteria were designed in the study protocol. The study fulfilled the validity criteria outlined in the study protocol, as detailed below:

- The mortality and sub-lethal effects in the controls (i.e. control and solvent controls) should not exceed 20% at the end of the test (actual: 6 and 12% in the negative and solvent controls, respectively, on day 28).
- At the end of the test/incubation period, the oxygen concentration should be at least 60% of the air saturation at the temperature used (actual: ≥ 88%).

## III. CONCLUSION

The chronic toxicity of mandestrobin to the aquatic isopod *Gammarus pulex* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations between 0.030 and 3.0 mg a.s./L.

100% mortality (statistically significant) occurred in the highest treatment level of 2.8 mg a.s./L. A significant effect on mortality was also observed at 0.91 mg a.s./L. A significant reduction in individual dry weight was observed at 0.91 mg a.s./L and a significant reduction in individual length was observed at 0.25 and 0.91 mg a.s./L. As all isopods had died by day 28 in the highest treatment level, no analysis of dry weight and length was performed for this treatment group.

The 28-day NOEC for survival was 0.25 mg a.s./L and the lowest NOEC for growth was 0.092 mg a.s./L, based on effects on individual length and growth in terms of mm/day.

Assessment and Conclusion by Applicant:	<p>Although not a standard test species, the study has been performed according to current standards and is considered to be fully valid.</p> <p>Based on the mean measured concentrations, the relevant endpoint derived from the study is:</p> <p>NOEC: 0.092 mg a.s./L (length of survivors)</p>
Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met. No deviations were noted.</p> <p>The following endpoints were derived: NOEC: 0.092 mg a.s./L (based on TWA concentration) EC<sub>10</sub> = 0.18 mg a.s./L(based on TWA concentration)</p>

#### A 2.2.1.6 Study 6

<b>Data point:</b>	KCP 10.2.1/06
<b>Report author:</b>	Roessink, I.
<b>Report year:</b>	2019d
<b>Report title:</b>	Chronic Effects of the Fungicide Mandestrobin to <i>Asellus aquaticus</i>
<b>Report No.:</b>	ALT.IR.2018.2
<b>Document No.:</b>	ROW-0104
<b>Guidelines followed in study:</b>	OECD 211 (2012); OECD 219 (2004); OECD 233 (2010)
<b>Deviations from current test guideline:</b>	None/not applicable
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to the aquatic isopod *Asellus aquaticus* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations between 0.030 and 3.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. Immobility, mortality and possible number of offspring were recorded on day 7, 14, 21 and 28. Length and dry weight biomass of survivors was assessed on day 28. The results of the study were reported based on the time weighted average (TWA) concentrations.

At test termination there was a statistically significant reduction on survival at the highest TWA treatment level of 2.8 mg a.s./L. There was a significant reduction in individual dry weight at 0.095, 0.25, 0.92 and 2.8 mg a.s./L and on mean growth rate (mg/day) at 0.25, 0.92 and 2.8 mg a.s./L. There was also a significant reduction in individual body length at 0.92 and 2.8 mg a.s./L and on mean growth rate (mm/day) at 0.095, 0.25, 0.92 and 2.8 mg a.s./L.

The 28-day NOEC for survival was 0.92 mg a.s./L and the lowest NOEC for growth was 0.029 mg a.s./L, based on effects on individual dry weight and growth in terms of mm/day.

### I. MATERIALS AND METHODS

#### A. MATERIALS

- Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.1%
- Controls:** Negative control: dilution water  
 Solvent control: dilution water + acetone
- Reference item:** Not applicable.

#### B. STUDY DESIGN AND METHODS

- Test animals:** Aquatic isopod (*Asellus aquaticus*)  
**Age:** Not reported

**Source:** Uncontaminated experimental freshwater ecosystem dominated by *Glyceria maxima* at Sinderhoeve experimental station.  
**Acclimation:** 5 days  
**Diet:** *Populus* leaves

**2. Dilution water:** Aerated groundwater

**3. Test vessels:** 1.5 L glass beakers filled with aerated groundwater and equipped with a small stainless steel mesh to provide additional substrate for the organisms. Test medium volume in each test vessel was 1000 mL.

**4. Environmental conditions:**

**Temperature:** 18.2 – 20.0°C

**pH:** 7.6 – 8.7

**Dissolved oxygen:** 7.6 – 9.6 mg/L (83.6 – 106% saturation)

**Conductivity:** 127.5 – 179.4 µS/cm

**Photoperiod:** 16 hours light : 8 hours dark (96.2 – 105.5 lux)

**5. Animal assignment and treatment:**

The chronic toxicity of mandestrobin to the aquatic isopod *Asellus aquaticus* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations of 0.030, 0.10, 0.30, 1.0 and 3.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Five replicate test vessels were set up for the control and solvent control, and three were set up per test item concentration. Each replicate contained ten individuals. Mandestrobin was applied once per week, immediately after the renewal of the test medium.

**6. Dose preparation:**

To dose the different treatment levels, different stock solutions were prepared. The preparation and concentrations of the stock solutions used to dose the test vessels is shown in Table 10.2.1/06-1 below.

The dose solutions were made once and stored in the freezer for later use. After manual shaking of the stock solution, a subsample of approximately 4.0 mL was transferred into a 4 mL wisp vial. Per treatment level, one vial was used for dosing the test systems. Each test system was dosed with 0.1 mL of the respective stock solution. The control systems received water only, while the solvent control systems were dosed with 0.1 mL acetone. All test systems, except the negative control, received an equal amount of acetone.

**Table 10.2.1/06-1: Preparation of stock solutions**

Code stock		Concentration (mg a.s./L)	Mass of mandestrobin TG added (g)	Mass acetone added (g)	Final volume stock (mL)
MDB-7 <sup>a)</sup>	Intended	100,000	3.222	23.73	30
	Actual	99,909.66	3.2227	23.7541	30.030
MDB-6	Intended	30,000	1.611	39.55	50
	Actual	29,986.64	1.6106	39.5536	50.005
MDB-5	Intended	10,000	0.537	39.55	50
	Actual	9985.95	0.5364	39.5572	50.009
MDB-4	Intended	3000	0.161	39.55	50
	Actual	3001.40	0.1612	39.5519	50.002
MDB-3	Intended	1000	0.054	39.55	50
	Actual	1021.87	0.0549	39.5641	50.018
		<b>Intended concentration (mg a.s./L)</b>	<b>Intended mass stock MDB-4 added (g)</b>	<b>Mass of final volume (after addition with acetone) (g)</b>	<b>Final volume stock</b>
MDB-2	Intended	300	3.955	39.55	50
	Actual	300.21	3.9577	39.5675	50.022

<sup>a)</sup> This solution was not used in this particular experiment since it was intended for a treatment level of 10 mg a.s./L.

## 7. Measurements and observations:

Observations for immobility, mortality and possible number of offspring were performed on day 7, 14, 21 and 28 in test vessels of all treatments. Effects were scored under mortality when no response of any kind was observed over a time period of 15 seconds after tactile stimulation. Effects were scored under immobility when after tactile stimulation only limited movement was visible.

At test termination, length and dry weight were determined for all surviving test organisms. For the length measurement, animals were photographed using a stereomicroscope, after which their length was measured using computer software ImageJ. For dry weight estimation, the surviving individuals from each test vessel were dried at 105°C for 24 hours. The average dry weight of these individuals per vessel was weighed on the micro-balance. On day 0, length and dry weight biomass was measured for 20 representative test individuals to provide these measurements for the start of the experiment.

Mandestrobin concentrations were monitored in the dosing solutions and in each test vessel immediately after each treatment and before refreshment of the medium. Analysis was performed using LC-QQQ.

Oxygen, temperature, pH and electrical conductivity were measured before and after each medium refreshment in test systems of controls and the highest treatment level.

## 8. Statistics:

NOEC and LC<sub>x</sub> values were calculated for mortality on days 7, 14, 21 and 28. Clear immobility effects could not be scored during the test, therefore NOEC and EC<sub>x</sub> values for immobility were not calculated.

28-day NOEC and EC<sub>x</sub> values were calculated for dry weight of survivors, length of survivors, daily growth in dry weight of survivors and daily growth in length of survivors.

E(L)C<sub>x</sub> values and 95% confidence limits were calculated by a log concentration-logit effect regression method. Within the regression, calculated E(L)C<sub>x</sub> values were corrected for mortality in the controls. The regression method was programmed in GENSTAT (version 18).

NOEC estimations for each endpoint ( $p \leq 0.05$ ) were carried out using the Williams test. The analyses were performed with the Community Analysis (CA) computer program.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

#### Mortality

There was a statistically significant increase in mortality at the highest treatment level of 2.8 mg a.s./L (TWA) compared to the pooled control. No significant effects on mortality were observed at any other treatment level (See Table 10.2.1/06-2).

**Table 10.2.1/06-2: Summary of effects on adult survival**

TWA concentration (mg a.s./L)	% Mortality (D28) <sup>a)</sup>
Control	19.0
Solvent control	8.7
0.029	3.33
0.095	16.67
0.25	13.33
0.92	0.00
2.8	93.33*

\* Significantly increased compared to the pooled controls.

<sup>a)</sup> Mortality values for the treatment levels calculated by the Applicant as the values were not presented in the report.

#### Reproduction

On day 28, juvenile *A. aquaticus* were observed in some of the test systems. The occurrence of offspring was very irregularly distributed over the replicate systems. In the highest treatment level no juveniles were found at all, while only one juvenile was observed in the 1.0 mg a.s./L treatment level. Therefore, it was not possible to estimate reliable effect concentrations and no further analysis of this measurement parameter was performed.

#### Growth

At test initiation, 20 isopods were separated into two groups of 10 to determine average dry weight per group. Reported dry weights per group of 10 individuals were 7.585 mg and 8.480 mg, corresponding to 0.759 mg and 0.848 mg per individual, respectively.

At test termination (day 28), the dry weight of surviving isopods was determined in groups (per replicate). A statistically significant reduction in individual dry weight was observed at the four highest treatment levels, compared to the pooled control. The mean individual dry weights per control and treatment group are reported in Table 10.2.1/06-3.

At test initiation, lengths of 20 isopods were measured individually. The mean individual length at test initiation was 4.95 mm.



At test termination (day 28), the length of surviving isopods was again determined individually. A statistically significant reduction in individual length was observed at the two highest treatment levels compared to the pooled control. The mean individual lengths of survivors per control and treatment group are reported in Table 10.2.1/06-3.

**Table 10.2.1/06-3: Summary of effects on growth on day 28 <sup>a)</sup>**

TWA concentration (mg a.s./L)	Mean individual length (mm)	Mean growth rate (mm/day)	Mean individual dry weight (mg)	Mean growth rate (mg/day)
Control	7.518	0.016	2.799	0.046
Solvent control	7.836	0.018	2.778	0.046
0.029	7.440	0.016	2.594	0.044
0.095	7.050	0.014*	2.299*	0.040
0.25	6.650	0.012*	1.964*	0.034*
0.92	5.737*	0.006*	1.004*	0.009*
2.8	4.220*	-0.004*	0.360*	-0.027*

\* Significantly reduced compared to the pooled controls.

<sup>a)</sup> Values were calculated by the Applicant as the mean values were not clearly presented in the report.

A summary of endpoints based on both the nominal and TWA concentrations is shown in Table 10.2.1/06-4 below.

**Table 10.2.1/06-4: Summary of endpoints based on nominal and TWA concentrations**

Parameter	Endpoint	Based on nominal concentrations (mg a.s./L)	Based on TWA concentrations (mg a.s./L)
28-day adult survival	LC <sub>10</sub>	1.9 <sup>a)</sup>	1.5 <sup>a)</sup>
	LC <sub>20</sub>	2.1 <sup>a)</sup>	1.7 <sup>a)</sup>
	LC <sub>50</sub>	2.4 <sup>a)</sup>	2.0 <sup>a)</sup>
	NOEC	1.0	0.92
Mean individual dry weight of survivors (mg)	EC <sub>10</sub> (95% CI)	0.060 (0.010 - 0.28)	0.050 (0.010 - 0.23)
	EC <sub>20</sub> (95% CI)	0.15 (0.060 - 0.39)	0.13 (0.050 - 0.34)
	EC <sub>50</sub> (95% CI)	0.63 (0.43 - 0.93)	0.57 (0.38 - 0.85)
	NOEC	0.030	0.029
Mean individual length of survivors (mm)	EC <sub>10</sub> (95% CI)	0.64 (0.27 - 1.5)	0.60 (0.25 - 1.4)
	EC <sub>20</sub> (95% CI)	0.90 (0.46 - 1.8)	0.84 (0.43 - 1.6)
	EC <sub>50</sub> (95% CI)	1.6 (1.1 - 2.4)	1.5 (1.0 - 2.2)
	NOEC	0.30	0.25
Mean individual daily growth in dry weight of survivors (mg/day)	EC <sub>10</sub> (95% CI)	0.22 (0.080 - 0.60)	0.18 (0.060 - 0.52)
	EC <sub>20</sub> (95% CI)	0.31 (0.14 - 0.68)	0.25 (0.11 - 0.60)
	EC <sub>50</sub> (95% CI)	0.54 (0.33 - 0.87)	0.47 (0.28 - 0.79)
	NOEC	0.10	0.095
Mean individual daily growth in length of survivors (mm/day)	EC <sub>10</sub>	0.88 <sup>a)</sup>	0.81 <sup>a)</sup>
	EC <sub>20</sub>	0.92 <sup>a)</sup>	0.85 <sup>a)</sup>
	EC <sub>50</sub>	0.99 <sup>a)</sup>	0.91 <sup>a)</sup>
	NOEC	0.030	0.029

CI: Confidence intervals.

<sup>a)</sup> No convergence of the model. No 95% confidence intervals could be determined.

## B. ANALYSIS

Measured mandestrobin concentrations in the dosing solutions ranged between 96 to 107% of nominal concentrations. In the test systems, mandestrobin concentrations remained relatively stable after each medium refreshment throughout the test period and overall did not decline below 80% of initial levels, except in the 0.30 mg a.s./L treatment immediately after the last refreshment. As the measurement at the end of the incubation period showed a correct concentration, this was likely due to incomplete mixing of the test volume just after the dosing. The time weighted average concentration (TWA) over the complete 28-day test period was calculated for each test vessel (see Table 10.2.1/06-5 below).

**Table 10.2.1/06-5: Calculated time weighted average concentrations of mandestrobin over the whole experimental period**

Nominal concentration (mg a.s./L)	Mean TWA concentration (mg a.s./L)	Mean TWA concentration (% of nominal)
Control	0	n.a.
Solvent control	0	n.a.
0.03	0.029	97.3
0.1	0.095	94.7
0.3	0.25	82.4
1.0	0.92	92.3
3.0	2.8	91.8

n.a.: not applicable.

## C. VALIDITY CRITERIA

The study was performed with *Asellus aquaticus*, which is not a standard test species in the EU. As such, there is no adopted guideline for this species. The study was designed based on a combination of OECD 211 (2012), OECD 219 (2004) and OECD 233 (2010), and appropriate validity criteria were designed in the study protocol. The study fulfilled the validity criteria outlined in the study protocol, as detailed below:

- The mortality and sub-lethal effects in the controls (i.e. control and solvent controls) should not exceed 20% at the end of the test (actual: 19.0 and 8.7% in the negative and solvent controls, respectively, on day 28).
- At the end of the test/incubation period, the oxygen concentration should be at least 60% of the air saturation at the temperature used (actual:  $\geq 83.6\%$ ).

## III. CONCLUSION

The chronic toxicity of mandestrobin to the aquatic isopod *Asellus aquaticus* was evaluated under semi-static conditions in the laboratory over 28 days, at five nominal concentrations between 0.030 and 3.0 mg a.s./L.

At test termination there was a significant reduction in survival at the highest TWA treatment level of 2.8 mg a.s./L. There was a significant reduction in individual dry weight at 0.095, 0.25, 0.92 and 2.8 mg a.s./L and on mean growth rate (mg/day) at 0.25, 0.92 and 2.8 mg a.s./L. There was also a significant reduction in individual body length at 0.92 and 2.8 mg a.s./L and on mean growth rate (mm/day) at 0.095, 0.25, 0.92 and 2.8 mg a.s./L.

The 28-day NOEC for survival was 0.92 mg a.s./L and the lowest NOEC for growth was 0.029 mg a.s./L, based on effects on individual dry weight and growth in terms of mm/day.

Assessment and Conclusion by Applicant:	<p>Although not a standard test species, the study has been performed according to current standards and is considered to be fully valid.</p> <p>Based on the mean measured concentrations, the relevant endpoint derived from the study is: NOEC: 0.029 mg a.s./L (growth)</p>
---	---

Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met. The minor deviations were noted, not ecotoxicologically relevant.</p> <p>The following endpoints were derived: NOEC: 0.029 mg a.s./L (based on TWA concentration) EC<sub>10</sub> = 0.050 mg a.s./L (based on TWA concentration)</p>
-------------------	--

#### A 2.2.1.7 Study 7

<b>Data point:</b>	KCP 10.2.1/07
<b>Report author:</b>	Shaw, A.C.
<b>Report year:</b>	2021b
<b>Report title:</b>	Mandestrobin – 42-Day Toxicity Test Exposing Freshwater Amphipods ( <i>Hyalella azteca</i> ) Under Static-Renewal Conditions
<b>Report No.:</b>	13048.7202
<b>Document No.:</b>	ROW-0127
<b>Guidelines followed in study:</b>	U.S. EPA Test Method 100.4; ASTM E1706-19; U.S. EPA OCSP 850.1000.
<b>Deviations from current test guideline:</b>	None/not applicable.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to the freshwater amphipod *Hyalella azteca* was determined over 42 days under semi-static conditions. *H. azteca* were exposed to five nominal concentrations between 0.063 and 1.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Eight replicates were set up per treatment group and control each containing 10 amphipods. Test medium was renewed three times per week. Observations for mortality and behaviour were performed daily. Offspring production was measured at each solution renewal. At test termination the sex of surviving amphipods was determined, and the growth (length) measured. The results of the study were reported based on the mean measured concentrations.

Based on the results of survival, reproduction, and growth (measured as length), the overall NOEC was determined to be 0.26 mg a.s./L (reproduction and length). The LC<sub>50</sub> value for survival was calculated to be 0.75 mg a.s./L, the EC<sub>50</sub> for reproduction was 0.38 mg a.s./L and the EC<sub>50</sub> for body length was not calculated.

### I. MATERIALS AND METHODS

## A. MATERIALS

1. **Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.7%
2. **Controls:** Negative control: dilution water  
Solvent control: dilution water + acetone
3. **Reference item:** Not applicable.

## B. STUDY DESIGN AND METHODS

1. **Test animals:** Freshwater amphipod (*Hyalella azteca*)  
**Age:** 8 days old at exposure initiation  
**Source:** In-house culture  
**Diet:** Flaked fish food suspension and *Rhaphidocelis subcapitata* ( $6 \times 10^7$  cells/mL) during holding and flaked fish food suspension and a suspension of YTC (yeast, trout chow, and cereal leaves) during testing at an increasing rate increasing with age.
2. **Dilution water:** Mixture of fortified well water and dechlorinated tap water  
**Total hardness:** 60 – 76 mg/L as  $\text{CaCO}_3$   
**Total alkalinity:** 20 – 26 mg/L as  $\text{CaCO}_3$   
**pH:** 7.0 – 7.3  
**Conductivity:** 390 – 520  $\mu\text{S}/\text{cm}$
3. **Test vessels:** 250 mL clear glass beakers each containing approximately 200 mL of test solution.
4. **Environmental conditions:**  
**Temperature:** 21 – 24°C (continuously monitored 21 – 24°C)  
**pH:** 6.4 – 7.2  
**Dissolved oxygen:** 2.6 – 9.5 mg/L  
**Photoperiod:** 16 hours light : 8 hours dark (500 – 1000 lux)

### 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to the freshwater amphipod *H. azteca* was determined over 42 days under semi-static conditions. *H. azteca* were exposed to five nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Eight replicates were set up per treatment group and control each containing 10 amphipods.

The test was initiated when amphipods were impartially distributed to each of the eight replicates for each nominal concentration and the controls. At exposure initiation and three times per week thereafter, fresh exposure solutions were prepared and added to a new set of test vessels before the organisms were transferred by pipette.

### 6. Dose preparation:

Prior to exposure initiation, a 10 mg/mL primary stock solution was prepared by placing 0.2664 g (0.2496 g as active ingredient) of mandestrobin in a volumetric flask and bringing it to a volume of 25 mL with acetone. After mixing by inversions, the resulting stock solution was observed to be yellow with no visible undissolved test substance.

Secondary stock solutions were prepared as dilutions at nominal concentrations of 5.0, 2.5, 1.3 and 0.63 mg/mL. All resulting stock solutions were observed to be yellow with no visible undissolved test substance. The yellow colour decreased with intensity with decreasing concentration. These stock solutions were used to prepare exposure solutions at test initiation and at each renewal interval. A volume of 0.20 mL of the appropriate stock solution was added to 2.0 L of dilution water to achieve the desired test concentrations.

A solvent control was established by adding 0.20 mL of acetone to 2.0 L of dilution water. Each test solution was mixed for approximately one minute using a glass rod. All test solutions were observed to be clear and colourless with no visible undissolved test substance present following preparation. Each exposure and control solution was divided into eight replicate vessels containing approximately 200 mL each. A small amount of quartz sand (approximately 5 g) was added to each replicate as substrate to mitigate organism stress.

## **7. Measurements and observations:**

Observations of organism behaviour (e.g., sublethal effects) and characteristics of the exposure solutions were recorded daily. Dead organisms were recorded for each exposure vessel. Dead organisms are defined as the lack of visible movement or lack of reaction of a test organism to gentle prodding. The day of appearance of the first offspring in each exposure vessel was recorded. Live offspring were counted and removed at each solution renewal interval beginning with the appearance of the first brood. The presence of immobilized or dead offspring was noted, if observed.

At test termination, survived adult males were identified by the enlarged second gnathopod and the numbers of males and females were recorded. Reproduction was evaluated as the cumulative number of offspring per female per vessel, as of test day 42. In addition, the number of gravid females recovered on test day 42 in each replicate was recorded. Following exposure termination, the length of each surviving adult amphipod was measured. The adults were preserved for up to two weeks in a buffered formalin solution prior to taking images for the determination of amphipod length. The growth of amphipods was determined by measuring body length from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface to the nearest 0.01 mm using a digital camera in combination with imaging software. Dissolved oxygen, temperature and pH were measured in each test and control solution at the beginning (new solutions) and end (aged solutions) of each renewal period, where appropriate. The temperature of the water bath was continuously monitored throughout the study in a satellite vessel.

During the definitive exposure, one sample was removed from each treatment level, negative control, and solvent control solution for analysis of mandestrobin concentration at day 0 (new), 2 (new & aged), 5 (aged), 7 (new), 9 (aged), 14 (new), 16 (aged), 23 (new), 26 (aged), 30 (new), 33 (aged), 37 (new), 40 (new & aged) and 42 (aged). New solutions were collected from intermediate mixing vessels before addition to individual tests vessels and old solutions were collected from composited (pooled) replicates. All samples were analysed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

## **8. Statistics:**

At the termination of the study, data obtained on organism survival, reproduction and growth (as total body length) were statistically analysed to identify significant treatment-related effects.

All statistical analyses were conducted at the 95% level of certainty except in the case of the qualifying tests for normality and homogeneity, in which the 99% level of certainty was applied. CETIS Version 1.9 was used to perform the statistical computations. The NOEC and LOEC were determined.

The negative and solvent controls were assessed for differences. Survival was assessed using Two-sample t-Test. Reproduction and growth were assessed for normality using Shapiro-Wilk's Test and homogeneity using Variance Ratio F Test before analysis with the Two-sample t-Test. Survival was compared to the solvent control for the treatment data and reproduction and growth were compared to the pooled control. For survival treatment analysis, Cochran-Armitage (O) Trend Test was used. For reproduction treatment

analysis, Shapiro-Wilk's Test was used for normality and Bartlett's Equality of Variance Test for homogeneity before analysis with Dunnett Multiple Comparison Test. For growth treatment analysis, Shapiro-Wilk's Test was used for normality and Bartlett's Equality of Variance Test for homogeneity before analysis with Jonckheere-Terpstra Step-Down Test.

Where appropriate, the data for survival, reproduction and growth were used to estimate the 42-day  $L/EC_{10/20/50}$  values and the corresponding 95% confidence intervals. If at least one test concentration caused a  $\geq 10$ , 20, or 50% reduction in survival, reproduction or growth of the test population, then CETIS Version 1.9 was used to calculate the  $L/EC_x$  values and 95% confidence intervals. Estimations were performed with a linear and nonlinear regression analysis. When linear and nonlinear regression do not meet the criteria, linear interpolation was performed.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

Results for adult survival, reproduction and growth (total body length) are shown in Table 10.2.1/07-1. A summary of endpoints is presented in Table 10.2.1/07-2. Test day 28 survival data was evaluated to establish that acceptability criteria of  $\geq 80\%$  survival in the control(s) was met but the day 28 data was not statistically analysed.

There was a statistically significant reduction in survival at day 42 among amphipods at 1.0 mg a.s./L compared to the solvent control. There was a statistically significant reduction in reproduction among amphipods at 0.14 and 0.53 mg a.s./L compared to the pooled control.

Although the 0.14 mg a.s./L treatment level was determined to have a statistically significant reduction compared to the pooled control, the higher treatment level (0.26 mg a.s./L) was not determined to have a significant effect and had the same average offspring per female as the low treatment level. Thus, the slightly lower reproduction in the 0.14 mg a.s./L treatment level was considered to be biological variability and the NOEC and LOEC for reproduction are reported as 0.26 and 0.53 mg a.s./L, respectively.

There was a statistically significant reduction in total body length among amphipods at 0.53 mg a.s./L compared to the pooled control. Due to significant mortality in the 1.0 mg a.s./L treatment, this treatment was not assessed for length.

Both the negative control and solvent control amphipods released their first offspring on test day 28. First offspring release also occurred on test day 28 in the 0.067, 0.14, 0.26, and 0.53 mg a.s./L treatment levels. No offspring were produced in the 1.0 mg a.s./L treatment level.

**Table 10.2.1/07-1: Summary of effects on adult survival, reproduction and growth**

Mean measured concentration (mg a.s./L)	Mean % adult survival $\pm$ SD (day 28)	Mean % adult survival $\pm$ SD (day 42)	Mean no. of offspring per female $\pm$ SD	Mean body length (mm) $\pm$ SD
Control	96 $\pm$ 5	96 $\pm$ 5	9 $\pm$ 5	6.21 $\pm$ 0.18
Solvent control	94 $\pm$ 7	78 $\pm$ 9	9 $\pm$ 3	6.22 $\pm$ 0.22
Pooled control	-	87 $\pm$ 12	9 $\pm$ 4	6.22 $\pm$ 0.20
0.067	91 $\pm$ 6	74 $\pm$ 13	8 $\pm$ 4	6.42 $\pm$ 0.22
0.14	100 $\pm$ 0	80 $\pm$ 11	5** $\pm$ 3	6.20 $\pm$ 0.42
0.26	81 $\pm$ 22	74 $\pm$ 19	8 $\pm$ 4	6.03 $\pm$ 0.23
0.53	91 $\pm$ 17	66 $\pm$ 33	3** $\pm$ 2	5.82*** $\pm$ 0.19
1.0	50 $\pm$ 20	14* $\pm$ 11	0 (n.a.)	5.28 $\pm$ 0.38

SD: Standard deviation; n.a.: not applicable.

\* Significantly reduced compared to the solvent control (Cochran-Armitage (O) Trend Test).

\*\* Significantly reduced compared to the pooled control (Dunnett's Multiple Comparison Test).

\*\*\* Significantly reduced compared to the pooled control (Jonckheere-Terpstra Step-Down Test).

**Table 10.2.1/07-2: Summary of endpoints**

<b>Endpoint (mg a.s./L)</b>	<b>Survival</b>	<b>Reproduction</b>	<b>Body length</b>
L/EC <sub>10</sub> (95% CI)	n.c.	n.c.	0.66 (0.53 – 0.78)
L/EC <sub>20</sub> (95% CI)	0.58 (0.43 – 0.67)	n.c.	n.c.
L/EC <sub>50</sub> (95% CI)	0.75 (0.67 – 0.85)	0.38 (0.14 – 0.49)	n.c.
NOEC	0.53	0.26	0.26
LOEC	1.0	0.53	0.53
<b>Overall NOEC = 0.26 mg a.s./L</b>			
<b>Overall LOEC = 0.53 mg a.s./L</b>			

CI: Confidence intervals; n.c.: not calculable/unreliable value.

## B. ANALYSIS

Measured concentrations were generally consistent between sampling intervals for both the newly prepared and aged solutions. Mean measured concentrations ranged from 100 to 110% of nominal concentrations (see Table 10.2.1/07-3).

**Table 10.2.1/07-3: Measured concentrations of mandestrobin in the exposure solutions**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)								
	Day 0 (new)	Day 2 (aged/new)	Day 5 (aged)	Day 7 (new)	Day 9 (aged)	Day 14 (new)	Day 16 (aged)	Day 23 (new)	Day 26 (aged)
Control	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011
Solvent control	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011
0.063	0.068	0.065/0.064	0.071	0.069	0.074	0.068	0.072	0.073	0.071
0.13	0.14	0.13/0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.15
0.25	0.25	0.24/0.24	0.28	0.27	0.27	0.27	0.28	0.26	0.29
0.50	0.54	0.48/0.49	0.55	0.54	0.56	0.51	0.54	0.55	0.54
1.0	1.0	0.94/0.94	1.1	1.1	1.1	1.1	1.1	1.2	1.1
Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)								% of nominal
	Day 30 (new)	Day 33 (aged)	Day 37 (new)	Day 40 (aged/new)	Day 42 (aged)	Mean	%CV		
Control	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	n.a.	n.a.	n.a.	
Solvent control	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011	n.a.	n.a.	n.a.	
0.063	0.070	0.062	0.063	0.061/0.065	0.059	0.067	6.8	110	
0.13	0.15	0.13	0.13	0.12/0.14	0.13	0.14	5.7	110	
0.25	0.28	0.24	0.26	0.24/0.27	0.23	0.26	6.8	100	
0.50	0.54	0.51	0.51	0.51/0.53	0.50	0.53	4.8	110	
1.0	1.0	1.0	1.0	0.99/1.0	1.0	1.0	6.4	100	

CV : Coefficient of variation; n.a.: not applicable.



### C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the study protocol, as detailed below and is considered valid:

- Test organisms should be 7 to 8 days old at exposure initiation (actual age: 8 days).
- All organisms in the exposure should be from the same source. All organisms were from the same in-house culture.
- All test vessels should be identical and/or contain approximately the same amount of solution. All test vessels were identical glass beakers (250 mL) containing the same quantity (200 mL) of solution.
- The mean survival on day 28 should be  $\geq 80\%$  in the control(s). The mean survival on day 28 was 96 and 94% in the negative control and solvent control, respectively.
- The mean length on day 42 should be  $\geq 3.2$  mm in the control(s). The mean length on day 42 was 6.21 and 6.22 mm in the negative control and solvent control, respectively.
- Reproduction by day 42 should be  $\geq 2$  young per surviving female in the control(s). Reproduction by day 42 was 9 young per surviving female both in the negative control and solvent control.

### III. CONCLUSION

The chronic toxicity of mandestrobin to the freshwater amphipod *H. azteca* was determined over 42 days under semi-static conditions. Results are reported based on the mean measured concentrations.

Based on the results of survival, reproduction and growth (measured as length), the overall NOEC was determined to be 0.26 mg a.s./L (reproduction and length). The  $LC_{50}$  value for survival was calculated to be 0.75 mg a.s./L, the  $EC_{50}$  for reproduction was 0.38 mg a.s./L and the  $EC_{50}$  for body length was not reported.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>Based on the mean measured concentrations, the relevant endpoint derived from the study is: NOEC: 0.26 mg a.s./L</p>
Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met. The minor deviations from study protocol were noted, no impact on exposure.</p> <p>The following endpoints were derived: NOEC: 0.26 mg a.s./L (reproduction; based on measured concentration) <math>EC_{10}</math> = 0.66 mg a.s./L (length; based on measured concentration; no <math>EC_{10}</math> for reproduction was reported)</p>

### A 2.2.1.8 Study 8

<b>Data point:</b>	KCP 10.2.1/08
<b>Report author:</b>	Shaw, A.C.
<b>Report year:</b>	2022
<b>Report title:</b>	Mandestrobin – 28-Day Toxicity Test Exposing Freshwater Decapods ( <i>Palaemonetes paludosus</i> ) Under Static-Renewal Conditions
<b>Report No.:</b>	13048.7203
<b>Document No.:</b>	ROW-0148
<b>Guidelines followed in study:</b>	U.S. EPA OCSPP 850.1000 and ASTM STP 667
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The chronic toxicity of mandestrobin to the freshwater decapod *Palaemonetes paludosus* was determined over 28 days under semi-static conditions. *P. paludosus* were exposed to five nominal concentrations between 0.13 and 2.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Four replicates were set up per treatment group and control each containing five decapods. Test medium was renewed three times per week. Observations of mortality and behaviour (e.g. sublethal effects) and characteristics of the exposure solutions were recorded daily. Dry weight of each surviving adult decapod was measured at test termination on day 28. The results of the study were reported based on the mean measured concentrations.

A significant effect on survival was observed at the highest treatment level of 2.1 mg a.s./L. No significant effects were observed at any other treatment level. Due to the effects on survival at 2.1 mg a.s./L, this treatment level was excluded from analysis of dry weight. No significant effects on dry weight were observed at any other treatment level.

The LC<sub>10/20/50</sub> for survival were determined to be 1.2, 1.5 and > 2.1 mg a.s./L, respectively, and the NOEC was 1.0 mg a.s./L. The EC<sub>10/20/50</sub> for dry weight were all determined to be > 2.1 mg a.s./L and the NOEC was 1.0 mg a.s./L.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Not reported  
**Lot/Batch:** ST-0811G  
**Purity:** 93.7%
- Controls:** Negative control: dilution water  
 Solvent control: dilution water + acetone
- Reference item:** Not applicable.

## B. STUDY DESIGN AND METHODS

- 1. Test animals:** Freshwater decapod/grass shrimp (*Palaemonetes paludosus*)  
**Age:** 1 – 2 weeks old at exposure initiation  
**Source:** Northeast Brine Shrimp LLC, Oak Hill, FL, USA  
**Diet:** During holding and acclimation, the freshwater decapods were fed shrimp food pellet diet, spirulina formulation flakes, and live brine shrimp.  
  
During the 28-day test, each replicate test vessel received a 0.50 or 0.60 mL aliquot of live brine shrimp and a 0.50 or 0.60 mL aliquot of a 10 mg/mL spirulina flake slurry daily.
- 2. Dilution water:** Laboratory well water  
**Total hardness:** 54 – 64 mg/L as CaCO<sub>3</sub>  
**Total alkalinity:** 24 – 30 mg/L as CaCO<sub>3</sub>  
**pH:** 6.6 – 7.5  
**Conductivity:** 350 – 500 mg/L as µS/cm
- 3. Test vessels:** 600 mL clear glass beakers, each containing approximately 500 mL of exposure solution.
- 4. Environmental conditions:**  
**Temperature:** 22 – 24°C  
**pH:** 6.8 – 8.0  
**Dissolved oxygen:** 6.6 – 9.3 mg/L (78 – 110% saturation)  
**Photoperiod:** 16 hours light : 8 hours dark (490 – 950 lux)

### 5. Animal assignment and treatment:

The chronic toxicity of mandestrobin to the freshwater decapod *Palaemonetes paludosus* was determined over 28 days under semi-static conditions. *P. paludosus* were exposed to five nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L. An untreated control and solvent (acetone) control were tested in parallel. Four replicates were set up per treatment group and control, each containing five decapods.

The test was initiated when decapods were impartially distributed to each of the four replicates for each nominal concentration and the controls. At exposure initiation and three times per week thereafter, fresh exposure solutions were prepared and added to a new set of test vessels before the organisms were transferred by pipette or soft mesh net.

### 6. Dose preparation:

Prior to exposure initiation, a 20 mg/mL primary stock solution was prepared by placing 0.5276 g (0.4944 g as active ingredient) of mandestrobin in a glass volumetric flask and bringing to a volume of 25 mL with acetone. After mixing by inversions, the resulting stock solution was observed to be yellow with no visible undissolved test substance.

Secondary stock solutions were prepared as dilutions at nominal concentrations of 10, 5.0, 2.5 and 1.3 mg/mL. The secondary stock solutions were observed to be clear with a yellow tint and no visible undissolved test substance following mixing by inversions of the glass volumetric flasks. The yellow colour decreased in intensity with each decreasing concentration. These stock solutions were used to prepare exposure solutions at test initiation and at each renewal interval. A volume of 0.25 mL of the appropriate stock solution was added to 2.5 L of dilution water to achieve the desired test concentrations.

A solvent control was established by adding 0.25 mL of acetone to 2.5 L of dilution water. Each test solution was mixed for approximately one minute using a glass rod. All test solutions were observed to be clear and

colourless with no visible undissolved test substance present following preparation. Each exposure and control solution was divided into four replicate vessels containing approximately 500 mL each.

## **7. Measurements and observations:**

Observations of organism behaviour (e.g. sublethal effects) and characteristics of the exposure solutions were recorded daily. Any dead organisms were recorded for each exposure vessel. Dead organisms are defined as the lack of visible movement or lack of reaction of a test organism to gentle prodding. Following exposure termination, the dry weight of each surviving adult decapod was measured to the nearest 0.01 mg. Dissolved oxygen, temperature, and pH were measured in each newly prepared test concentration and control solution at exposure initiation, in both new and aged solutions at each renewal interval, and in aged solutions at exposure termination. Water quality was measured in a representative replicate in each treatment level or control. The temperature of the water bath was continuously monitored in a satellite vessel throughout the study.

During the in-life phase of the definitive exposure, new solution samples were removed from each treatment level, negative control, and solvent control solution for analysis of mandestrobin concentration at days 0 (exposure initiation), 2, 7, 14, 23, and 26. Aged solution samples were removed from each treatment level, negative control, and solvent control solution for analysis of mandestrobin concentration at days 2, 5, 9, 16, 26, and 28 (exposure termination). New solution samples were removed from the intermediate mixing vessel prior to division into the replicate test vessels. Aged solution samples were removed from a composite of the replicate solutions of each test concentration and the control. All samples were analysed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

## **8. Statistics:**

At the termination of the study, data obtained on organism survival and growth (as dry weight) were statistically analysed to identify significant treatment-related effects.

All statistical analyses were conducted at the 95% level of certainty except in the case of the qualifying tests for normality and homogeneity, in which the 99% level of certainty was applied. CETIS Version 1.9 was used to perform the statistical computations. The NOEC and LOEC were determined.

The negative and solvent controls were assessed for differences. Survival was assessed using Two-sample t-Test. Dry weight data were assessed for normality using Shapiro-Wilk's Test and homogeneity using Variance Ratio F Test before analysis with the Two-sample t-Test. Treatment survival was compared to the pooled control and dry weight data were compared to the solvent control. For survival treatment analysis, Cochran-Armitage (O) Trend Test was used. For dry weight, treatments were compared to the solvent control using the Jonckheere-Terpstra Step-Down Test.

Where appropriate, the data for survival and dry weight were used to estimate the 28-day  $L/EC_{10/20/50}$  values and the corresponding 95% confidence intervals. If at least one test concentration caused a  $\geq 10$ , 20, or 50% reduction in survival or growth of the test population, then CETIS Version 1.9 was used to calculate the  $L/EC_x$  values and 95% confidence intervals. Estimations were performed with a linear and nonlinear regression analysis. When linear and nonlinear regression did not meet the criteria, linear interpolation was performed.

# **II. RESULTS AND DISCUSSION**

## **A. BIOLOGICAL EFFECTS**

Results for adult survival and growth (dry weight) are shown in Table 10.2.1/08-1. A summary of endpoints is presented in Table 10.2.1/08-2. Day 28 survival data was evaluated to establish that the acceptability criteria of  $\geq 80\%$  survival in the control(s) was met.

There was a statistically significant reduction in survival at day 28 among decapods at 2.1 mg a.s./L compared to the pooled control. Based on a significant effect determined at 2.1 mg a.s./L, this treatment

level was excluded from the comparison statistics for dry weight. There were no statistically significant reductions in dry weight in any of the other treatment levels analysed compared to the solvent control.

**Table 10.2.1/08-1: Summary of effects on survival and growth**

Mean measured concentration (mg a.s./L)	Mean % adult survival $\pm$ SD (day 28)	Mean dry weight (mg) $\pm$ SD (day 28)
Control	100 $\pm$ 0	2.00 $\pm$ 0.18
Solvent control	100 $\pm$ 0	1.58 $\pm$ 0.10
Pooled control	100 $\pm$ 0	1.79 $\pm$ 0.26
0.13	95 $\pm$ 10	1.99 $\pm$ 0.16
0.26	100 $\pm$ 0	1.75 $\pm$ 0.22
0.54	100 $\pm$ 0	1.62 $\pm$ 0.17
1.0	95 $\pm$ 10	1.61 $\pm$ 0.34
2.1	65* $\pm$ 19	1.73 $\pm$ 0.04

SD: Standard deviation.

\* Statistically significant reduction compared to the pooled control, based on Cochran-Armitage (O) Trend Test.

**Table 10.2.1/08-2: Summary of endpoints**

Endpoint (mg a.s./L)	Survival	Growth (dry weight)
EC/LC <sub>10</sub> (95% CI)	1.2 (0.61 – 1.5)	> 2.1 (n.a.)
EC/LC <sub>20</sub> (95% CI)	1.5 (1.1 – 2.5)	> 2.1 (n.a.)
EC/LC <sub>50</sub> (95% CI)	> 2.1 (n.a.)	> 2.1 (n.a.)
NOEC	1.0	1.0
LOEC	2.1	> 1.0

CI: Confidence intervals.

n.a.: not applicable. As the LC/EC value was empirically estimated to be greater than the highest treatment level tested, a 95% confidence interval could not be determined.

## B. ANALYSIS

Results of the analyses established that the measured concentrations approximated nominal concentrations in both new and aged solutions and the expected concentration gradient was maintained during the test. Mean measured concentrations ranged from 100 to 110% of nominal concentrations (see Table 10.2.1/08-3).

**Table 10.2.1/08-3: Measured concentrations of mandestrobin in the exposure solutions**

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)							
	Day 0 (new)	Day 2 (aged)	Day 2 (new)	Day 5 (aged)	Day 7 (new)	Day 9 (aged)	Day 14 (new)	Day 16 (aged)
Control	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020
Solvent control	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020
0.13	0.13	0.13	0.15	0.13	0.13	0.13	0.14	0.14
0.25	0.24	0.25	0.27	0.25	0.25	0.26	0.26	0.27
0.50	0.50	0.48	0.56	0.51	0.51	0.51	0.54	0.54
1.0	1.0	1.0	1.1	1.0	0.99	1.0	1.1	1.1
2.0	2.1	2.0	2.2	2.3	2.0	2.1	2.2	2.2
Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)							
	Day 23 (new)	Day 26 (aged)	Day 26 (new)	Day 28 (aged)	Mean	%CV	% of nominal	
Control	< 0.020	< 0.020	< 0.020	< 0.020	n.a.	n.a.	n.a.	
Solvent control	< 0.020	< 0.020	< 0.020	< 0.020	n.a.	n.a.	n.a.	
0.13	0.14	0.12	0.12	0.14	0.13	5.4	100	
0.25	0.29	0.26	0.28	0.30	0.26	6.9	110	
0.50	0.56	0.54	0.61	0.62	0.54	7.8	110	
1.0	1.1	1.0	0.86	1.2	1.0	7.3	100	
2.0	2.2	2.0	2.1	2.2	2.1	4.5	110	

CV : Coefficient of variation; n.a.: not applicable.

### C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the study protocol, as detailed below:

- All organisms in the exposure should be from the same source. All adult shrimp were purchased from the same original source and test organisms were produced from these adult shrimp at the test facility.
- All test vessels should be identical and/or contain approximately the same amount of solution. All test vessels were identical glass beakers (600 mL) containing the same quantity (500 mL) of solution.
- The mean survival on day 28 should be  $\geq 80\%$  in the control(s). The mean survival on day 28 was 100% in both the negative and solvent controls.

### III. CONCLUSION

The chronic toxicity of mandestrobin to the freshwater decapod *Palaemonetes paludosus* was determined over 28 days under semi-static conditions. The results of the study were reported based on the mean measured concentrations.

The LC<sub>10/20/50</sub> for survival were determined to be 1.2, 1.5 and > 2.1 mg a.s./L, respectively, and the NOEC was 1.0 mg a.s./L. The EC<sub>10/20/50</sub> for dry weight were all determined to be > 2.1 mg a.s./L and the NOEC was 1.0 mg a.s./L.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>Based on the mean measured concentrations, the relevant endpoint derived from the study is: 28-day NOEC: 1.0 mg a.s./L</p>
---	---

Comments of zRMS:	The study was not evaluated as it was performed with active substance. The
-------------------	--

	<p>study should be submitted and evaluated at the EU level during active substance renewal.</p> <p>In accordance with Central Zone Manual v. 3.0, December 2024, the study was submitted for risk refinement. The study was evaluated and accepted.</p> <p>The validation criteria were met. The minor deviations were noted, not ecotoxicologically relevant.</p> <p>The following endpoints were derived: NOEC: 1.00 mg a.s./L (based on mean measured concentration)) EC<sub>10</sub> = 1.2 mg a.s./L(based on mean measured concentration)</p>
--	--

#### A 2.2.1.9 Study 9

<b>Data point:</b>	KCP 10.2.1/09
<b>Report author:</b>	White, K., Lopez Mangas, A. and Eck, G.
<b>Report year:</b>	2024
<b>Report title:</b>	Mandestrobin: Species Sensitivity Distribution for the Refinement of the Chronic Risk to Aquatic Invertebrates Based on Two Approaches
<b>Report No.:</b>	2202311.UK0.6874
<b>Document No.:</b>	ROW-0158
<b>Guidelines followed in study:</b>	Not applicable
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Not applicable
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The aquatic risk assessment for the active substance mandestrobin is driven by toxicity to aquatic invertebrates. In order to refine the risk, a Tier 2B approach is taken with a Species Sensitivity Distribution (SSD).

In accordance with the Central Zone Evaluation Manual (v2.0, August 2023) available estimates (e.g. NOEC and EC<sub>10</sub> values) should not be mixed in the SSD, and the EC<sub>10</sub> is the preferable estimate for use. However, in two of the available studies for mandestrobin, no reliable EC<sub>10</sub> values could be calculated. One of these studies is with the standard species *Daphnia magna*, and the other is the original study with *Americamysis bahia* which represents the Tier 1 RAC in the aquatic risk assessment.

By contrast, the EFSA Aquatic Guidance Document (2013) indicates that for chronic SSDs with aquatic invertebrate data it is acceptable to combine estimate types, e.g. NOECs and EC<sub>10</sub> values.

In this report, two separate SSDs were calculated, in order to fulfil the differing requirements of the Central Zone Evaluation Manual (v2.0, August 2023) and the EFSA Aquatic Guidance Document (2013).

With the first approach, only EC<sub>10</sub> estimates were included, and a HC<sub>5</sub> value of 0.060 mg a.s./L and RAC of 0.020 mg a.s./L were calculated (assessment factor of 3).

In the second approach, EC<sub>10</sub> estimates were included where available or the NOEC where EC<sub>10</sub> values were not available. In this approach, a geometric mean of the NOECs from the two available *Americamysis* studies were included, to account for both studies. A HC<sub>5</sub> value of 0.056 mg a.s./L and RAC of 0.019 mg a.s./L were calculated (assessment factor of 3).

Regardless of the approach taken to application of the available data, a very similar Tier 2B RAC is derived for the chronic risk to aquatic invertebrates. The available results therefore provide additional reassurance and certainty as to the reliability of the Tier 2B RACs applied to the risk assessment.

## I. INTRODUCTION

In the original EFSA Conclusion (2015) for mandestrobin, the relevant NOEC from the chronic study with *Americamysis bahia* (ROW-0063) was listed as 0.0056 mg a.s./L, in contrast to the original reported NOEC of 0.049 mg a.s./L determined by the Study Director. This endpoint is considered to be unduly conservative by the Applicant, who argues that the original endpoint of 0.049 mg a.s./L is the most relevant and reliable for use from this study. This argumentation is based on the fact that a statistically significant effect found for the next higher concentration of 0.011 mg a.s./L tested in the study is not considered to be treatment related, since there were no statistically significant effects at the following concentrations of 0.024 and 0.049 mg a.s./L. This was, accordingly, discussed in the study report and 0.049 mg a.s./L was concluded as the appropriate NOEC for the study. A reliable EC<sub>10</sub> value could not be calculated.

In order to address the uncertainties, a new chronic study with *Americamysis bahia* (ROW-0096) was performed in 2016. A NOEC of 0.13 mg a.s./L and an EC<sub>10</sub> of 0.15 mg a.s./L were obtained from this study. These new data support the interpretation of the original study results by the Study Director and Applicant, with the NOEC of 0.049 mg a.s./L being comparable to the NOEC and EC<sub>10</sub> of the new study.

Since aquatic invertebrates are the most sensitive organism group driving the aquatic risk assessment for mandestrobin, and to refine the chronic risk, data on additional species were generated in order to perform a Species Sensitivity Distribution (SSD) and to derive a Tier 2B Regulatory Acceptable Concentration (RAC) based on the calculated median HC<sub>5</sub> value. The median HC<sub>5</sub> value is the concentration that with 50% certainty is lower than the toxicity values (e.g. EC<sub>x</sub> or NOECs) for 95% of the species tested. The generated data is focused on crustacean species which were considered to be sensitive taxa based on the existing data. The available dataset for mandestrobin (shown in Table 10.2.1/09-1 below) comprises ten studies covering nine species (since two of the available studies are with *Americamysis bahia*).



**Table 10.2.1/09-1: Available studies**

Author (date)	Report No.	Title
Sayers, L.E. (2010)	ROW-0020	S-2200 Technical Grade - Full Life Cycle Toxicity Test with Water Fleas ( <i>Daphnia magna</i> ) Under Static Renewal Conditions
Roessink, I. (2019)	ROW-0103	Chronic effects of the fungicide Mandestrobin to <i>Daphnia pulex</i>
Shaw, A.C. (2021)	ROW-0126	Mandestrobin - Full Life-Cycle Toxicity Test with Daphnids ( <i>Ceriodaphnia dubia</i> ) Under Static-Renewal Conditions
Claude, M.B., Kendall, T.Z., Gallagher, S.P. & Krueger, H.O (2012)	ROW-0063	S-2200: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid ( <i>Americamysis bahia</i> )
Urann, K. (2016)	ROW-0096	Mandestrobin (S-2200) - Life-Cycle Toxicity Test with Mysids ( <i>Americamysis bahia</i> )
Roessink, I. (2019)	ROW-0106	Chronic effects of the fungicide Mandestrobin to <i>Caridina parvidentata</i>
Roessink, I. (2019)	ROW-0105	Chronic effects of the fungicide Mandestrobin to <i>Gammarus pulex</i>
Roessink, I. (2019)	ROW-0104	Chronic effects of the fungicide Mandestrobin to <i>Asellus aquaticus</i>
Shaw, A.C. (2021)	ROW-0127	Mandestrobin – 42-Day Toxicity Test Exposing Freshwater Amphipods ( <i>Hyalella azteca</i> ) Under Static-Renewal Conditions
Shaw, A.C. (2022)	ROW-0148	Mandestrobin – 28-Day Toxicity Test Exposing Freshwater Decapods ( <i>Palaemonetes paludosus</i> ) Under Static-Renewal Conditions

In accordance with the Central Zone Evaluation Manual (v2.0, August 2023) available estimates (e.g. NOEC and EC<sub>10</sub> values) should not be mixed in the SSD, and the EC<sub>10</sub> is the preferable estimate for use. However, in two of the available studies, no reliable EC<sub>10</sub> values could be calculated (see Table 10.2.1/09-3). One of these studies is with the standard species *Daphnia magna* (ROW-0020), and the other is the original study with *Americamysis bahia* (ROW-0063) which represents the Tier 1 RAC in the aquatic risk assessment. Nevertheless, in order to comply with the CZ Evaluation Manual (v2.0, 2023), an SSD has been performed taking into account the available EC<sub>10</sub> estimates only. This is still feasible even with the omission of the two aforementioned studies, as the required number of eight endpoints from eight different species is available. Moreover, the species *Americamysis bahia* is still accounted for by using the EC<sub>10</sub> derived from the more recent study. The method for conducting this SSD is reported in Section II, and the results are described in Section III.

It is noted by the Applicant however, that the approach to the SSD outlined above, while in line with the requirements of the CZ Evaluation Manual (2023), leads to the omission of two valuable data points, considering that one of these studies is with the standard species *Daphnia magna*, and the other is the original invertebrate study with the disputed endpoint which led to the initiative to conduct this SSD.

In contrast to the CZ Evaluation Manual (v2.0, 2023) and in accordance with the EFSA Aquatic GD (2013), for chronic SSDs with aquatic invertebrate data it is acceptable to combine estimate types, e.g. NOECs and EC<sub>10</sub> values (see Table 10.2.1/09-2 below, reproduced based on Table 5 of the EFSA Aquatic GD).

**Table 10.2.1/09-2: Proposal for the derivation of a RAC in edge-of-field surface waters, based on hazardous concentrations derived from SSDs with aquatic invertebrates (reproduced based on Table 5 of the EFSA 2013 Aquatic GD).**

Type of effect/risk assessment	Relevant PEC	Hazardous concentration	AF to derive RAC from hazardous concentration
Chronic effect/risk assessment for invertebrates and long-term exposure	PEC <sub>sw,max</sub> or PEC <sub>sw,twa</sub>	Median chronic HC <sub>5</sub> (based on chronic NOEC and/or EC <sub>10</sub> data)	3

PEC: Predicted environmental concentration; AF: Assessment Factor; RAC: Regulatory Acceptable Concentration.

Therefore, in order to be inclusive of the two otherwise omitted studies with *Daphnia magna* and *Americamysis bahia*, a second SSD was performed, taking the approach to combine estimates. In this case, where EC<sub>10</sub> estimates were available, these were applied to the SSD, and where no reliable EC<sub>10</sub> estimates could be calculated, the NOEC values were applied instead. To avoid use of two separate endpoints for the same species, the available NOECs from the two studies on *Americamysis bahia* (both based on the growth parameter) were combined using a geometric mean. This geometric mean NOEC was calculated based on the NOEC of 0.049 mg a.s./L from the original study (argued as the most reliable by the Applicant) and 0.13 mg a.s./L from the repeat study. A resulting geometric mean of 0.08 mg a.s./L was calculated. The method for conducting this SSD is reported in Section II, and the results are described in Section III.

## II. METHODS

The SSDs were calculated using the tool “MOSAIC” (Modelling and Statistical Tools for Ecotoxicology), which was developed especially for this purpose by the University of Lyon (<https://mosaic.univ-lyon1.fr/ssd>) which is based on the R-package *fitdistrplus* that uses the maximum likelihood method to fit the distribution and bootstrap to calculate the 95% confidence intervals. As the R-script for the MOSAIC web tool is provided, this script was also run in R to reproduce the analysis performed with MOSAIC. Afterwards, additional code lines were included in the script to calculate further goodness of fit parameters and generate graphs.

The log-normal and the log-logistic distributions are the most widely used, as parameter estimation appears robust enough to accommodate for most datasets, as they contain only two parameters. In order to select which distribution best describes the data, in the first step a qualitative assessment, by visually assessing the representative curves, was performed. The value of the likelihood function and the Akaike's Information Criterion (AIC) for each distribution were then used as further decision criteria. The log-likelihood value and AIC are measures of goodness of fit for any model. The log-likelihood is the logarithm of the likelihood which corresponds to the probability of observing the data for a given set of parameters and therefore, the higher the value, the better the model fit. The AIC corresponds to deviance penalized by the complexity of the model and therefore, the smaller the value the better the model fit. The log-logistic distribution has heavier tails than the log-normal and is therefore more conservative in the determination of the HC<sub>5</sub>. As the MOSAIC web tool only provides log-likelihood values, the AIC values were calculated using the R-package *fitdistrplus*. This R package also allows the generation of several goodness of fit plots (e.g. histogram against fitted density functions; Q-Q plot; Empirical Cumulative Distribution Function against fitted distribution functions and P-P plot). Finally, the SSD curve was plotted for a better visualisation.

As outlined in Section I above, two separate SSDs were calculated with differing approaches. The combinations of endpoints applied to each SSD are shown in Tables 3 and 4. These approaches are outlined again below:

- Approach 1: Use of EC<sub>10</sub> estimates only (in line with the CZ Evaluation Manual v2.0, August 2023), see Table 10.2.1/09-3.
- Approach 2: Use of a combination of NOEC and EC<sub>10</sub> estimates (EC<sub>10</sub> where available, NOEC where there is no reliable EC<sub>10</sub>), see Table 10.2.1/09-4.

Log-logistic and log-normal distributions were used to derive a median HC<sub>5</sub> value and associated 95% confidence intervals for each approach. As a result, an SSD-RAC was derived in each case and discussed in the context of the higher-tier aquatic risk assessment.

**Table 10.2.1/09-3: Endpoints included in SSD Approach 1, EC<sub>10</sub> estimates only (shown in bold)**

Report No.	Test species	Timescale	Endpoint type		Endpoint value (mg a.s./L)
ROW-0020	<i>Daphnia magna</i>	21 d	NOEC	Mortality, growth and reproduction	0.56
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0103	<i>Daphnia pulex</i>	28 d	NOEC	Growth and reproduction	0.92
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.54</b>
ROW-0126	<i>Ceriodaphnia dubia</i>	7 d	NOEC	Growth and reproduction	0.63
			<b>EC<sub>10</sub></b>	<b>Reproduction</b>	<b>0.69</b>
ROW-0063	<i>Americamysis bahia</i>	36 d	NOEC	Mortality and growth	0.049
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0096	<i>Americamysis bahia</i>	28 d	NOEC	Growth and reproduction	0.13
			<b>LC<sub>10</sub></b>	<b>Mortality</b>	<b>0.15</b>
ROW-0106	<i>Caridina parvidentata</i>	28 d	NOEC	Mortality and growth	2.7
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>2.6</b>
ROW-0105	<i>Gammarus pulex</i>	28 d	NOEC	Growth	0.092
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.18</b>
ROW-0104	<i>Asellus aquaticus</i>	28 d	NOEC	Growth	0.029
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.050</b>
ROW-0127	<i>Hyalella azteca</i>	42 d	NOEC	Growth and reproduction	0.26
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.66</b>
ROW-0148	<i>Palaemonetes paludosus</i>	28 d	NOEC	Mortality and growth	1.0
			<b>LC<sub>10</sub></b>	<b>Mortality</b>	<b>1.2</b>

Note: **bold** values are used in the SSD. Studies greyed out were omitted from the SSD as no reliable EC<sub>10</sub> could be calculated.  
n.d.: not determined.

<sup>a)</sup> EC<sub>10</sub> values were not reported in the original study, and reliable values could not be calculated.

**Table 10.2.1/09-4: Endpoints included in SSD Approach 2, NOEC and EC<sub>10</sub> (shown in bold)**

Report No.	Test species	Timescale	Endpoint type		Endpoint value (mg a.s./L)
ROW-0020	<i>Daphnia magna</i>	21 d	NOEC	<b>Mortality, growth and reproduction</b>	<b>0.56</b>
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0103	<i>Daphnia pulex</i>	28 d	NOEC	Growth and reproduction	0.92
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.54</b>
ROW-0126	<i>Ceriodaphnia dubia</i>	7 d	NOEC	Growth and reproduction	0.63
			<b>EC<sub>10</sub></b>	<b>Reproduction</b>	<b>0.69</b>
ROW-0063	<i>Americamysis bahia</i>	36 d	NOEC	Mortality and growth	0.049
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0096	<i>Americamysis bahia</i>	28 d	NOEC	Growth and reproduction	0.13
			LC <sub>10</sub>	Mortality	0.15
ROW-0063/ ROW-0096	<i>Americamysis bahia</i>	n/a	<b>Geometric mean NOEC</b>	<b>Growth</b>	<b>0.08</b>
ROW-0106	<i>Caridina parvidentata</i>	28 d	NOEC	Mortality and growth	2.7
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>2.6</b>
ROW-0105	<i>Gammarus pulex</i>	28 d	NOEC	Growth	0.092
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.18</b>
ROW-0104	<i>Asellus aquaticus</i>	28 d	NOEC	Growth	0.029
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.050</b>
ROW-0127	<i>Hyalella azteca</i>	42 d	NOEC	Growth and reproduction	0.26
			<b>EC<sub>10</sub></b>	<b>Growth</b>	<b>0.66</b>
ROW-0148	<i>Palaemonetes paludosus</i>	28 d	NOEC	Mortality and growth	1.0
			<b>LC<sub>10</sub></b>	<b>Mortality</b>	<b>1.2</b>

Note: **bold** values are used in the SSD. n.d.: not determined.

<sup>a)</sup> EC<sub>10</sub> values were not reported in the original study, and reliable values could not be calculated.

### III. RESULTS AND DISCUSSION

#### A. SSD Approach 1 (EC<sub>10</sub> estimates only)

The results of this SSD are presented in Table 10.2.1/09-5, and goodness of fit plots for the log-normal and log-logistic fitted distributions are shown in Figure 10.2.1/09-1. Visual assessment of the fitted curves (Figure 10.2.1/09-1) indicates that both distribution fits are very close. The log-likelihood and AIC values are presented in Table 10.2.1/09-6. The log-likelihood value for the log-normal distribution is a little higher and the AIC value is slightly lower, indicating that this distribution is more appropriate for this data set. The SSD curve with the HC<sub>5</sub> value for mandestrobin based on the log-normal distribution is shown in Figure 10.2.1/09-2.

**Table 10.2.1/09-5: Median HC<sub>p</sub> values and their 95% confidence limits for the fitted log-logistic and log-normal distributions**

Distribution	mg a.s./L	
	Log-logistic	Log-normal
Median HC <sub>5</sub> (95% CI)	0.057 (0.013 – 0.25)	0.06 (0.021 – 0.25)
Median HC <sub>10</sub> (95% CI)	0.097 (0.028 – 0.33)	0.093 (0.036 – 0.32)
Median HC <sub>20</sub> (95% CI)	0.17 (0.06 – 0.48)	0.16 (0.067 – 0.44)
Median HC <sub>50</sub> (95% CI)	0.45 (0.19 – 1.1)	0.42 (0.19 – 0.95)

CI: confidence interval.

The goodness of fit criteria for the log-logistic and log-normal distributions are shown in Table 10.2.1/09-6.

**Table 10.2.1/09-6: Goodness of fit criteria for the log-logistic and log-normal distributions**

	Log-logistic	Log-normal
Log-likelihood	-6.08	-5.84
AIC	16.16	15.68

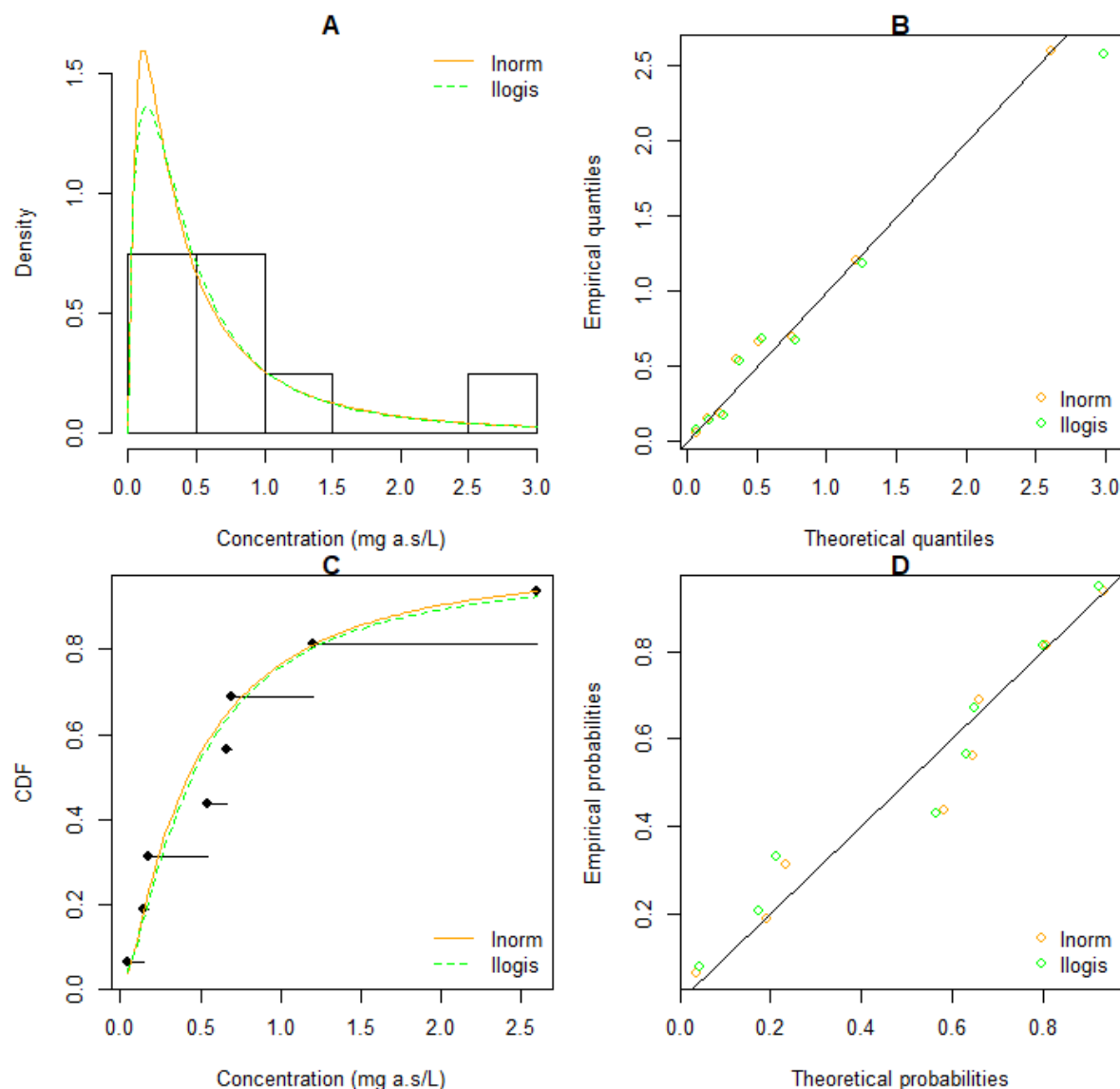
The median HC<sub>5</sub> estimate of 0.06 mg a.s./L is slightly greater than the endpoint for the most sensitive invertebrate species included in the SSD (i.e. *Asellus aquaticus* with a NOEC of 0.050 mg a.s./L) but lower than the endpoints for all other species included in the SSD.

Moreover, since the lower limit HC<sub>5</sub> (0.021 mg a.s./L) is  $\geq 1/3$  of the median HC<sub>5</sub>, the Assessment Factor (AF) of 3 is justified.

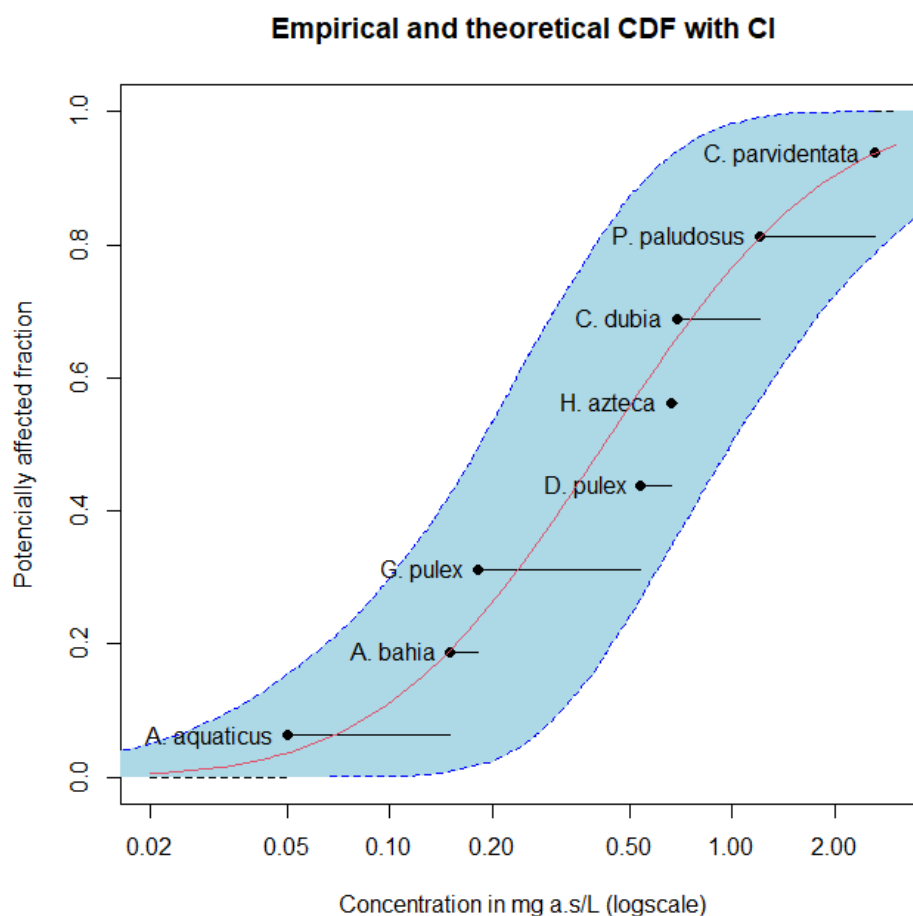
Based on the PPR panel recommendations to calculate the SSD-RAC on the basis of the median HC<sub>5</sub> and the application of an AF of 3 (EFSA Aquatic Guidance, 2013), the results are shown in the following table.

**Table 10.2.1/09-7: SSD-RAC derived from the relevant EC<sub>10</sub> data for aquatic invertebrate species**

Parameter	Result (n = 8)
Median HC <sub>5</sub> (mg a.s./L)	0.06
95% Confidence Intervals (mg a.s./L)	0.021 – 0.25
Assessment Factor	3
<b>Regulatory Acceptable Concentration (mg a.s./L)</b>	<b>0.020</b>



**Figure 10.2.1/09-1:** A: Histogram against fitted density functions; B: Theoretical quantiles against empirical ones (Q-Q plot); C: Empirical Cumulative Distribution Function (CDF) against fitted distribution functions; D: Theoretical probabilities against empirical ones (P-P plot).



**Figure 10.2.1/09-2: SSD plot for mandestrobin based on the log-normal distribution**

#### B. SSD Approach 2 (EC<sub>10</sub> and NOECs)

The results of this approach to the SSD are presented in Table 10.2.1/09-8, and goodness of fit plots for the log-normal and log-logistic fitted distributions are shown in Figure 10.2.1/09-3. In this case, the same HC<sub>5</sub> values were estimated regardless of the distribution used (i.e. log-normal or log-logistic). Visual assessment of the fitted curves (Figure 10.2.1/09-3) indicates that both distribution fits are very close. The log-likelihood and AIC values are presented in Table 10.2.1/09-9. The log-likelihood value for the log-normal distribution is a little higher and the AIC value is slightly lower, indicating that this distribution is more appropriate for this data set. The SSD curve with the HC<sub>5</sub> value for mandestrobin based on the log-normal distribution is shown in Figure 10.2.1/09-4.

**Table 10.2.1/09-8: Median HC<sub>p</sub> values and their 95% confidence limits for the fitted log-logistic and log-normal distributions**

	mg a.s./L	
	Log-logistic	Log-normal
Median HC <sub>5</sub> (95% CI)	0.056 (0.015 – 0.23)	0.056 (0.02 – 0.21)
Median HC <sub>10</sub> (95% CI)	0.095 (0.031 – 0.31)	0.087 (0.034 – 0.28)
Median HC <sub>20</sub> (95% CI)	0.17 (0.065 – 0.45)	0.15 (0.064 – 0.40)
Median HC <sub>50</sub> (95% CI)	0.45 (0.2 – 0.99)	0.41 (0.18 – 0.88)

CI: confidence interval.

The goodness of fit criteria for the log-logistic and log-normal distributions are shown in Table 9.

**Table 10.2.1/09-9: Goodness of fit criteria for the log-logistic and log-normal distributions**

	mg a.s./L	
	Log-logistic	Log-normal
Loglikelihood	-6.61	-6.35
AIC	17.21	16.69

The median HC<sub>5</sub> estimate of 0.056 mg a.s./L is slightly greater than the endpoint for the most sensitive invertebrate species included in the SSD (i.e. *Americamysis bahia* with a NOEC of 0.049 mg a.s./L) and slightly greater than the endpoint for the next most sensitive species (*Asellus aquaticus*, NOEC = 0.050 mg a.s./L) but lower than the endpoints for all other species included in the SSD.

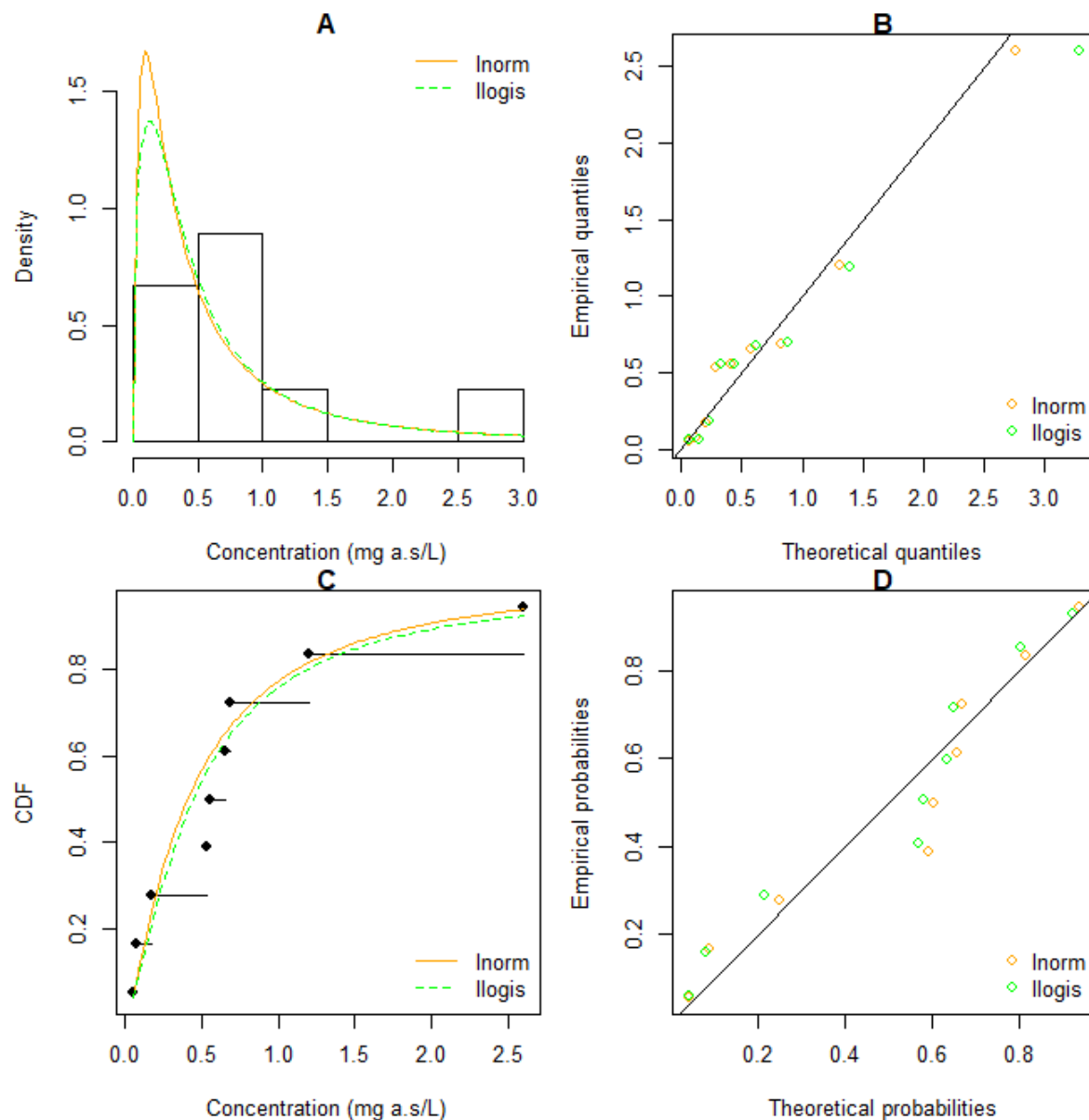
Moreover, since the lower limit HC<sub>5</sub> (0.02 mg a.s./L) is  $\geq 1/3$  of the median HC<sub>5</sub>, the Assessment Factor (AF) of 3 is justified.

Based on the PPR panel recommendations to calculate the SSD-RAC on the basis of the median HC<sub>5</sub> and the application of an AF of 3 (EFSA Aquatic Guidance, 2013), the results are shown in the following table. Detailed results can be found in the Appendix.

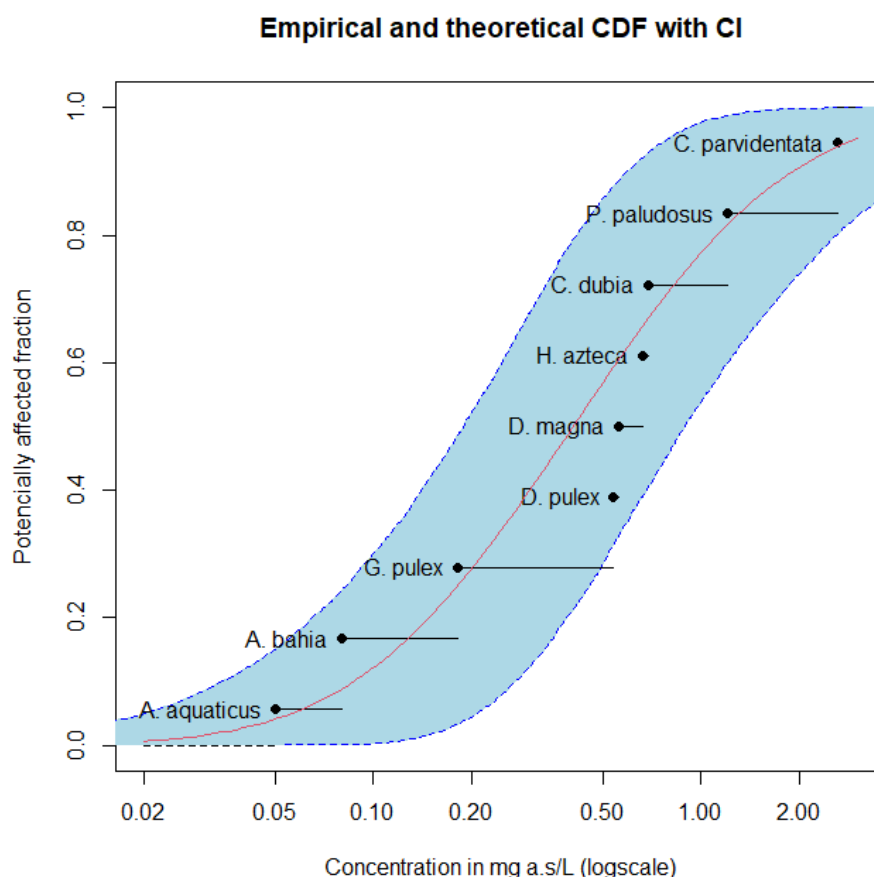
**Table 10.2.1/09-10: SSD-RAC derived from the relevant EC<sub>10</sub>/NOEC data for aquatic invertebrate species**

Parameter	Result (n = 9)
Median HC <sub>5</sub> (mg a.s./L)	0.056
95% Confidence Intervals (mg a.s./L)	0.02 – 0.21
Assessment Factor	3
<b>Regulatory Acceptable Concentration (mg a.s./L)</b>	<b>0.019</b>





**Figure 10.2.1/09-3:** A: Histogram against fitted density functions; B: Theoretical quantiles against empirical ones (Q-Q plot); C: Empirical Cumulative Distribution Function (CDF) against fitted distribution functions; D: Theoretical probabilities against empirical ones (P-P plot).



**Figure 10.2.1/09-4: SSD plot for mandestrobin based on the log-normal distribution**

### III. CONCLUSION

In this report, two separate SSDs were calculated, in order to fulfil the differing requirements of the Central Zone Evaluation Manual (v2.0, August 2023) and the EFSA Aquatic Guidance Document (2013).

In the first approach, only EC<sub>10</sub> estimates were included in the SSD, as required by the CZ Evaluation Manual (v2.0, 2023). With this approach, a HC<sub>5</sub> value of 0.060 mg a.s./L and RAC of 0.020 mg a.s./L were calculated (using an assessment factor of 3).

In the second approach, EC<sub>10</sub> estimates were included where available, and in cases where an EC<sub>10</sub> was not available, the NOEC was applied (*Daphnia magna*), or in the case of *Americamysis bahia*, a geometric mean NOEC for the two available studies was applied. With this approach, a HC<sub>5</sub> value of 0.056 mg a.s./L and RAC of 0.019 mg a.s./L were calculated (using an assessment factor of 3).

Therefore, regardless of the approach taken to application of the available data, a very similar Tier 2B RAC is derived for the chronic risk to aquatic invertebrates. The available results provide additional reassurance and certainty as to the reliability of the Tier 2B RACs applied to the risk assessment.

Assessment and Conclusion by Applicant:	SSD-RAC (based on EC <sub>10</sub> estimates only): 19 µg a.s./L (Assessment Factor: 3) SSD-RAC (combining EC <sub>10</sub> and NOEC estimates): 20 µg a.s./L (Assessment Factor: 3)
Comments of zRMS:	The study was not evaluated as all relevant studies were not evaluated. In accordance with Central Zone Manual v. 3.0, December 2024, the additional

	chronic studies on aquatic invertebrates were submitted as necessary for risk refinement.
	The submitted report was accepted.
	The following endpoints were derived: HC <sub>5</sub> = 0.060 mg a.s./L (based on EC <sub>10</sub> estimates only) HC <sub>5</sub> = 0.056 mg a.s./L (based on combining EC <sub>10</sub> and NOEC estimates)
	The SSD-RAC = 0.020 mg a.s./L (EC <sub>10</sub> estimates only) and SSD-RAC = 0.019 mg a.s./L (combining EC <sub>10</sub> and NOEC estimates) These values were accepted and can be used in higher tier risk assessment for aquatic invertebrates.

#### A 2.2.1.10 Study 10

<b>Data point:</b>	KCP 10.2.1/10
<b>Report author:</b>	Obert-Rausser, P.
<b>Report year:</b>	2023a
<b>Report title:</b>	Mandestrobin 400 g/L: Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test -Static)
<b>Report No.:</b>	S22-06619
<b>Document No.:</b>	ROW-0155
<b>Guidelines followed in study:</b>	OECD 202 (2004)
<b>Deviations from current test guideline:</b>	Compared to OECD 202 (2004): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The acute toxicity of Mandestrobin 40SC to *Daphnia magna* was determined over 48 hours under static conditions at five nominal concentrations between 0.500 and 8.00 mg product/L. An untreated control was tested in parallel. Four replicates were set up per treatment and control, each containing five daphnids (20 per treatment). Observations for immobility were performed after 24 and 48 hours. Results are reported based on the nominal test item concentrations and the geometric mean measured active substance concentrations.

The 48-hour EC<sub>50</sub> was calculated to be 2.55 mg product/L and the NOEC was determined to be 1.00 mg product/L corresponding to EC<sub>50</sub> of 0.973 mg a.s./L and a NOEC of 0.393 mg a.s./L.

### I. MATERIALS AND METHODS

#### A. MATERIALS

- Test material:** Mandestrobin 400 g/L (Mandestrobin 40SC)  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L (nominal), 403.3 g/L (analysed)

2. **Control:** Untreated test medium
3. **Reference item:** Potassium dichromate, tested in a separate study

## B. STUDY DESIGN AND METHODS

1. **Test organism:** *Daphnia magna*  
**Age:** < 24 hours old  
**Source:** In-house culture  
**Diet:** *Desmodesmus subspicatus*, formerly *Scenedesmus subspicatus* during culture. Daphnids were not fed over the duration of the study.
2. **Test Medium:** Elendt M4 medium  
**Hardness:** 214 mg/L as CaCO<sub>3</sub>  
**Alkalinity:** Not reported  
**pH:** 7.91  
**Conductivity:** Not reported
3. **Test vessels:** 100 mL glass vessels, each containing ≥ 50 mL of test solution. The test units were covered with a glass plate to reduce evaporation.
4. **Environmental conditions:**  
**Temperature:** 19.8 – 20.8°C  
**pH:** 7.60 – 7.96  
**Dissolved oxygen:** 6.70 – 9.05 mg/L  
**Photoperiod:** 16 hours light : 8 hours dark (mean 1044 lux)

### 5. Animal assignment and treatment:

The acute toxicity of Mandestrobin 40SC to *Daphnia magna* was determined over 48 hours under static conditions at five nominal concentrations of 0.500, 1.00, 2.00, 4.00 and 8.00 mg product/L corresponding to geometric mean measured active substance concentrations of 0.201, 0.393, 0.756, 1.55 and 3.03 mg a.s./L. An untreated control was tested in parallel. Four replicates were set up per treatment and control, each containing five daphnids (20 per treatment). Additionally, a separate reference test with potassium dichromate was performed at the test facility to assess the performance and sensitivity of the test organism.

### 6. Dose preparation:

The necessary amount of test item for preparing the stock solution S1 was weighed in and transferred to a volumetric flask. Test medium (Elendt M4 medium) was added up to the mark and the solution was treated with 5 minutes of ultrasonication. Afterwards the solution was clear and transparent. Lower test solutions were prepared by dilution of the appropriate solution with test medium. ≥ 50 mL of the prepared solutions were transferred to each test vessel. Control group vessels contained test medium only.

### 7. Measurements and observations:

The number of immobilised daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure. Immobilisation was defined as those animals not able to swim within 15 seconds after gentle agitation of the test vessel. If present, behavioural changes of daphnids were recorded 24 and 48 hours after starting the test.

The pH, dissolved oxygen concentrations and temperature were measured at 0, 24 and 48 hours in one separate replicate per treatment without test organisms in all treatment levels and the control. Light intensity was measured at test start in five different spots. Appearance of test solutions was also recorded daily. At test initiation and test termination, analytical samples were removed from each test solution and the control

for analysis of test substance concentration. All samples were analysed by high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS).

## 8. Statistics:

The EC<sub>50</sub> values for immobilisation were determined by Weibull analysis using linear max. likelihood regression. The evaluation of data was performed by ToxRat Professional 3.3.0. ToxRat Professional, Version 3.3.0.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

Following 24 hours of exposure, no immobilisation was observed among daphnids exposed to the 0.500 mg product/L treatment. No immobilisation higher than the allowed control immobilisation was observed at 1.00 mg product/L and 2.00 mg product/L. 70% and 100% immobilisation were observed in daphnids exposed to test item concentrations of 4.00 mg product/L and 8.00 mg product/L, respectively.

After 48 hours of exposure no immobilisation was observed in the control or at 0.500 mg product/L. No immobilisation higher than the allowed control immobilisation was observed at 1.00 mg product/L. No behavioural changes were observed during the study.

A summary of the cumulative immobilisation is shown in Table 10.2.1/10-1 below.

**Table 10.2.1/10-1: Summary of cumulative percent of immobilised organisms**

Nominal concentration (mg product/L)	Geometric mean measured conc. (mg a.s./L)	Cumulative immobilisation (%)	
		24 hours	48 hours
Control	0	0	0
0.500	0.201	0	0
1.00	0.393	5	5
2.00	0.756	10	15
4.00	1.55	70	100
8.00	3.03	100	100

A summary of the endpoints is presented in Table 10.2.1/10-2.

**Table 10.2.1/10-2: Summary of endpoints**

	Test item (mg/L) <sup>a)</sup>		Active substance (mg a.s./L) <sup>b)</sup>	
	24 h	48 h	24 h	48 h
NOEC	2.00	1.00	0.756	0.393
EC <sub>50</sub> (95% CI)	3.34 (2.75 – 4.03)	2.55 (2.12 – 3.01)	1.29 (1.06 – 1.55)	0.973 (0.808 – 1.16)

<sup>a)</sup> Based on nominal test item concentrations.

<sup>b)</sup> Based on geometric mean measured concentrations of the active substance mandestrobin.

In the separate reference test with potassium dichromate, the EC<sub>50</sub> values were within the expected range of 0.10 – 10 mg/L (actual: 1.075 mg/L), thus demonstrating the appropriate sensitivity of the daphnid population used in the current study.

### B. ANALYSIS

The measured concentrations of mandestrobin in samples taken at test start ranged from 100 to 106% of nominal. In the samples taken from aged solutions, the measured concentrations were between 102 and 113% of nominal. Toxicological endpoints were evaluated using the nominal test item concentrations and the mean measured active substance concentrations (based on the geometric mean of the analytical

recoveries for each concentration level). A summary of the analytical results is shown in Table 10.2.1/10-3 below.

**Table 10.2.1/10-3: Measured concentrations of Mandestrobin 40SC in the exposure solutions**

Nominal concentration (mg product/L)	Nominal mandestrobin conc. (mg a.s./L)	Measured concentration of mandestrobin (mg/L)				Geometric mean measured conc. (mg a.s./L)
		0 hours	% of nominal	48 hours	% of nominal	
Control	0	< LOD	-	< LOD	-	-
0.500	0.186	0.192	103	0.211	113	0.201
1.00	0.373	0.394	106	0.392	105	0.393
2.00	0.745	0.744	100	0.768	103	0.756
4.00	1.49	1.49	100	1.61	108	1.55
8.00	2.98	3.03	102	3.04	102	3.03

-.: not calculated. LOD: limit of detection (0.00520 mg/L mandestrobin); LOQ: limit of quantification (0.0186 mg/L mandestrobin).

### C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (OECD 202, 2004) as detailed below:

- In the controls,  $\leq 10\%$  of daphnids should have been immobilised by the end of the test. 0% immobility was observed in the untreated control.
- The dissolved oxygen concentration at the end of the test should be  $\geq 3$  mg/L in the control and test vessels. Dissolved oxygen concentrations were  $\geq 6.7$  mg/L at all times during the study.

### III. CONCLUSION

The acute toxicity of Mandestrobin 40SC to *Daphnia magna* was determined over 48 hours under static conditions. Results are reported based on the nominal product concentrations and the geometric mean measured active substance concentrations.

The 48-hour  $EC_{50}$  was calculated to be 2.55 mg product/L and the NOEC was determined to be 1.00 mg product/L corresponding to  $EC_{50}$  of 0.973 mg a.s./L and a NOEC of 0.393 mg a.s./L.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>48-hour <math>EC_{50}</math>: 2.55 mg product/L (based on nominal test item concentrations)</p> <p>48-hour <math>EC_{50}</math>: 0.973 mg a.s./L (based on geometric mean measured active substance concentrations)</p>
---	--

Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>No deviation was noted.</p> <p>The proposed endpoints:</p> <p><math>EC_{50}</math> = 2.55 mg product/L (0.973 mg a.s./L)</p> <p>NOEC = 1.0 mg product/L (0.393 mg a.s./L)</p> <p>were accepted.</p>
-------------------	--

### A 2.2.1.11 Study 11

<b>Data point:</b>	KCP 10.2.1/11
<b>Report author:</b>	Obert-Rausser, P.
<b>Report year:</b>	2023b
<b>Report title:</b>	Mandestrobin 400 g/L: Toxicity to the Single Cell Green Alga <i>Pseudokirchneriella subcapitata</i> Hindak under Laboratory Conditions
<b>Report No.:</b>	S22-06618
<b>Document No.:</b>	ROW-0154
<b>Guidelines followed in study:</b>	OECD 201 (2011)
<b>Deviations from current test guideline:</b>	Compared to OECD 201 (2011): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The 72-hour toxicity of Mandestrobin 40SC to the green alga *Pseudokirchneriella subcapitata* was determined in the laboratory under static conditions. Algae were exposed to five nominal concentrations between 0.286 and 30.0 mg product/L, corresponding to geometric mean measured active substance concentrations between 0.0877 and 11.4 mg a.s./L. An untreated control was tested in parallel. Three replicates were set up per treatment group and six were prepared for the control, with initial nominal cell densities of  $0.5 \times 10^4$  cells/mL. Cell counts and observations of the health of the algal cells were performed at 24-hour intervals.

Results are reported based on the nominal test item concentrations and the geometric mean measured active substance concentrations.

The 72-hour  $E_rC_{10/20/50}$  values were calculated to be 1.95, 3.69 and 11.0 mg product/L, corresponding to 0.655, 1.27 and 3.94 mg a.s./L, respectively. The NOEC for growth rate was 0.916 mg product/L (0.287 mg a.s./L).

The 72-hour  $E_yC_{10/20/50}$  values were calculated to be 0.933, 1.45 and 3.08 mg product/L, corresponding to 0.315, 0.497 and 1.08 mg a.s./L, respectively. The NOEC for yield was 0.916 mg product/L (0.287 mg a.s./L).

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** Mandestrobin 400 g/L (Mandestrobin 40SC)  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L (nominal), 403.3 g/L (analysed)
- Control:** Untreated test medium
- Reference item:** Potassium dichromate, tested in a separate study

## B. STUDY DESIGN AND METHODS

1. **Test organism:** Green algae (*Pseudokirchneriella subcapitata*)  
**Source:** In-house culture  
**Pre-culture:** 3 – 4 days  
**Initial cell density:**  $0.5 \times 10^4$  cells/mL
2. **Test medium:** Algal Assay Procedure (AAP) medium  
**pH:**  $7.5 \pm 0.1$
3. **Test vessels:** 100 mL Erlenmeyer flasks with aluminium caps containing ~ 50 mL test medium.
4. **Environmental conditions:**  
**Temperature:**  $22.7 - 23.3^\circ\text{C}$   
**pH:**  $7.52 - 8.62$   
**Photoperiod:** Continuous illumination  $96.3 \mu\text{Em}^{-2}\text{s}^{-1}$  (mean light intensity)

### 5. Test organism set up and treatment:

The 72-hour toxicity of Mandestrobin 40SC to the green alga *Pseudokirchneriella subcapitata* was determined in the laboratory under static conditions. Algae were exposed to five nominal concentrations of 0.286, 0.916, 2.93, 9.38 and 30.0 mg product/L. An untreated control was tested in parallel. Three replicates were set up per treatment group and six were prepared for the control, with initial nominal cell densities of  $0.5 \times 10^4$  cells/mL.

After the test solutions were added to the test flasks, the inoculum of *P. subcapitata* cells took place in a temperature controlled light incubator. Carbon dioxide was supplied by continuous agitation; test vessels were placed on a pivoted bogie which turned around and induced shaking by regular sudden stops.

Additionally, a separate reference test with potassium dichromate was performed at the test facility to assess the performance and sensitivity of the test organism.

### 6. Dose preparation:

A 30.0 mg a.s./L primary stock solution was prepared prior to test initiation, the necessary amount of test item for preparing the stock solution was weighed in and transferred to a volumetric flask. Test medium (AAP medium) was added up to the mark and the solution was treated with 5 minutes of ultrasonication. Afterwards the solution was turbid.

Lower stock solutions were prepared by dilution of appropriate solutions with test medium. In this process a stock solution for each test item concentration was produced. For the preparation of the test solutions, defined volumes of the prepared stock solutions were added to test medium followed by homogenization by shaking. The resulting solutions was observed to be clear and transparent.

### 7. Measurements and observations:

At test start (0 hours) the number of cells in each control replicate was determined in duplicate. At each subsequent 24-hour interval (24, 48 and 72 hours), the number of cells in each replicate was determined in duplicate by fluorescence measurement. Additionally, the morphological appearance of the algae cells was assessed microscopically at the end of the test.

Water quality parameters (pH and conductivity) were measured at test initiation and termination. Temperature was measured continuously and recorded at 24-hour intervals. The light intensity of all positions of the incubator is measured once a year and was confirmed for one representative position at test start. Appearance of test solution was assessed and documented on a daily basis.



At test initiation (0 hours) and test termination (72 hours), analytical samples of all test item concentrations and control were verified. All samples were analysed using high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS).

## 8. Statistics:

The  $EC_{10/20/50}$  values for growth rate and yield were determined with a non-linear regression using a 3 parametric logistic cumulative distribution function (optimisation method: Levenberg- Marquardt).

For the NOEC/LOEC determination for growth rate and yield, the data was transformed to  $y'=\ln(y)$ . Afterwards a test for normality of the data was performed by calculating the Shapiro-Wilk's statistic and the homogeneity of variance of the data was evaluated by Levene's test. The monotonicity of the concentration response relation was assessed by trend analysis by contrasts. The NOEC and LOEC were determined by using a multiple comparison method (Williams' test).

The statistical evaluation was performed using ToxRat Professional, Version 3.3.0.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

At test termination, cells exposed to all treatment levels and the controls were observed to be normal. After 72 hours, at termination of the test a concentration response was observed for the inhibition of growth rate and yield from nominal test item concentrations of 0.286 to 30.0 mg product/L. The inhibition of growth rate peaked at 77.1% and the inhibition of yield peaked at 97.9% at a nominal test item concentration of 30.0 mg product/L. Cell density and yield data are shown in Table 10.2.1/11-1 and a summary of endpoints is shown in Table 10.2.1/11-2.

**Table 10.2.1/11-1: Summary of effects on cell density, yield and growth rate**

Nominal concentration (mg product/L)	Cell density ( $\times 10^4$ cells/mL)			Yield (0 – 72 hours)		Growth rate (0 – 72 hours)
	24 hours	48 hours	72 hours	Total ( $\times 10^4$ cells days/mL)	% Inhibition	% Inhibition
Control	2.07	11.16	51.08	50.50	0.0	0.0
0.286	2.17	11.25	51.66	51.07	-1.1	0.1
0.916	2.22	11.38	46.68	46.09	8.7	2.3
2.93	1.69	7.10	27.05	26.46	47.6	14.5
9.38	0.92	2.51	6.64	6.05	88.0	45.9
30.0	0.60	1.01	1.64	1.05	97.9	77.1

**Table 10.2.1/11-2: Summary of endpoints**

	Test item (mg/L) <sup>a)</sup>	Active substance (mg a.s./L) <sup>b)</sup>
E <sub>r</sub> C <sub>10</sub> (95% CI)	1.95 (1.70 – 2.19)	0.655 (0.562 – 0.746)
E <sub>r</sub> C <sub>20</sub> (95% CI)	3.69 (3.35 – 4.02)	1.27 (1.14 – 1.40)
E <sub>r</sub> C <sub>50</sub> (95% CI)	11.0 (10.3 – 11.6)	3.94 (3.69 – 4.19)
E <sub>y</sub> C <sub>10</sub> (95% CI)	0.933 (0.620 – 1.21)	0.315 (0.204 – 0.415)
E <sub>y</sub> C <sub>20</sub> (95% CI)	1.45 (1.09 – 1.76)	0.497 (0.368 – 0.608)
E <sub>y</sub> C <sub>50</sub> (95% CI)	3.08 (2.64 – 3.54)	1.08 (0.922 – 1.25)
NOEC	0.916	0.287
LOEC	2.93	1.04

CI: Confidence intervals.

<sup>a)</sup> Based on nominal test item concentrations.

<sup>b)</sup> Based on geometric mean measured concentrations of the active substance mandestrobin.

In the separate reference test with potassium dichromate, the 72-hour E<sub>r</sub>C<sub>50</sub>, E<sub>y</sub>C<sub>50</sub> and E<sub>b</sub>C<sub>50</sub> values based on growth rate, yield and biomass were determined to be 1.30 mg/L, 0.611 mg/L and 0.608 mg/L respectively, thus demonstrating appropriate sensitivity of the culture used in the current study.

## B. ANALYSIS

The measured concentrations of mandestrobin in samples taken at test start ranged from 84 to 102% of nominal. In the samples taken from aged solutions, the measured concentrations were between 80 and 102% of nominal. Toxicological endpoints were evaluated using the nominal test item concentrations and the mean measured active ingredient concentrations (based on the geometric mean of the analytical recoveries for each concentration level) of the test item. A summary of the analytical results is shown in Table 10.2.1/11-3 below.

**Table 10.2.1/11-3: Measured concentrations of mandestrobin in the exposure solutions**

Nominal concentration (mg prod./L)	Nominal mandestrobin concentration (mg a.s./L)	Measured concentration (mg a.s./L)				Geometric mean measured concentration (mg a.s./L)
		0 hours	% of nominal	72 hours	% of nominal	
Control	0	< LOD	-	< LOD	-	-
0.286	0.107	0.0901	84	0.0853	80	0.0877
0.916	0.341	0.291	85	0.284	83	0.287
2.93	1.09	1.04	95	1.04	95	1.04
9.38	3.49	3.24	93	3.34	96	3.29
30.0	11.2	11.4	102	11.4	102	11.4

- = not calculated; LOD: limit of detection (0.00320 mg/L mandestrobin); LOQ: limit of quantification (0.0107 mg/L mandestrobin).

## C. VALIDITY CRITERIA

The study was compared to the validity criteria outlined in the most recent EU test guideline (OECD 201, 2011), as detailed below:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. Cell numbers, measured in the control between 0 and 72 hours, were found to increase by a factor of 86.6.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. The mean coefficient of variation for the section-by-section specific growth rates was 14% in the control.

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The coefficient of variation of average growth rate was 2.2% in the control.

### III. CONCLUSION

The 72-hour toxicity of Mandestrobin 40SC to the green alga *Pseudokirchneriella subcapitata* was determined in the laboratory under static conditions. Results are reported based on the nominal test item concentrations and the geometric mean measured active substance concentrations.

The 72-hour  $E_rC_{10/20/50}$  values were calculated to be 1.95, 3.69 and 11.0 mg product/L, corresponding to 0.655, 1.27 and 3.94 mg a.s./L, respectively. The NOEC for growth rate was 0.916 mg product/L (0.287 mg a.s./L).

The 72-hour  $E_yC_{10/20/50}$  values were calculated to be 0.933, 1.45 and 3.08 mg product/L, corresponding to 0.315, 0.497 and 1.08 mg a.s./L, respectively. The NOEC for yield was 0.916 mg product/L (0.287 mg a.s./L).

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>Based on the on the nominal test item concentrations and the geometric mean measured active substance concentrations, the relevant endpoints derived from the study are:</p> <p><math>E_rC_{10}</math>: 1.95 mg product/L (0.655 mg a.s./L)  <math>E_rC_{20}</math>: 3.69 mg product/L (1.27 mg a.s./L)  <math>E_rC_{50}</math>: 11.0 mg product/L (3.94 mg a.s./L)  <math>NOE_rC</math>: 0.916 mg product/L (0.287 mg a.s./L)</p>
---	---

Comments of zRMS:	<p>The submitted study was accepted.  The validity criteria were met.  No deviation was noted.</p> <p>The proposed endpoints:  <math>E_rC_{10}</math> = 1.95 mg product/L (0.655 mg a.s./L)  <math>E_rC_{20}</math> = 3.69 mg product/L (1.27 mg a.s./L)  <math>E_rC_{50}</math> = 11.0 mg product/L (3.94 mg a.s./L)  <math>NOE_rC</math> = 0.916 mg product/L (0.287 mg a.s./L)  were accepted.</p>
-------------------	---

### A 2.2.1.12 Study 12

<b>Data point:</b>	KCP 10.2.1/12
<b>Report author:</b>	White, K. and Eck, G.
<b>Report year:</b>	2024
<b>Report title:</b>	Mandestrobin Renewal: Position Paper on the Relevant Tier 1 Chronic Endpoint for <i>Americamysis bahia</i>
<b>Report No.:</b>	1810776.UK0 - 5829
<b>Document No.:</b>	ROW-0159
<b>Guidelines followed in study:</b>	Not applicable
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Not applicable
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

In the original submission of the fungicidal active substance mandestrobin in November 2012, a chronic mysid study with *Americamysis bahia* (Claude *et al.*, 2012) was submitted to address the risk to additional aquatic invertebrate species. In this study, significant reductions in adult first-generation survival were observed compared to the pooled control at concentrations of 0.011 and 0.084 mg a.s./L. The supposed effects at 0.011 mg a.s./L were concluded by the Study Director in the original study report to be not related to treatment, as the difference from the controls was slight and not dose responsive (higher rates of survival were actually observed at the next two treatment levels of 0.024 and 0.049 mg a.s./L, with no statistically significant reductions compared to the control).

However, in contrast to the findings of the study report, in the Addendum to the DAR (2015), the RMS Austria suggested that the relevant endpoint from this study was actually a NOEC of 0.0056 mg a.s./L, based on the statistically significant reduction in survival at 0.011 mg a.s./L. Consequently, the result of this evaluation was that the final chronic endpoint for *Americamysis bahia* was listed in the EFSA Conclusion (2015) as 0.0056 mg a.s./L.

The Applicant firmly maintains that the endpoint of 0.0056 mg a.s./L is unduly conservative and not scientifically reliable nor robust, and that the NOEC of 0.049 mg a.s./L is the most relevant endpoint from the study concerned.

In this position paper, weight of evidence is put forth which demonstrates that the NOEC of 0.049 mg a.s./L is sufficiently conservative for use in the aquatic risk assessment (and in turn, towards the classification of mandestrobin). This evidence includes:

- The effects seen on adult first-generation survival at 0.011 mg a.s./L in the 2012 study were slight, not dose responsive and, accordingly, not treatment related.
- A second chronic study with *A. bahia* is available (Urann, 2016) which identified no significant effects on survival, growth or reproduction at concentrations up to and including 0.13 mg a.s./L (NOEC). Significant effects were observed at a LOEC of 0.24 mg a.s./L. The lowest of the estimated L/EC<sub>10</sub> values was an LC<sub>10</sub> of 0.15 mg a.s./L. These endpoints are in agreement with the NOEC of 0.049 mg a.s./L from the original study, which was based on mortality and growth. Moreover, the lower 95% confidence interval of this LC<sub>10</sub> (0.071 mg a.s./L) provides further reassurance that a NOEC of 0.049 mg a.s./L is sufficiently protective of *Americamysis bahia*.
- The median time to first brood release in the two main mysid studies suggest that the mysids used in the 2016 study were in better health conditions than those used in the 2012 study. In the 2012 study,

median time to first brood release in the combined controls was 27 days, leading to the study duration being extended to 36 days (standard duration is 28 days). Comparatively, in the 2016 study, median time to first brood release in the combined controls was 20.5 days, and the study was terminated on schedule at 28 days. These results indicate that the findings of the 2016 study may be more reliable and that a NOEC/EC<sub>10</sub> of 0.13 and 0.15 mg a.s./L, respectively may even be argued to be more appropriate for the species.

- A well-performed non-GLP range finding study is available, performed prior to the 2016 study. The findings further support the conclusions noted above, with a NOEC of 0.160 mg a.s./L (nominal) based on significant effects observed at the highest test concentration of 0.500 mg a.s./L (nominal).
- Studies on eight species in addition to *Americamysis bahia* are available. The most closely related species to *A. bahia* of these species are *Asellus aquaticus*, *Gammarus pulex* and *Hyalella azteca*. The available endpoints from these studies are all highly comparable and in line with the NOEC of 0.049 mg a.s./L, further demonstrating that this NOEC is sufficiently conservative of the risk to this species.

## I. INTRODUCTION

In the original evaluation of mandestrobin, two studies with the technical active substance mandestrobin were submitted to address the chronic risk to aquatic invertebrates. These studies were performed with the standard species *Daphnia magna* (Sayers, 2010; ROW-0020) and the additional species *Americamysis bahia* (Claude *et al.*, 2012; ROW-0063).

In the original study report for *Americamysis bahia* (ROW-0063) a NOEC of 0.049 mg a.s./L was concluded by the Study Director due to statistically significant effects on first-generation adult mortality and growth (male total body length) at the highest concentration tested (0.084 mg a.s./L).

However, in contradiction to the findings of the study report, in the Addendum to the DAR (2015), the RMS Austria suggested that the relevant endpoint was actually a NOEC of 0.0056 mg a.s./L, due to a statistically significant decrease in adult survival observed at 0.011 mg a.s./L. This supposed effect on survival, however, was concluded by the Study Director to be not treatment related, as the difference was slight and not dose responsive; indeed, a higher rate of survival was observed at the next two treatment levels of 0.024 and 0.049 mg a.s./L (77.3 and 74.5%, respectively, compared to survival of 72.9% at 0.011 mg a.s./L), and no significant difference from the control was observed in either of these two higher treatments.

Consequently, the result of this evaluation was that the final chronic endpoint for *Americamysis bahia* was listed in the EFSA Conclusion as 0.0056 mg a.s./L.

The Applicant firmly maintains that the endpoint of 0.0056 mg a.s./L is unduly conservative and not scientifically reliable nor robust, and that the NOEC of 0.049 mg a.s./L is the most relevant and reliable endpoint from the study concerned. Consequently, a second study with *Americamysis bahia* was performed in 2016 (detailed in Section II below), which provides further evidence that the NOEC of 0.0056 mg a.s./L is overly conservative.

This position paper outlines the justifications as to why the original NOEC of 0.049 mg a.s./L is most relevant for the Tier 1 aquatic risk assessment as well as for use in the classification of mandestrobin, using the available data for *Americamysis bahia*, as well as available studies on additional aquatic invertebrate species generated for the 2022 renewal.

## II. OVERVIEW OF AVAILABLE AMERICAMYSIS STUDIES

### A. Claude *et al.* (2012) – ROW-0063

Here, a summary of the results of the study is presented, to accompany the weight of evidence detailed in the position paper.

At test termination, the mysids in the negative and solvent controls met the performance criteria outlined in OCSPP Guideline 850.1350.

The results for survival and reproduction are shown in Table 10.2.1/12-1 below, while results for growth parameters (total length and dry weight) are shown in Table 10.2.1/12-2.

Surviving mysids in the control and treatment groups appeared normal during the period from test initiation to pairing on day 14, with no signs of toxicity observed. There was a statistically significant difference in juvenile G1 survival (day 0 – 14) between the negative and solvent control groups, therefore treatments were compared to the negative control. There was a statistically significant decrease in survival at 0.024 mg a.s./L compared to the negative control (see Table 10.2.1/12-1) which was not considered to be treatment related, as the decrease was slight and not dose responsive (100% survival was observed at the two highest treatment groups of 0.049 and 0.084 mg a.s./L). Consequently, the NOEC for G1 juvenile survival up to day 14 was concluded to be 0.084 mg a.s./L, the highest concentration tested.

Since there were no statistically significant differences in adult (day 15 – 36) G1 survival after pairing between the negative and solvent controls, the control data were pooled for comparison with the treatments. Statistically significant decreases in survival were observed at 0.011 and 0.084 mg a.s./L. Survival at 0.011 mg a.s./L was 72.9% (see Table 10.2.1/12-1) while survival at the following two concentrations of 0.024 and 0.049 mg a.s./L was greater (77.3 and 74.5%, respectively) and without statistical significance. Therefore, while statistically significant, the decrease in survival at 0.011 mg a.s./L was not considered to be treatment related, since the decrease was slight and was not dose responsive.

Conversely, the marked decrease in survival among mysids at the highest treatment of 0.084 mg a.s./L (66.7%) was in fact concluded to be treatment related. Consequently, the NOEC for adult G1 survival (days 15 – 36) was determined to be 0.049 mg a.s./L.

There were no statistically significant differences in reproduction between the negative and solvent controls, therefore the control data were pooled for comparisons with the treatment groups. No significant differences in reproduction between the treatment groups and pooled control were observed. Consequently, the NOEC for reproduction was 0.084 mg a.s./L, the highest concentration tested.

**Table 10.2.1/12-1: Survival and reproduction**

Mean measured concentration (mg a.s./L)	Juvenile G1 survival to pairing on day 14 (%)	Adult G1 survival after pairing (D15 – D36) (%)	Mean no. young produced per reproductive day $\pm$ SD	% of females producing young	Average no. young per female
Negative control	98.3	91.1	0.398 $\pm$ 0.192	88.9	7.6
Solvent control	86.7	81.0	0.491 $\pm$ 0.157	100	9.5
Pooled control	--	86.2	0.445 $\pm$ 0.170	94.6	8.5
0.0056	98.3	81.1	0.457 $\pm$ 0.236	94.1	9.2
0.011	91.7	72.9**	0.481 $\pm$ 0.152	88.2	8.8
0.024	88.3*	77.3	0.238 $\pm$ 0.106	63.2	4.2
0.049	100	74.5	0.357 $\pm$ 0.196	80.0	7.1
0.084	100	66.7**	0.197 $\pm$ 0.163	57.9	3.9
<b>Endpoints (<math>\mu</math>g a.s./L)</b>					
NOEC	0.084	0.049	0.084	--	--

\* Statistically significant difference to the negative control (Fisher's Exact test) which was not considered treatment related as the decrease was slight and not dose responsive whereas no mortality was reported for the two highest concentrations tested.

\*\* Statistically significant difference to the pooled control (Fisher's Exact Test). The decrease in adult G1 survival at 0.011 mg a.s./L was not considered to be treatment related, as the decrease was slight and not dose responsive. The marked decrease in survival among mysids in the 0.084 mg a.s./L treatment group was considered to be treatment related.

Growth parameters were assessed separately for males and females. Since there were no statistically significant differences between the negative and solvent controls for either body length or dry weight for either males or females, the pooled controls were used for treatment comparisons for all four parameters.

There was a statistically significant decrease in mean total body length of males in the 0.084 mg a.s./L treatment group, which was considered to be treatment related (see Table 10.2.1/12-2). There were no significant differences in total body length of females compared to the pooled control.

There was a statistically significant decrease in mean dry weight of males in the 0.0056 mg a.s./L treatment group (Table 10.2.1/12-2). While statistically significant, this was not considered to be a treatment related effect, since the decrease was slight and not dose responsive (greater mean dry weights were in fact observed in all four of the higher treatment levels, see Table 10.2.1/12-2).

Consequently, due to the significant treatment related effect on male total body length observed at the highest treatment level of 0.084 mg a.s./L, the NOEC for growth of mysids was concluded to be 0.049 mg a.s./L.

**Table 10.2.1/12-2: Total length and dry weight**

Mean measured concentration (mg a.s./L)	Growth parameters at termination on day 36			
	Mean total length $\pm$ SD (mm)		Mean dry weight $\pm$ SD (mg)	
	Males	Females	Males	Females
Negative control	8.54 $\pm$ 0.054	8.41 $\pm$ 0.297	0.98 $\pm$ 0.058	1.12 $\pm$ 0.115
Solvent control	8.50 $\pm$ 0.153	8.67 $\pm$ 0.255	0.99 $\pm$ 0.068	1.22 $\pm$ 0.125
Pooled control	8.52 $\pm$ 0.109	8.54 $\pm$ 0.292	0.98 $\pm$ 0.059	1.17 $\pm$ 0.124
0.0056	8.26 $\pm$ 0.392	8.83 $\pm$ 0.089	0.85* $\pm$ 0.151	1.20 $\pm$ 0.075
0.011	8.59 $\pm$ 0.147	8.79 $\pm$ 0.217	1.00 $\pm$ 0.082	1.23 $\pm$ 0.218
0.024	8.40 $\pm$ 0.182	8.76 $\pm$ 0.150	0.98 $\pm$ 0.062	1.25 $\pm$ 0.078
0.049	8.59 $\pm$ 0.143	8.91 $\pm$ 0.166	0.96 $\pm$ 0.061	1.30 $\pm$ 0.093
0.084	8.04* $\pm$ 0.414	8.50 $\pm$ 0.039	0.94 $\pm$ 0.070	1.24 $\pm$ 0.172
Endpoints (mg a.s./L)				
NOEC	0.049			

\* Statistically significant decrease in comparison to the pooled control (Dunnett's test). While the decrease in male dry weight at 0.0056  $\mu$ g a.s./L was statistically different to the pooled control, it was not considered to be treatment related since the difference was slight and was not dose responsive. The decrease in male length at 0.084 mg a.s./L was considered to be treatment related.

A summary of endpoints for all parameters is shown in Table 10.2.1/12-3.

**Table 10.2.1/12-3: Summary of endpoints (all parameters)**

	NOEC (mg a.s./L)
Juvenile G1 survival to pairing (day 0 – day 14)	0.084 <sup>a)</sup>
Adult G1 survival after pairing (day 15 – day 36)	0.049
Reproduction	0.084 <sup>a)</sup>
Growth	0.049
Overall	0.049

<sup>a)</sup> Highest concentration tested.

### Previous evaluation of the study

During Austria's (RMS) evaluation of this study in the 2015 Addendum to the DAR, an endpoint of 0.0056 mg a.s./L, the lowest concentration tested, was selected as the NOEC from this study. This was based on the significant decrease in first-generation adult survival (post-pairing) observed at 0.011 mg a.s./L, even though this decrease was clearly not related to treatment, considering the higher rate of survival observed at the next two treatment levels of 0.024 and 0.049 mg a.s./L, with no statistically significant difference observed in either of these two treatments compared to the pooled control.

As a result of this evaluation, an endpoint of 0.0056 mg a.s./L was reported in the EFSA Conclusion (2015) List of Endpoints (LoEP).

In order to address these apparent uncertainties, a second chronic study with *Americamysis bahia* (ROW-0096) was subsequently performed in 2016. An overall NOEC of 0.13 mg a.s./L and lowest EC<sub>10</sub> of 0.15 mg a.s./L were obtained from this study. The findings of this study strongly support the interpretation of the original study results by the Study Director and Applicant, being in agreement with the NOEC of 0.049 mg a.s./L from the original study. Details of this second study are outlined below.

## B. Urann (2016) – ROW-0096

Here, a summary of the results of the study is presented, to accompany the weight of evidence detailed in the position paper.

At test termination, the mysids in the negative and solvent controls met the performance criteria outlined in OCSPP Guideline 850.1350.

The results for F0 survival and reproduction are shown in Table 10.2.1/12-4 below, while results for growth parameters (total length and dry weight) are shown in Table 10.2.1/12-5. Results for 96-hour survival (F1 generation) are shown in Table 10.2.1/12-6.

In the F0 generation, no behavioural abnormalities were observed during the exposure period.

Male and female post-pairing survival were assessed separately for significant differences from the pooled controls in addition to being combined for analysis. Male and female survival data were also combined for analysis of survival on day 28. There were no significant differences in male or female survival compared to the pooled control at any treatment level. There were also no significant differences in combined male and female survival post-pairing at any treatment level compared to the pooled control.

On day 28, there was a significant reduction in combined male and female survival at the highest mean measured treatment level of 0.48 mg a.s./L compared to the pooled control, which was considered to be treatment related. There were no significant reductions in survival at any other treatment level (see Table 10.2.1/12-4). Consequently, the NOEC for survival (post-pairing) was 0.48 mg a.s./L and the NOEC for survival (day 28) was 0.24 mg a.s./L. The LC<sub>10</sub> for 28-day survival was calculated to be 0.15 mg a.s./L (see Table 10.2.1/12-7).

Due to the significantly reduced survival in the 0.48 mg a.s./L treatment, this group was excluded from statistical analyses of reproduction and growth parameters.

There was a significant difference in the mean number of offspring per female among organisms exposed to the 0.24 mg a.s./L treatment level compared to the pooled control (see Table 10.2.1/12-4). Consequently, the NOEC for reproduction (offspring per female) was determined to be 0.13 mg a.s./L.

**Table 10.2.1/12-4: First-generation (F<sub>0</sub>) survival and reproduction**

Mean measured concentration (mg a.s./L)	Survival (post-pairing, day 13 – 28)				Reproduction	
	Male survival ± SD (%) <sup>a)</sup>	Female survival ± SD (%) <sup>a)</sup>	Combined M/F survival (post-pairing) ± SD (%) <sup>b)</sup>	Combined M/F survival (day 28) ± SD (%) <sup>b)</sup>	% of Females producing young ± SD	Number of offspring/female ± SD
Negative control	80 ± 18	94 ± 11	89 ± 4	86 ± 7	95 ± 10	11.2 ± 3.0
Solvent control	90 ± 7	82 ± 8	86 ± 7	81 ± 10	100 ± 0	9.8 ± 1.4
Pooled control	85 ± 14	88 ± 11	88 ± 6	83 ± 8	98 ± 7	10.5 ± 2.3
0.035	73 ± 24	95 ± 6	84 ± 9	78 ± 14	100 ± 0	8.5 ± 2.1
0.058	86 ± 10	97 ± 6	89 ± 7	77 ± 3	90 ± 12	9.4 ± 0.7
0.130	84 ± 14	89 ± 21	86 ± 10	82 ± 10	95 ± 10	8.0 ± 3.2
0.240	73 ± 10	80 ± 14	74 ± 11	72 ± 13	88 ± 15	5.5* ± 0.8
0.480 <sup>c)</sup>	86 ± 10	87 ± 16	86 ± 4	56* ± 18	31 ± 10	2.0 ± 1.6

<sup>a)</sup> Calculations of male and female survival began after pairing.

<sup>b)</sup> Calculations of survival are of both male and female mysids combined.

<sup>c)</sup> Due to significantly reduced survival, this treatment level was excluded from statistical analyses (other than survival).

\* Significantly reduced compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment for 28-day survival, Dunnett's Multiple Comparison Test for number of offspring per female).

There was a significant difference in the average total body length of both male and female mysids exposed to the 0.24 mg a.s./L treatment compared to the pooled control (males) and solvent control (females). Consequently, the NOEC for both male and female length was determined to be 0.13 mg a.s./L.

There was no significant difference in male dry weight compared to the pooled control. Consequently, the NOEC for male dry weight was determined to be 0.24 mg a.s./L, the highest concentration included in the



analysis. There was a significant difference in female dry weight compared to the pooled control at 0.24 mg a.s./L, therefore the NOEC for female dry weight was determined to be 0.13 mg a.s./L.

**Table 10.2.1/12-5: First-generation (F<sub>0</sub>) mean body length and dry weight (day 28)**

Mean measured concentration (mg a.s./L)	Mean body length (mm) ± SD		Mean dry body weight (mg) ± SD	
	Males	Females	Males	Females
Negative control	7.09 ± 0.16	7.28 ± 0.06	0.79 ± 0.04	1.06 ± 0.08
Solvent control	7.03 ± 0.13	7.14 ± 0.07	0.82 ± 0.02	1.02 ± 0.04
Pooled control	7.06 ± 0.14	n.a. <sup>a)</sup>	0.80 ± 0.03	1.04 ± 0.06
0.035	7.05 ± 0.10	7.19 ± 0.16	0.79 ± 0.02	1.03 ± 0.09
0.058	6.92 ± 0.13	6.99 ± 0.11	0.82 ± 0.04	1.06 ± 0.06
0.130	6.99 ± 0.15	7.09 ± 0.06	0.79 ± 0.03	1.06 ± 0.04
0.240	6.81* ± 0.09	6.90* ± 0.21	0.80 ± 0.05	0.93* ± 0.07
0.480	7.18 ± 0.14	7.21 ± 0.18	0.75 ± 0.02	0.87 ± 0.06

n.a.: not applicable.

<sup>a)</sup> Female length data was not pooled.

\* Statistically significant difference compared to the pooled control (male length and female dry weight) and solvent control (female length), based on Dunnett's Multiple Comparison Test.

There was no significant difference in 96-hour F<sub>1</sub> survival compared to the pooled control at any treatment level and consequently the NOEC for F<sub>1</sub> survival at 96 hours was 0.24 mg a.s./L, the highest treatment included in the analysis for this parameter (see Table 10.2.1/12-6).

**Table 10.2.1/12-6: F<sub>1</sub> survival at 96 hours post-release**

Mean measured concentration (mg a.s./L)	Mean F <sub>1</sub> survival at 96 hours post-release ± SD (%)
Negative control	95 ± 6
Solvent control	98 ± 5
Pooled control	96 ± 5
0.035	88 ± 19
0.058	90 ± 14
0.130	98 ± 5
0.240	94 ± 8
0.480 <sup>a)</sup>	n.a.

n.a.: not applicable.

<sup>a)</sup> No F<sub>1</sub> populations were established at this treatment level.

**Table 10.2.1/12-7: Summary of endpoints**

Endpoint	Based on mean measured concentrations (mg a.s./L)			
	LC <sub>10</sub> /EC <sub>10</sub> (95% CL)	LC <sub>20</sub> /EC <sub>20</sub> (95% CL)	LC <sub>50</sub> /EC <sub>50</sub> (95% CL)	NOEC
Male survival (pairing to D28)	█	█	█	0.48
Female survival (pairing to D28)	█	█	█	0.48
28-day survival	0.15 (0.071 – 0.23)	0.31 (0.18 – 0.45)	> 0.48 (n.d.)	0.24
Offspring per female	█	0.110 (0.049 – 0.17)	0.260 (0.19 – 0.32)	0.13
Male length	█	█	█	0.13
Female length	█	█	█	0.13
Male body weight	█	█	█	0.24 <sup>a)</sup>
Female body weight	0.26 (0.18 – 0.35)	█	█	0.13
F <sub>1</sub> survival at 96 hours post-release	█	█	█	0.24 <sup>a)</sup>
Overall NOEC	0.13			

█: not determined.

<sup>a)</sup> Highest concentration included in analysis of this parameter.

### C. Urann (2015) – Range finding study reported within ROW-0096

In this non-GLP range finding study, performed based on the US EPA OCSPP 850.1350 guideline, *Americamysis bahia* were exposed to mandestrobin (analysed purity 93.5%, batch ST-0811G) under flow-through conditions for 28 days (approximately one generation). The test was performed with nominal concentrations of 0.0052, 0.016, 0.051, 0.16 and 0.50 mg a.s./L, plus a negative and solvent (DMF) control. No analytical verification of test concentrations was conducted. The test medium used was diluted and filtered natural seawater. Two replicates were set up per treatment group and control, each containing 20 mysids (40 per treatment), less than 24 hours old at test initiation. The mysids were maintained in these replicates until the start of the reproduction phase. When sexual maturity was reached (day 13), one mature male and one mature female were assigned to each of the pairing chambers (with a minimum of five male/female pairs per replicate).

At test termination, the mysids in the negative and solvent controls met the performance criteria outlined in OCSPP Guideline 850.1350.

The results for F0 survival and reproduction are shown in Table 10.2.1/12-8 below, while results for growth parameters (total length and dry weight) are shown in Table 10.2.1/12-9. Results for 96-hour survival of the second-generation are shown in Table 10.2.1/12-10 and a summary of endpoints is provided in Table 10.2.1/12-11.

No behavioural abnormalities were observed during the exposure period.

At test termination, there was a significant difference in male survival at the highest treatment level of 0.50 mg a.s./L (67%) compared to the pooled control (91%). There were no significant differences in female survival in any of the treatments compared to the pooled control.

When male and female survival data were combined, at day 28 there was a significant difference in survival of organisms at the highest treatment level of 0.50 mg a.s./L compared to the pooled control. There were no significant effects on survival observed in any other treatment level. An LC<sub>50</sub> value of 0.33 mg a.s./L was calculated for this parameter (see Table 10.2.1/12-11).

At test termination, there was also a statistically significant difference in the mean number of offspring per female at the highest treatment level of 0.50 mg a.s./L compared to the pooled control. There were no effects on reproduction in any other treatment group.

**Table 10.2.1/12-8: First-generation (F<sub>0</sub>) survival and reproduction**

Nominal concentration (mg a.s./L)	Survival (post-pairing, day 13 – 28)				Reproduction	
	Male survival ± SD (%) <sup>a)</sup>	Female survival ± SD (%) <sup>a)</sup>	Combined M/F survival (post-pairing) ± SD (%) <sup>b)</sup>	Combined M/F survival (day 28) ± SD (%) <sup>b)</sup>	% of Females producing young ± SD	Number of offspring/female ± SD
Negative control	82 ± 7	100 ± 0	89 ± 7	84 ± 0	100 ± 0	11.6 ± 0
Solvent control	100 ± 0	95 ± 6	94 ± 0	94 ± 0	90 ± 14	10.3 ± 4.3
Pooled control	91 ± 11	98 ± 5	92 ± 5	89 ± 6	95 ± 10	11.0 ± 2.6
0.0052	79 ± 30	79 ± 13	79 ± 21	71 ± 19	90 ± 14	9.7 ± 0.7
0.016	100 ± 0	94 ± 9	98 ± 4	98 ± 4	100 ± 0	11.7 ± 2.4
0.051	93 ± 10	89 ± 16	90 ± 0	90 ± 0	100 ± 0	7.8 ± 0
0.16	100 ± 0	95 ± 7	97 ± 4	95 ± 7	100 ± 0	8.9 ± 1.6
0.50	67* ± NA	90 ± 14	78 ± 4	18* ± 4	50 ± 71	3.0** ± 4.2

<sup>a)</sup> Calculations of male and female survival began after pairing.

<sup>b)</sup> Calculations of survival are of both male and female mysids combined.

\* Significant difference compared to the pooled control, based on Bonferroni's Adjusted t-Test.

\*\* Significant difference compared to the pooled control, based on Dunnett's Multiple Comparison Test.

There were no significant differences in mean total body length of male or female mysids compared to the pooled control at any of the tested treatment levels.

A significant difference in mean dry body weight of both male and female mysids was observed at the highest treatment level of 0.50 mg a.s./L, compared to the pooled control (see Table 10.2.1/12-9).

**Table 10.2.1/12-9: First-generation (F<sub>0</sub>) mean body length and dry weight (day 28)**

Nominal concentration (mg a.s./L)	Mean body length (mm) ± SD		Mean dry body weight (mg) ± SD	
	Males	Females	Males	Females
Negative control	7.28 ± 0.04	7.01 ± 0.74	0.81 ± 0.02	1.05 ± 0.03
Solvent control	7.07 ± 0.01	7.23 ± 0.03	0.83 ± 0.04	1.01 ± 0.01
Pooled control	7.18 ± 0.13	7.21 ± 0.45	0.82 ± 0.03	1.03 ± 0.03
0.0052	6.71 ± 0.23	6.98 ± 0	0.84 ± 0.07	1.08 ± 0.06
0.016	6.81 ± 0.09	6.99 ± 0.08	0.77 ± 0.06	1.04 ± 0
0.051	6.67 ± 0.07	6.83 ± 0.13	0.79 ± 0.04	1.03 ± 0.06
0.16	6.71 ± 0.01	6.81 ± 0.04	0.79 ± 0.02	1.01 ± 0.05
0.50	6.29 ± (NA)	6.96 ± 0.23	0.67* ± (NA)	0.88* ± 0.14

n.a.: not applicable.

\*Significant difference compared to the pooled control, based on Dunnett's Multiple Comparison Test.

No F<sub>1</sub> populations could be established for the 0.50 mg a.s./L treatment level, therefore, the highest treatment level included in this analysis was 0.16 mg a.s./L. No significant differences in 96-hour F<sub>1</sub> survival were observed in any of the remaining treatment levels compared to the pooled control (Table 10.2.1/12-10).

**Table 10.2.1/12-10: F<sub>1</sub> survival at 96 hours post-release**

Nominal concentration (mg a.s./L)	Mean F <sub>1</sub> survival at 96 hours post-release ± SD (%)
Negative control	100 ± 0
Solvent control	95 ± 7
Pooled control	98 ± 5
0.0052	90 ± 14
0.016	95 ± 7
0.051	100 ± 0
0.16	95 ± 7
0.50 <sup>a)</sup>	n.a.

n.a.: not applicable.

<sup>a)</sup> No F<sub>1</sub> populations were established at this treatment level.

**Table 10.2.1/12-11: Summary of endpoints**

Endpoint	Based on nominal concentrations (mg a.s./L)	
	L/EC <sub>50</sub> (95% CL)	NOEC
28-day survival (F0)	0.33 (0.29 – 0.37)	0.16
Male dry weight	n.r.	0.16
Female dry weight	n.r.	0.16
Male survival post-pairing	n.r.	0.16
Offspring per female	n.r.	0.16
Male length	n.r.	0.50 <sup>a)</sup>
Female length	n.r.	0.50 <sup>a)</sup>
Female survival post-pairing	n.r.	0.50 <sup>a)</sup>

n.r.: not reported.

<sup>a)</sup> Not reported in the study, but determined to be the highest concentration tested for this position paper based on an absence of statistically significant effects at any treatment level.

### III. WEIGHT OF EVIDENCE

#### A. Comparison of available studies for *Americamysis bahia*

A table summarising the significant effects observed for each parameter in the two full chronic mysid studies is provided below (Table 10.2.1/12-12). Together, the 2012 and 2016 studies covered test concentrations between 0.0052 and 0.480 mg a.s./L (5.2 and 480 µg a.s./L). Both studies were performed with the same batch of test item (ST-0811G) with almost identical analysed purities of 93.4 and 93.5%, respectively. In addition to providing a colour-coded overview of observed significant differences from control groups in each study, the table also indicates the percentage reductions compared to the controls used for statistical analyses of each parameter (the pooled control was used in all cases except for two instances which are indicated in the table).

To enable a full overview of the effects observed in these studies and any observable trends across the entire dataset, all parameters which were statistically analysed in at least one study are included in the table. From visual inspection, it is clearly evident that statistically significant, treatment related effects are not identified up to a test item concentration of 0.084 mg a.s./L. Indeed, the significant, treatment related effects observed at this concentration in the 2012 study led to the derivation of the original NOEC of 0.049 mg a.s./L. The absence of treatment related effects at 0.049 mg a.s./L, and even up to 0.24 mg a.s./L in the second study from 2016 which suggests that the NOEC might even be higher.

While the NOEC still remains the most common endpoint applied to chronic risk assessments for aquatic invertebrates, it is pertinent to note that this endpoint can have severe limitations and cannot always be considered a reliable indicator of true treatment related effects, since they are highly dependent on the study design (for example, spacing of test concentrations, as well as number of replications, since increasing

replication reduces variability). As a result, expert scientific judgement is required in order to differentiate between treatment and non-treatment related effects in these instances. In the case of this study, the Study Director concluded that this effect was not treatment related, leading to the NOEC of 0.049 mg a.s./L, due to the confirmed treatment related effects at 0.084 mg a.s./L. This effect was concluded to be not treatment related as the difference was slight (15.43% reduction compared to the pooled control), and as there was no dose response at the next treatment levels of 0.024 and 0.049 mg a.s./L.

Furthermore, the control data (see Table 10.2.1/12-12) shows that reduced survival was observed in the solvent control compared to the negative control in both juveniles (survival to pairing on day 14) and adults (post-pairing). As this difference was not statistically significant in the adult survival data, the treatments were compared to the pooled control. However, the reduction of 11.1% in survival in the solvent control suggests that there may have been some effect of the solvent on survival. This is supported by the data for juvenile survival, where a significant difference between the controls was observed, and controls were accordingly not pooled for treatment comparisons. In consideration of this, it is reasonable to suggest that that it would be more applicable to compare the treatments to the solvent control instead of the pooled control, which would also be in line with OECD 54 (2006)<sup>3</sup>, which states that the solvent control group is the appropriate control group for comparisons with treated groups. When compared to the solvent control instead, percent reductions in adult survival in the treatments of 0.0056, 0.011, 0.024, 0.049 and 0.084 mg a.s./L are -0.12, 10.0, 4.57, 8.02 and 17.65%, respectively. The reductions up to 0.049 mg a.s./L are all  $\leq$  10% and do not follow a concentration response (with only 4.6% mortality at 0.024 mg a.s./L) which is considered to be not biologically relevant and therefore further supporting that 0.049 mg a.s./L is the relevant NOEC from this study.

In Table 10.2.1/12-12, the findings of the 2016 study can be observed in parallel with the results of the 2012 study. In the 2016 study, two of the concentrations tested (0.035 and 0.058 mg a.s./L) were within the range of the original test concentrations (0.0056 and 0.084 mg a.s./L), while the remaining three were greater than 0.084 mg a.s./L. With the addition of the results for these two test concentrations, the absence of treatment related effects within this range of treatments is reaffirmed, with no reductions >7.5% occurring in either of these treatments, and no statistically significant effects observed against the respective pooled controls.

Up to the concentration of 0.084 mg a.s./L, reductions in survival compared to the pooled controls in both studies are variable and show no clear trend or dose response. In the 2016 study, no significant effects upon this parameter were observed until the highest treatment level of 0.480 mg a.s./L, where 32.16% reduction in survival was observed, leading to a NOEC of 0.24 mg a.s./L (43 times greater than the disputed NOEC of 0.0056 mg a.s./L based on the same parameter in the 2012 study). It is also notable that in the 2016 study, while the number of replications was the same as in the 2012 study (four per treatment), a greater number of individuals was used per replicate (20 per replicate, 80 per treatment) compared to the 2012 study (15 per replicate, 60 per treatment). The greater number of individuals tested increases the reliability of the dataset, since a larger dataset leads to reduced variability and more reliable results. Moreover, in this study, due to a well-described dose response curve around the 10 and 20% mark, it was possible to calculate both an LC<sub>10</sub> and LC<sub>20</sub> (0.15 and 0.31 mg a.s./L, respectively), which are inherently more reliable than NOECs. Even the lower 95% confidence interval of the calculated LC<sub>10</sub> (0.071 mg a.s./L) provides reassurance that the NOEC of 0.049 mg a.s./L is sufficiently conservative, and that a NOEC of 0.0056 mg a.s./L is overly and unduly conservative.

Expanding observations across the remaining parameters, in the 2016 study, the lowest concentration tested was 0.035 mg a.s./L, where no significant effects were observed on any measured parameter (including survival, reproduction, length and dry weight). An absence of effects on any of these parameters was further demonstrated at the next two concentrations of 0.058 and 0.130 mg a.s./L. In fact, the first concentration where any significant effects were observed in the 2016 study was 0.240 mg a.s./L, where significant treatment related effects were observed on reproduction, male and female length, and female dry weight. As already noted above, in this study, a significant effect on survival was not observed until the highest test concentration of 0.480 mg a.s./L.

<sup>3</sup> OECD (2006). Number 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. ENV/JM/MONO(2006)18.

In the accompanying range finder for the 2016 study, although fewer replicates were utilised (two replicates per treatment, each containing 20 individuals), similar observations were made, which reaffirm the results of this 2016 main study. No significant effects compared to the controls were observed in any parameter at 0.160 mg a.s./L (nominal), which was concluded to be the NOEC from the range finding study, due to significant effects observed at the next (and highest) test concentration of 0.500 mg a.s./L (nominal), with an LC<sub>50</sub> calculated to be 0.33 mg a.s./L (based on nominal test concentrations). While the endpoints derived from this study cannot be considered fully reliable for use (the study was conducted with too few replications, and not under GLP), they do provide further support for the use of 0.049 mg a.s./L as the relevant NOEC for *Americamysis bahia*.

**Table 10.2.1/12-12: Summary of effects (as % reduction of controls) observed in the 2012 and 2016 full chronic *Americamysis* studies**

Mean measured concentration (mg a.s./L)	% Reduction vs control									
	Combined first-generation survival at pairing	Male survival at pairing	Female survival at pairing	First-generation survival test end	96-hour second-generation survival	Offspring per female	Male length	Female length	Male dry weight	Female dry weight
0.0056	0.00 <sup>a)</sup>			5.92		-8.24	3.05	-3.40	13.27	-2.56
0.011	6.71 <sup>a)</sup>			15.43		-3.53	-0.82	-2.93	-2.04	-5.13
0.024	10.17 <sup>a)</sup>			10.32		50.59	1.41	-2.58	0.00	-6.83
0.035	4.55	14.12	-7.95	5.71	9.09	18.85	0.05	-0.77 <sup>b)</sup>	1.71	0.72
0.049	-1.73 <sup>a)</sup>			13.57		16.47	-0.82	-4.33	2.04	-11.11
0.058	-1.14	-1.18	-10.23	7.47	6.49	10.26	1.86	2.07 <sup>b)</sup>	-1.09	-2.66
0.084	-1.73 <sup>a)</sup>			22.62		54.12	5.63	0.47	4.08	-5.98
0.130	2.27	1.18	-1.14	0.98	-1.09	24.11	0.94	0.60 <sup>b)</sup>	2.02	-2.90
0.240	15.91	14.12	9.09	13.70	1.57	47.97	3.49	3.36 <sup>b)</sup>	1.40	10.14
0.480	2.27	-1.18	1.14	32.16						

Note: All values are reductions compared to the pooled control as per the Study Report, unless otherwise indicated.

<sup>a)</sup> Compared to the negative control, as per the Study Report.

<sup>b)</sup> Compared to the solvent control, as per the Study Report.

#### Key

	2012 study (ROW-0063)
	2016 study (ROW-0096)
	No significant effect
	Significant effect not considered to be treatment related in the study
	Significant effect considered to be treatment related in the study
	Not assessed

Furthermore, an important observation when comparing the two full mysid studies is the difference in test duration (36 days and 28 days in the 2012 and 2016 studies, respectively), which results from the differing reproductive performance of the control organisms in the studies. The standard test duration in accordance with OCSPP 850.1350 is 28 days.

In the 2012 study, time to first brood release was 17 days; however first brood was actually released in the 0.084 mg a.s./L treatment (i.e. the highest concentration tested) rather than a control indicating that time to first brood release was not affected by the treatment. In the negative and solvent controls, first brood were released on days 22 and 18, respectively. Overall, the median time to first brood release was 29 days (negative control), 25 days (solvent control) and 27 days (combined negative and solvent controls). As a result, the 2012 study was prolonged to day 36 (at least 7 days after the median time of first brood release of 27 days in the combined controls). These delays in brood release being more pronounced in the controls suggest that the mysids used in the study generally may not have been in good health conditions prior to use in the study.

By contrast, in the 2016 study, first brood was released in the negative control. The median time to first brood release was 19.5 days (negative control), 21.5 days (solvent control) and 20.5 days (combined negative and solvent controls). Accordingly, the study was terminated on schedule and in accordance with the guideline on day 28. These results indicate that the mysids used in the 2016 study were in better health conditions prior to initiating the study and were therefore more appropriate for testing. The use of test organisms which are in good health is a key requirement for laboratory testing in order to eliminate variability in the results and to ensure that any effects observed can actually be attributed to the test item and not suboptimal or poor pre-existing health. Therefore, the results of the 2016 study are considered to be more reliable and that a NOEC and EC<sub>10</sub> of 0.13 and 0.15 mg a.s./L, respectively, would actually be more appropriate for this species.

A comparison of endpoints derived from the chronic mysid studies is shown in Table 10.2.1/12-13 below.

**Table 10.2.1/12-13: Comparison of chronic endpoints for *Americamysis bahia* (as defined in the study reports)**

Endpoint	Based on mean measured concentrations (mg a.s./L)				Based on nominal concentrations (mg a.s./L)	
	Claude <i>et al.</i> (2012)		Urann (2016)		Urann (2015) Non-GLP range finder	
	LC/EC <sub>10</sub> (95% CL)	NOEC	LC/EC <sub>10</sub> (95% CL)	NOEC	LC <sub>50</sub>	NOEC
Survival (first-generation, test end)	-	0.049	0.15 (0.071 – 0.23)	0.24	0.33 (0.29 – 0.37)	0.16
Reproduction	-	0.084 <sup>c)</sup>	-	0.13	-	0.16
Growth	-	0.049	0.26 (0.18 – 0.35) <sup>b)</sup>	0.13	-	0.16
Second-generation survival at 96 hours post-release	- <sup>a)</sup>	- <sup>a)</sup>	-	0.24	-	n.r.
Overall NOEC	0.049		0.13		0.16	

-: not determined; n.r.: not reported.

<sup>a)</sup> Not assessed.

<sup>b)</sup> Calculated based on effects on female body weight.

<sup>c)</sup> Highest concentration tested in the study.

The overall lowest NOECs from the studies are 0.049 and 0.13 mg a.s./L, respectively, which are comparable. The results of the non-GLP range finder provide further confidence in this conclusion, with a NOEC of 0.160 mg a.s./L (based on nominal concentrations). Therefore, based on the available chronic data for *Americamysis*, the NOEC of 0.049 mg a.s./L is considered to be sufficiently conservative and protective of the risk to this species, and even more so when considering the additionally protective assessment factor of 10 applied to this endpoint in the Tier 1 risk assessment, especially in light of additional species testing being available (see below under Point B).



## **B. Available data for other aquatic invertebrate species**

Further evidence that the NOEC of 0.049 mg a.s./L is sufficiently conservative can be identified within the available studies on additional aquatic invertebrate species (all of the subphylum crustacea).

Studies on the chronic toxicity of mandestrobin to additional aquatic invertebrate species were generated for the purpose of a Species Sensitivity Distribution (SSD), i.e. a Tier 2B Regulatory Acceptable Concentration (RAC) for the aquatic risk assessment. Combined with the available studies on *Americamysis bahia* and *Daphnia magna*, a total of ten chronic studies are available which cover nine different species. These studies can be used in this case to show the relative sensitivities of the eight other species in comparison to *Americamysis bahia*.

The available endpoints from these studies are shown in the following table, with the species most closely related to *Americamysis bahia* (as demonstrated by the phylogenetic tree in Figure 10.2.1/12-1) indicated in bold. These closely related species include *Asellus aquaticus*, *Gammarus pulex* and *Hyalella azteca*, which are all from the class Malacostraca and the superorder Peracarida.

The available endpoints, in particular those for *Asellus aquaticus* and *Gammarus pulex*, provide additional confidence that the selected NOEC of 0.049 mg a.s./L for *Americamysis bahia* is sufficiently conservative.

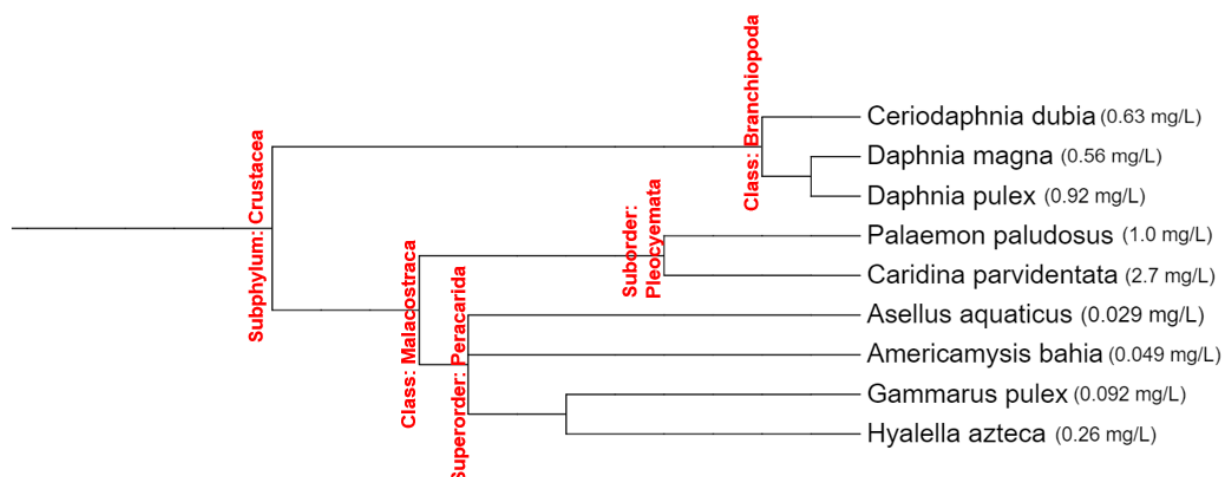
Among the most closely related species to *Americamysis* in the dataset, the NOEC (0.029 mg a.s./L) and EC<sub>10</sub> (0.050 mg a.s./L) for *Asellus aquaticus*, are both in the range of the NOEC of 0.049 mg a.s./L obtained from the 2016 mysid study. The NOEC for *Gammarus pulex* (0.092 mg a.s./L) is also highly comparable, while the calculated EC<sub>10</sub> value for growth (0.18 mg a.s./L) is similar to the EC<sub>10</sub> for growth obtained from the 2016 *Americamysis* study (0.26 mg a.s./L), thus demonstrating very similar toxicity responses among these related species.

**Table 10.2.1/12-14: Available endpoints for all species (most closely related to *Americamysis bahia* shown in bold)**

Report No.	Test species	Timescale	Endpoint type		Endpoint value (mg a.s./L)
ROW-0063	<i>Americamysis bahia</i>	36 d	NOEC	Mortality and growth	0.049
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0096	<i>Americamysis bahia</i>	28 d	NOEC	Growth and reproduction	0.13
			LC <sub>10</sub>	Mortality	0.15
			EC <sub>10</sub>	Growth	0.26
ROW-0104	<i>Asellus aquaticus</i>	28 d	NOEC	Growth	0.029
			EC <sub>10</sub>	Growth	0.050
ROW-0105	<i>Gammarus pulex</i>	28 d	NOEC	Growth	0.092
			EC <sub>10</sub>	Growth	0.18
ROW-0127	<i>Hyalella azteca</i>	42 d	NOEC	Growth and reproduction	0.26
			EC <sub>10</sub>	Growth	0.66
ROW-0020	<i>Daphnia magna</i>	21 d	NOEC	Mortality, growth and reproduction	0.56
			EC <sub>10</sub>	-	n.d. <sup>a)</sup>
ROW-0103	<i>Daphnia pulex</i>	28 d	NOEC	Growth and reproduction	0.92
			EC <sub>10</sub>	Growth	0.54
ROW-0126	<i>Ceriodaphnia dubia</i>	7 d	NOEC	Growth and reproduction	0.63
			EC <sub>10</sub>	Reproduction	0.69
ROW-0106	<i>Caridina parvidentata</i>	28 d	NOEC	Mortality and growth	2.7
			EC <sub>10</sub>	Growth	2.6
ROW-0148	<i>Palaemonetes paludosus</i>	28 d	NOEC	Mortality and growth	1.0
			LC <sub>10</sub>	Mortality	1.2

n.d.: not determined.

<sup>a)</sup> EC<sub>10</sub> values were not reported in the original study, and reliable values could not be calculated.



**Figure 10.2.1/12-1: Phylogenetic tree showing the relation of each additional test species to *Americamysis bahia*, with respective lowest NOECs from each study indicated for each species**

#### IV. CONCLUSIONS

During the initial evaluation of mandestrobin, the chronic NOEC for *Americamysis bahia* was listed in the EFSA Conclusion (2015) as 0.0056 mg a.s./L, which was in contrast to the findings of the original study report and the opinion of the Applicant, which firmly maintains that the original NOEC of 0.049 mg a.s./L for this species is the most reliable from the study in question, and that the endpoint of 0.0056 mg a.s./L is unduly conservative.

In this position paper, weight of evidence has been put forth which demonstrates that the NOEC of 0.049 mg a.s./L is sufficiently reliable and conservative for use in the aquatic risk assessment (and in turn, towards the classification of mandestrobin). This evidence includes:

- The effects seen on adult first-generation survival at 0.011 mg a.s./L in the 2012 study were slight, not dose responsive and, accordingly, not treatment related.
- A second chronic study with *A. bahia* is available (Urann, 2016) which identified no significant effects on survival, growth or reproduction at concentrations up to and including 0.13 mg a.s./L (NOEC). Significant effects were observed at a LOEC of 0.24 mg a.s./L. The lowest of the estimated L/EC<sub>10</sub> values was an LC<sub>10</sub> of 0.15 mg a.s./L. These endpoints are in agreement with the NOEC of 0.049 mg a.s./L from the original study, which was based on mortality and growth. Moreover, the lower 95% confidence interval of this LC<sub>10</sub> (0.071 mg a.s./L) provides further reassurance that a NOEC of 0.049 mg a.s./L is sufficiently protective of *Americamysis bahia*.
- The median time to first brood release in the two main mysid studies suggest that the mysids used in the 2016 study were in better health conditions than those used in the 2012 study. In the 2012 study, median time to first brood release in the combined controls was 27 days, leading to the study duration being extended to 36 days (standard duration is 28 days). Comparatively, in the 2016 study, median time to first brood release in the combined controls was 20.5 days, and the study was terminated on schedule at 28 days. These results indicate that the findings of the 2016 study may be more reliable and that a NOEC/EC<sub>10</sub> of 0.13 and 0.15 mg a.s./L, respectively may even be argued to be more appropriate for the species.
- A well-performed non-GLP range finding study is available, performed prior to the 2016 study. The findings further support the conclusions noted above, with a NOEC of 0.160 mg a.s./L (nominal) based on significant effects observed at the highest test concentration of 0.500 mg a.s./L (nominal).

- Studies on eight species in addition to *Americamysis bahia* are available. The most closely related species to *A. bahia* of these species are *Asellus aquaticus*, *Gammarus pulex* and *Hyalella azteca*. The available endpoints from these studies are all highly comparable and in line with the NOEC of 0.049 mg a.s./L, further demonstrating that this NOEC is sufficiently conservative of the risk to this species.

**Assessment and conclusion by applicant:**

The report outlines the main findings of the available studies with *Americamysis bahia* and based on the available data for this species and additional species, provides a weight of evidence justifying the relevance of the originally defined NOEC of 0.049 mg a.s./L for this species, as opposed to the NOEC of 0.0056 mg a.s./L defined in the EFSA Conclusion (2015), which is considered to be unduly conservative and not representative of the sensitivity of *Americamysis bahia* to mandestrobin.

**Assessment and conclusion by zRMS:** zRMS agrees with the Applicant that the NOEC of 0.0056 mg/L determined for *Americamysis bahia* in the DAR of Mandestrobin is conservative.

Considering the discussion on submitted studies for aquatic invertebrates: Claude et al. (2012). S-2200: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Americamysis bahia*), Urann, K. (2016). Mandestrobin (S-2200) – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) and Urann, K. (2015). Mandestrobin (S-2200) – Range-Finding Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) it can be concluded that the NOEC of 0.049 mg a.s./L is sufficiently reliable and conservative for use in the aquatic risk assessment.

Additionally, studies on other species are available. The most closely related species to *A. bahia* of these species are *Asellus aquaticus*, *Gammarus pulex* and *Hyalella azteca*. The available endpoints from these studies are all highly comparable and in line with the NOEC of 0.049 mg a.s./L, further demonstrating that this NOEC is sufficiently conservative of the risk to this species.

The presented evidence is acceptable.

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

**A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms**

**A 2.3 KCP 10.3 Effects on arthropods**

**A 2.3.1 KCP 10.3.1 Effects on bees**

**A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees**

**A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees**

**A 2.3.1.1.1.1 Study 1**

<b>Data point:</b>	KCP 10.3.1.1.1/01 and KCP 10.3.1.1.2/01
<b>Report author:</b>	Ansaloni, T.
<b>Report year:</b>	2023
<b>Report title:</b>	Mandestrobin 40 SC: Acute Oral and Contact Toxicity to the Honeybees ( <i>Apis mellifera</i> L.), under Laboratory Conditions.
<b>Report No.:</b>	S22-07815
<b>Document No.:</b>	ROW-0157
<b>Guidelines followed in study:</b>	OECD 213 (1998) and OECD 214 (1998)
<b>Deviations from current test guideline:</b>	<p>Compared to OECD 213: None.</p> <p>Compared to OECD 214 (1998): The test guideline recommends a volume of 1 µL of solution containing the test substance be administered however, 2 µL dose was administered in this study.</p> <p>These deviations are not considered to have an impact on the acceptability of this study since all validity was met and the test facility experience has shown that higher volumes are suitable and produce no adverse effects on the outcome of studies.</p>
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

**Executive Summary**

The acute oral and contact toxicity of Mandestrobin 40SC to the honeybee (*Apis mellifera*) was determined in the laboratory over 48 hours. Both toxicity tests were performed with five nominal doses between 268.38 and 1358.70 µg product/bee (between 100.00 and 506.25 µg a.s./bee) and an untreated control, with five replicates per treatment group, each containing ten bees. Dimethoate 400 g/L EC was used as a toxic

reference item and tested at four nominal doses with four replicates per treatment. Observations of mortality were performed after 4, 24 and 48 hours.

The 48-hour oral LD<sub>50</sub> was determined to be > 864.31 µg product/bee (> 322.04 µg a.s./bee) based on actual consumption. The 48-hour contact LD<sub>50</sub> was determined to be > 1358.70 µg product/bee (> 506.25 µg a.s./bee).

## I. MATERIALS AND METHODS

### A. MATERIALS

- 1. Test material:** Mandestrobin 40SC (S-2200 40SC)  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L nominal, 37.26% w/w (403.3 g/L) analysed
- 2. Controls:** Oral test: Untreated 50% w/v aqueous sucrose solution  
Contact test: 0.1% (v/v) Triton X solution in deionised water
- 3. Reference item:** BAS 152 65 I (420.1 g dimethoate/L)

### B. STUDY DESIGN AND METHODS

- 1. Test organism:** Honeybee *Apis mellifera* (Hymenoptera, Apidae)  
**Age:** Adult worker bees  
**Source:** Healthy colony, obtained from a commercial beehive maintained by the test facility.  
**Diet:** 50% aqueous sucrose solution
- 2. Test units:** Stainless steel cages (8.2 × 4.5 × 6.0 cm; width, depth, height). The front side of the cages was equipped with a transparent pane so that the bees could be observed. Test cages were lined with filter paper.
- 3. Environmental conditions:**  
**Temperature:** Oral test: 24.8 – 25.6°C  
Contact test: 24.8 – 25.6°C  
**Relative humidity:** Oral test: 62.9 – 66.5%  
Contact test: 62.4 – 66.5%  
**Photoperiod:** 24-hour darkness except during handling procedures and assessment.
- 4. Animal assignment and treatment:**

The acute oral and contact toxicity of Mandestrobin 40SC to the honeybee (*Apis mellifera*) was determined in the laboratory over 48 hours. One day before the start of the test the bees were collected randomly from the outer combs of the beehive and distributed into test units and kept under test conditions until the start of the test.

#### Oral toxicity test

The oral toxicity test was performed with five nominal doses of 268.38, 402.58, 603.86, 905.80 and 1358.70 µg product/bee, equivalent to 100.00, 150.00, 225.00, 337.50 and 506.25 µg a.s./bee. An untreated control was tested in parallel. There were five replicates per treatment group, each containing ten bees. Dimethoate was used as a toxic reference item, tested at four nominal doses of 0.062, 0.093, 0.140 and 0.210 µg a.s./bee with four replicates per reference group.

A quantity of 200 µL/replicate (corresponding to 20 µL/bee) of test item or reference item was offered for 6 hours to each cage of ten bees to ensure sufficient consumption of test or reference item. Bees were starved for 2 hours before they were fed with the solutions. Bees within a cage shared the test solution and therefore are assumed to have received a similar dose.

The amount of test solution consumed by each replicate was determined by weighing the feeders before and after feeding. After the feeding period, bees were supplied *ad libitum* with untreated 50% aqueous sucrose solution. In the control group, the bees were fed pure untreated 50% (w/v) sucrose solution.

### **Contact toxicity test**

The contact toxicity test was performed with five nominal doses of 268.38, 402.58, 603.86, 905.80, 1358.70 µg product/bee, equivalent to 100.00, 150.00, 225.00, 337.50 and 506.25 µg a.s./bee and an untreated control, with five replicates per treatment group, each containing ten bees.

Dimethoate was used as a toxic reference item, tested at four test concentrations of 0.062, 0.093, 0.140 and 0.271 µg a.s./bee, with four replicates per reference group.

Bees were anaesthetized with carbon dioxide, then treated individually by topical application to the thorax. A 2 µL droplet was applied to each bee. After the application the bees were returned to the test cages and fed with untreated 50% aqueous sucrose solution, *ad libitum*.

## **5. Dose preparation:**

### **Oral toxicity test**

The final doses were prepared by mixing an appropriate amount of the stock solution or higher solution with an appropriate amount of 50% (w/v) aqueous sucrose solution, so that 20 µL contained the required amount of test or reference item per bee.

### **Contact toxicity test**

The final doses of the test item or reference item were prepared by mixing an appropriate amount of the stock solution with an appropriate amount of tap water, so that 2 µL contained the required amount of test item per bee. 0.1% (v/v) Triton X solution only, was applied to control bees.

## **6. Measurements and observations:**

The number of dead bees in the individual test cages was recorded after 4, 24 and 48 hours in both the oral and contact toxicity test. Behavioural abnormalities, such as symptoms of poisoning or lack of coordination, were evaluated at each observation time. In the reference item group, behavioural abnormalities assessments were not conducted, as it could be assumed that moribund and affected bees of the reference item group died by the end of the test.

Behavioural abnormalities were recorded according to the following categories:

- Affected: bees still upright and attempting to walk but showing signs of reduced coordination.
- Apathetic: bees show only low or delayed reactions to stimulation, e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly.
- Cramps: bees contracting abdomen or entire body).
- Moribund: bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation, e.g. light or blowing; bees may recover but usually die.

For analytical verification of the test item, samples of the test item concentrations feeding solution (oral test) and application solution (contact test) were taken on the application day (D0), directly after preparation. Analytical analysis was performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

## **7. Statistics:**

The 24- and 48-hour LD<sub>50</sub> values were empirically estimated since virtually no mortality occurred in the test item treatments of the oral and contact test.

The 24-hour LD<sub>50</sub> values of the reference item treatment were calculated by means of a probit analysis using linear max. likelihood regression.

The 24- and 48-hour NOED values for the oral test were determined by Fisher's Exact Binomial Test with Bonferroni Correction, ( $\alpha = 0.05$ , one-sided greater).

Statistical calculations were made using the statistical program ToxRatPro Version 3.3.0.

## II. RESULTS AND DISCUSSION

### A. ORAL TOXICITY TEST

A summary of the mortalities observed throughout the exposure period is shown in Table 10.3.1.1.1/01-1.

**Table 10.3.1.1.1/01-1: Mortality in the oral toxicity test**

Target dose (µg a.s./bee)	Actual uptake (µg a.s./bee)	Mortality (%)		
		4 hours	24 hours	48 hours
Control	-	0.0	0.0	0.0
<b>Test item: Mandestrobin 40SC</b>				
100.00	90.50	0.0	4.0	4.0
150.00	130.34	0.0	0.0	0.0
225.00	198.91	0.0	0.0	0.0
337.50	248.73	0.0	0.0	0.0
506.25	322.04	0.0	0.0	4.0
<b>Reference item: Dimethoate</b>				
0.062	0.051	0.0	2.5	2.5
0.093	0.083	0.0	2.5	5.0
0.140	0.113	0.0	30.0	35.0
0.210	0.140	0.0	67.5	72.5

### B. CONTACT TOXICITY TEST

In the water control group 0.0% mortality was observed during the 48-hour observation period. In the test item nominal doses of 100.00, 150.00, 225.00, 337.50 and 506.25 µg a.s./bee, the cumulative mean mortality was 2.0, 0.0, 0.0, 0.0 and 0.0%, respectively, 48 hours after start of exposure. No sublethal effects were observed.

Mortality observed in the contact toxicity test is shown in Table 10.3.1.1.1/01-2.



**Table 10.3.1.1.1/01-2: Mortality in the contact toxicity test**

Treatment (µg a.s./bee)	Mortality (%)		
	4 Hours	24 Hours	48 Hours
Control	0.0	0.0	0.0
<b>Test item: Mandestrobin 40SC</b>			
100.00	0.0	0.0	2.0
150.00	0.0	0.0	0.0
225.00	0.0	0.0	0.0
337.50	0.0	0.0	0.0
506.25	0.0	0.0	0.0
<b>Reference item: Dimethoate</b>			
0.080	0.0	0.0	0.0
0.120	0.0	7.5	7.5
0.180	0.0	45.0	47.5
0.270	0.0	90.0	92.5

<sup>a)</sup> Since no control mortality occurred, the cumulative mortalities for each test item group and reference item group were not corrected.

The endpoints for Mandestrobin 40SC and the reference item are presented in Table 10.3.1.1.1/01-3.

**Table 10.3.1.1.1/01-3: Summary of endpoints**

Endpoints	µg product/bee	µg a.s./bee <sup>a)</sup>
24-hour oral LD <sub>50</sub>	> 864.31	> 322.04
48-hour oral LD <sub>50</sub>	> 864.31	> 322.04
24-hour oral NOED	864.31	322.04
48-hour oral NOED	864.31	322.04
24-hour contact LD <sub>50</sub>	> 1358.70	> 506.25
48-hour contact LD <sub>50</sub>	> 1358.70	> 506.25
24-hour contact NOED	1358.70	506.25
48-hour contact NOED	1358.70	506.25

<sup>a)</sup> Endpoints equivalences based on the actual content of the active substance (mandestrobin: 37.26% w/w) according to the certificate of analysis.

The 24-hour oral LD<sub>50</sub> with 95% confidence limits for the reference item was 0.127 (0.119 – 0.136) µg dimethoate/bee. The 24-hour contact LD<sub>50</sub> with 95% confidence limits for the reference item was 0.186 (0.171 – 0.202) µg dimethoate/bee.

## C. ANALYTICAL RESULTS

Samples of the test item treatments in both the oral and contact test gave recoveries within the 80 – 120% of the nominal concentrations (actual mean recoveries between 90 and 101%). Therefore, all endpoints are based on nominal concentrations.

**Table 10.3.1.1/01-4: Analytical results**

Active substance nominal concentration (mg/L)	Calculated (mean) concentration of active substance (mg/L)	% of target concentration
<b>Oral test <sup>a)</sup></b>		
5000.00	4689.91	94
16875.00	15378.95	91
25312.50	22880.16	90
<b>Contact test <sup>b)</sup></b>		
50000.00	50280.28	101
168750.00	166444.89	99
253125.00	243962.87	96

<sup>a)</sup> Corresponding to the Stock Solution. LOQ = 500 mg/L.

<sup>b)</sup> Corresponding to the Stock Solution. LOQ = 5000 mg/L.

## D. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent versions of the EU test guidelines (OECD 213, 1998 and OECD 214, 1998) as detailed below:

- The average mortality for the total number of controls must not exceed 10% at the end of the test. 0% mortality were observed in the controls in both the oral and contact toxicity tests, by the end of the test.
- The 24-hour LD<sub>50</sub> of the toxic standard dimethoate must meet the specified range of 0.10 – 0.35 µg dimethoate/bee (oral test) and 0.10 – 0.30 µg dimethoate/bee (contact test). The 24-hour LD<sub>50</sub> of dimethoate in the oral test was 0.127 µg dimethoate/bee and the 24-hour LD<sub>50</sub> of dimethoate in the contact test was 0.186 µg dimethoate/bee.

## III. CONCLUSION

The acute oral and contact toxicity of Mandestrobin 40SC to the honeybee (*Apis mellifera*) was determined in the laboratory over 48 hours (oral and contact test).

The 48-hour oral LD<sub>50</sub> was determined to be > 864.31 µg product/bee (> 322.04 µg a.s./bee) based on actual consumption. The 48-hour contact LD<sub>50</sub> was determined to be > 1358.70 µg product/bee (> 506.25 µg a.s./bee).

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>48-hour oral LD<sub>50</sub>: &gt; 864.31 µg product/bee (&gt; 322.04 µg a.s./bee)</p> <p>48-hour contact LD<sub>50</sub>: &gt; 1358.70 µg product/bee (&gt; 506.25 µg a.s./bee)</p>
---	---

Comments of zRMS:	The submitted study was accepted.											
	The validity criteria were met.											
	The following deviation was noted: <i>Behavioural abnormalities in the reference item treatment were not recorded since the reference item is known to be toxic to honeybees and therefore effects are expected.</i>											
	Mortality in reference item groups was observed and noted in oral and contact toxicity tests.											
	The following endpoints were proposed:											
	<table><tr><th>Endpoint</th><th>µg product/bee</th><th>µg a.s./bee</th></tr><tr><td>48 h Oral LD<sub>50</sub></td><td>&gt; 864.31</td><td>&gt; 322.04</td></tr><tr><td>48 h Oral NOED</td><td>864.31</td><td>322.04</td></tr></table>	Endpoint	µg product/bee	µg a.s./bee	48 h Oral LD <sub>50</sub>	> 864.31	> 322.04	48 h Oral NOED	864.31	322.04		
Endpoint	µg product/bee	µg a.s./bee										
48 h Oral LD <sub>50</sub>	> 864.31	> 322.04										
48 h Oral NOED	864.31	322.04										

		48 h Contact LD <sub>50</sub>	> 1358.70	> 506.25	
		48 h Contact NOED	1358.70	506.25	

#### A 2.3.1.1.1.2 Study 2

<b>Data point:</b>	KCP 10.3.1.1.1/02 and KCP 10.3.1.1.2/02
<b>Report author:</b>	Aguilar-Alberola, J.A.
<b>Report year:</b>	2022
<b>Report title:</b>	S-2200 (Mandestrobin) Technical Grade: Acute Oral and Contact Toxicity Test to the Bumblebee ( <i>Bombus terrestris</i> L.), under Laboratory Conditions
<b>Report No.:</b>	S21-04906
<b>Document No.:</b>	ROW-0112
<b>Guidelines followed in study:</b>	OECD 246 (2017), OECD 247 (2017), SANTE/2020/12830, rev. 1 (2021)
<b>Deviations from current test guideline:</b>	Compared to OECD 246 (2017) and OECD 247 (2017): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The acute oral and contact toxicity of mandestrobin to the bumblebee (*Bombus terrestris* L.) was determined in the laboratory over 48 hours. Both the oral and contact tests were carried out with five different test item doses between 39.51 to 200 µg a.s./bee. Negative and solvent (acetone) control groups were tested in parallel, plus dimethoate was tested as a reference item at one dose. Each treatment group consisted of 30 and 35 replicates of one bumblebee each for the contact and oral tests, respectively. Assessments of mortality were performed after 4, 24 and 48 hours.

The results of the oral test were based on actual intake values. One mortality occurred in the oral test at the nominal treatment 133.33 µg a.s./bee. No mortalities occurred in any other treatment group or in the control, and no mortalities occurred in the contact test. The 48-hour LD<sub>50</sub> values were determined to be > 193.03 µg a.s./bee and > 200 µg a.s./bee in the oral and contact toxicity tests, respectively.

### I. MATERIALS AND METHODS

#### A. MATERIALS

- Test material:** S-2200 Technical grade (mandestrobin)  
**Description:** Very pale yellow crystalline powdery solid  
**Lot/Batch:** ST-0811G  
**Purity:** 93.7% w/w
- Controls:**

Oral test  
 Control: untreated 50% aqueous sucrose solution  
 Solvent control: untreated 50% aqueous sucrose solution + acetone:Tween 80 (4:1)

Contact test  
 Control: 0.1 % (w/v) Triton X solution  
 Solvent control: pure acetone

- 3. Reference item:** BAS 152 65 I (dimethoate, 40.9% w/v)

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

- 1. Test organism:** Bumble bee (*Bombus terrestris* L.)  
**Age:** Young adult worker bees  
**Source:** Commercial queen-right colonies  
**Diet:** 50% (w/v) aqueous sucrose solution. Feeding was done *ad libitum* during acclimatisation and the test period for both the oral and contact tests, except during starvation and feeding of application solutions for the oral test.
- 2. Test units:** Bumblebees were kept individually in Nicot cages and fed via 1 mL syringes deprived of the tip.
- 3. Environmental conditions:**  
**Temperature:** Oral test: 25.0 – 25.3°C  
Contact test: 25.0 – 25.3°C  
**Relative humidity:** Oral test: 55.9 – 58.7%  
Contact test: 54.4 – 59.7%  
**Photoperiod:** Constant darkness except for applications and observations

### **4. Animal assignment and treatment:**

The acute oral and contact toxicity of mandestrobin to the bumblebee (*Bombus terrestris* L.) was determined in the laboratory over 48 hours. Both tests were performed with five nominal different test item doses of 42.16, 63.24, 94.87, 142.30 and 213.45 µg/bee (equivalent to 39.51, 59.26, 88.89, 133.33 and 200.00 µg a.s./bee, corrected for purity). An untreated control, solvent control, and reference item were tested in parallel.

#### **Oral test**

Each treatment group consisted of 35 replicates, each containing one bee. The test item was administered in the diet (50 % (w/v) aqueous sucrose solution) using 1 mL syringes (feeders). The application volume was 40 µL/replicate, corresponding to 40 µL/bee. The bees were starved for approximately 2 hours prior to application. Each replicate was provided with the application solution for up to 4 hours to ensure sufficient uptake. The feeders were then removed and the bees were offered untreated diet for the remainder of the test.

#### **Contact test**

Each treatment group consisted of 30 replicates, each containing one bee. The test item was administered as a 4 µL droplet using a hand operated micro-applicator. The droplet was applied individually to the dorsal side of the thorax of each bumblebee. The application amount of 4 µL instead of 2 µL ensures a more reliable dispersion of the application solution. After application, the bees were returned to the test units and provided with untreated diet for the remainder of the test.

### **5. Dose preparation:**

#### **Oral toxicity test**

For the preparation of the stock solution for the oral test, a defined amount of test item was mixed with acetone:Tween 80 (4:1) and aliquots were added to 50% (w/v) aqueous sucrose solution. These aliquots represented the 5% of the feeding solution volume. The solvent control group was prepared in the same way but without test item. For the preparation of the reference item feeding solution, 50% (w/v) aqueous sucrose solution was used as solvent. The negative control group was fed with pure 50% (w/v) aqueous sucrose solution.

## Contact toxicity test

A defined amount of test item was mixed with acetone. This solution was used as stock solution and the highest concentration application solution. For the preparation of the other treated application solutions, aliquots of the stock solution were taken and filled up with acetone to the final volume required. The solvent control group was applied with pure acetone. For the preparation of the reference item application solution, 0.1% (w/v) Triton X solution was used as solvent. The negative control group was applied with 0.1% (w/v) Triton X solution in deionised water.

## 6. Measurements and observations:

Observations for mortality and behavioural abnormalities were performed after 4, 24 and 48 hours. Behavioural abnormalities were recorded according to two categories:

A = Affected. Bumblebees still upright and attempting to walk but showing signs of reduced coordination.  
M = Moribund. Bumblebees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation, e.g. light or blowing; bees may recover but usually die.

Analytical data were required by the guidelines to verify the actual concentration of the test item (representative sample) and its solubility in the solvent. Samples of the test item treated solutions from the lowest and highest concentrations were taken directly after its preparation for both, oral and contact solutions. As the oral test stock solution was different from any other test solution, samples were also taken. Analysis was performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

## 7. Statistics:

Since no/little mortality was observed in the test item groups across both tests (only one individual was registered as dead in the oral test), the NOED and LD<sub>50</sub> values were empirically estimated from the results. Statistical calculations were made with MS Excel 2016 v.16.

# II. RESULTS AND DISCUSSION

## A. ORAL TOXICITY TEST

The mean consumption of feeding solution and the mean intake of test item (both uncorrected and corrected for purity of the test item) are presented in Table 10.3.1.1.1/02-1 below.

By the end of the test (48 hours after dosing) one dead bumblebee was observed in the treated group T4, which was equal to 3.03% mortality. No other mortalities occurred in any other test item treatment, or in the controls. For this reason, no statistical analysis was performed for this parameter. No individuals were recorded as having behavioural abnormalities (i.e. bumblebees affected or moribund) during the whole test. Endpoints for the oral toxicity test are shown in Table 10.3.1.1.1/02-2.

**Table 10.3.1.1.1/02-1: Mean consumption per treatment and actual intake for feeding individuals**

Nominal dose		Consumed solution		Consumed dose/bee		Number of feeders
µg test item/bee	Treatment group	mg	%	µg a.s./bee	µg a.s./bee <sup>a)</sup>	
--	C1	47.3	99.4	--	--	35
--	C2	47.3	99.4	--	--	35
42.16	T1	47.2	99.1	41.79	39.16	35
63.24	T2	44.5	93.4	59.07	55.35	34
94.87	T3	45.3	95.3	90.36	84.66	35
142.30	T4	46.0	96.7	137.55	128.89	33
213.45	T5	45.9	96.5	206.01	193.03	34

<sup>a)</sup> Corrected for purity.

**Table 10.3.1.1.1/02-2: Endpoints for the oral toxicity test**

Endpoint	µg test item/bee	µg a.s./bee <sup>a)</sup>
24-hour NOED	206.01	193.03
48-hour NOED	206.01	193.03
24-hour LD <sub>50</sub>	> 206.01	> 193.03
48-hour LD <sub>50</sub>	> 206.01	> 193.03

<sup>a)</sup> Corrected for purity.

## B. CONTACT TOXICITY TEST

No mortality occurred in the control groups and in the test item treated groups of the contact toxicity test. For this reason, no statistical analysis was performed on mortality data. No individuals were recorded as having behavioural abnormalities (i.e. bumblebees affected or moribund) during the whole test. Endpoints for the contact toxicity test are shown in Table 10.3.1.1.1/02-3.

**Table 10.3.1.1.1/02-3: Endpoints for the contact toxicity test**

Endpoint	µg test item/bee	µg a.s./bee <sup>a)</sup>
24-hour NOED	213.45	200.00
48-hour NOED	213.45	200.00
24-hour LD <sub>50</sub>	> 213.45	> 200.00
48-hour LD <sub>50</sub>	> 213.45	> 200.00

<sup>a)</sup> Corrected for purity.

## C. ANALYSIS

The measured concentration in the samples was within 20% of nominal test concentration used, thus the concentrations of the test item were confirmed, and the endpoints are based on nominal concentrations. Results are shown in Table 10.3.1.1.1/02-4.

**Table 10.3.1.1.1/02-4: Analytical results**

Sample type	Nominal concentration (g test item/L)	Nominal concentration (g a.s./L) <sup>a)</sup>	Analysed concentration (g a.s./L)	Recovery (%)
Stock solution	106.7	100	108	108
Feeding solution	1.05406	0.988	0.794	80
Feeding solution	5.33618	5.00	5.97	119
Contact test solution	10.54060	9.88	9.96	101
Contact test solution	53.36179	50.0	53.8	108

<sup>a)</sup> Calculations performed considering the purity of 93.7%.

## D. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent versions of the EU test guidelines (OECD 246, 2017 and OECD 247, 2017) as detailed below:

- Mortality in the water control should be ≤ 10% at the end of the test. If included, also solvent control mortality should be ≤ 10% at the end of the test. No mortality occurred in either the negative or solvent controls, in either the oral or contact tests.
- Mortality in the toxic reference substance group should be ≥ 50% at the end of the test. Mortality in the toxic reference was 100% and 60% in the oral and contact tests, respectively.

### III. CONCLUSION

The acute oral and contact toxicity of mandestrobin to the bumblebee (*Bombus terrestris* L.) was determined in the laboratory over 48 hours.

One mortality occurred in the oral test at the nominal treatment 133.33 µg a.s./bee. No mortalities occurred in any other treatment group or in the control, and no mortalities occurred in the contact test. The 48-hour LD<sub>50</sub> values were determined to be > 193.03 µg a.s./bee and > 200 µg a.s./bee in the oral and contact toxicity tests, respectively.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>48-hour oral LD<sub>50</sub>: &gt; 193.03 µg a.s./bee</p> <p>48-hour contact LD<sub>50</sub>: &gt; 200 µg a.s./bee</p>
---	---

Comments of zRMS:	<p>The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.</p>
-------------------	--

#### A 2.3.1.1.2      KCP 10.3.1.1.2      Acute contact toxicity to bees

Refer to A 2.3.1.1.1.1 (Study 1) and A 2.3.1.1.1.2 (Study 2) above, which contains details of contact toxicity studies with the honeybee (Mandestrobin 40SC) and bumblebee (mandestrobin), respectively.

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

### A 2.3.1.2.1 Study 1

<b>Data point:</b>	KCP 10.3.1.2/01
<b>Report author:</b>	Picard, C.
<b>Report year:</b>	2018a
<b>Report title:</b>	S-2200 (Mandestrobin): 10-Day Oral Toxicity Test with the Adult Honey Bee ( <i>Apis mellifera</i> )
<b>Report No.:</b>	201800153 / 12709.6461
<b>Document No.:</b>	ROW-0102
<b>Guidelines followed in study:</b>	Draft of OECD 245 dated 2016.
<b>Deviations from current test guideline:</b>	Compared to OECD 245 (2017): The dietary concentrations were expressed as µg a.s./mL diet rather than µg a.s./kg diet. This had no impact on the validity or reliability of the study, as the relevant endpoints from the study are reported in the correct units (µg a.s./bee/day).
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The chronic oral toxicity of mandestrobin to the honeybee (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test. The test item was administered in the diet at five nominal concentrations between 320 and 5000 µg a.s./mL diet (50% sucrose solution), equivalent to dose rates of 6.3 to 100 µg a.s./bee/day. An untreated (negative) control and solvent (acetone) control were tested in parallel. Dimethoate was used as a toxic reference item at a single concentration of 1.0 mg dimethoate/kg diet. Three replicates were set up per test item treatment and control, each containing ten bees (30 bees per treatment group). Observations for mortality and behavioural abnormalities were performed daily. All bees were weighed (frozen) at test end. Food consumption was determined, and additional replicates were set up without bees to determine the evaporation rate of the test diet.

The 10-day NOED values for survival and body weight were both determined to be 45 µg a.s./bee/day. The 10-day NOEC for the same parameters was determined to be 1900 µg a.s./mL diet.

The 10-day LD<sub>10/20/50</sub> values for survival were determined to be 31, > 45 and > 45 µg a.s./bee/day, respectively. The 10-day ED<sub>10/20/50</sub> values for body weight were all determined to be > 45 µg a.s./bee/day.

The 10-day LC<sub>10/20/50</sub> values for survival were determined to be 1300, > 1900 and > 1900 µg a.s./mL diet, respectively. The 10-day EC<sub>10/20/50</sub> values for body weight were all determined to be > 1900 µg a.s./mL diet.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** S-2200 Technical (mandestrobin)  
**Description:** White powder  
**Lot/Batch:** 21201  
**Purity:** 93.7% w/w
- Controls:** Control: 50% aqueous sucrose solution  
 Solvent control: 50% aqueous sucrose solution + acetone
- Reference item:** Dimethoate (purity not reported)



## B. STUDY DESIGN AND METHODS

1. **Test organism:** Honeybee (*Apis mellifera* L.)  
**Age:** ≤ 2-day-old emerged adult bees  
**Source:** Wood's Beekeeping Supply, Lincoln, Rhode Island, USA  
**Acclimation:** 1 day  
**Diet:** 50% aqueous sucrose solution
2. **Test units:** 120 mL glass jars with screw top lids. Ventilation holes and a larger hole for insertion of the feeding syringe were drilled in the lid. These vessels and feeding syringes were also used during the acclimation phase.
3. **Environmental conditions:**  
**Temperature:** 32 – 34°C  
**Relative humidity:** 59 – 61%  
**Photoperiod:** Darkness except during observations (approximately 30 minutes laboratory lighting per day).

### 4. Animal assignment and treatment:

The chronic oral toxicity of mandestrobin to honey bees (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test. The test item was administered in the diet at five nominal concentrations of 320, 630, 1300, 2500 and 5000 µg a.s./mL diet, equivalent to dose rates of 6.3, 13, 25, 50 and 100 µg a.s./bee/day. An untreated control and solvent (acetone) control were tested in parallel. Additionally, dimethoate was used as a toxic reference item, tested at a single concentration of 1.0 mg dimethoate/kg diet. Three replicates were set up per test item treatment and control, each containing ten bees (30 bees per treatment group). Three additional vessels were established without honeybees, which each contained one syringe filled with untreated sucrose solution. The syringe weight was monitored daily for evaporative loss.

A theoretical feeding rate of 200 µL per ten bees per day was assumed based on past consumption rates and published data. Complete consumption of 200 µL per vessel per day for the ten bees along with analytical recoveries that closely approximated nominal values would provide the expected nominal dose. Sucrose solution diet was administered to each test vessel using a 3.0 mL plastic syringe with the tip removed, containing approximately 2.0 mL of the appropriate diet. Syringes were refilled with the appropriate diet on a daily basis. The actual amount of diet consumed per vessel was verified throughout the test by weighing each syringe before and after each daily feeding. Bees were allowed to feed on the diets *ad libitum* each day during the 10-day exposure.

### 5. Dose preparation:

Prior to exposure initiation, a 100 mg a.s./mL primary stock solution was prepared by dissolving 10.8851 g of mandestrobin (10.1993 g as active ingredient) in 100 mL of acetone. The resulting stock solution was observed to be a clear, slightly yellow solution with no visible undissolved material following preparation. The acetone stock solution was stored refrigerated when not in use. The diet for the highest nominal dose rate was prepared from the primary stock solutions.

The nominal diet solution was allowed to settle for approximately 24 hours following a prolonged mixing process. The supernatant (functionally soluble portion) from the 5000 µg a.s./mL diet concentration was used as the highest diet concentration and to prepare the remaining diet concentrations. Following the removal of the functionally soluble portion of the diet solutions, the solutions were observed to be hazy, cloudy, and pale amber in colour with no visible undissolved material.

The solvent control solution was prepared with 22.5 mL of acetone per 427.5 mL of untreated sucrose diet solution resulting in a 5% acetone diet solution. The negative control vessels received only fresh, untreated, sucrose solution.

## **6. Measurements and observations:**

Observations for mortality and unusual behaviour (e.g. apathy) were performed daily. Test organisms were considered dead if observed to be immobile on the bottom of the vessel, exhibiting no response to gentle prodding. At termination (day 10), all surviving bees were frozen and weighed.

Feeding syringes containing dosing solutions were weighed daily before and after filling with freshly prepared diet solution. Additionally, three vessels were established, each with one syringe filled with untreated sucrose solution and without honey bees, and the syringe weight monitored daily for evaporative loss. Daily honeybee dose was then calculated from daily diet consumption, daily evaporative loss, and daily mortality to reflect the daily dose more accurately.

Temperature and relative humidity were monitored continuously.

A sample of the stock solution used to dose the sucrose solution diets was collected on day 3 and measured for mandestrobin concentrations using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) to confirm that the stock solution was correctly prepared. Additionally, samples of each treated, negative control, and solvent control diets were collected for analysis of test substance concentrations on days 3, 6, and 8. Archive samples were also collected from the diets at each sampling interval and stored frozen for possible future analysis.

## **7. Statistics:**

A two-tailed Fisher's Exact Test was used to compare the day 10 survival of the negative control to that of the solvent control. A two-tailed Equal Variance Two Sample t-Test was used to compare the 10-day body weight of the two controls. No significant difference was observed between the controls for either endpoint; therefore the test item treatments were compared to the negative control.

The treatment data were tested for normality and homogeneity of variance using the Shapiro-Wilks Test and Bartlett's Test, respectively.

The Fisher's Exact Test with Bonferroni Holm's Adjustment was used to determine statistically significant differences in mortality between the test item treatments and the negative control. Dunnett's Multiple Comparison Test was used to determine statistically significant differences in body weight of surviving bees between the test item treatments and negative control.

Based on the mortality data and measured diet concentration, the 10-day LC/LD<sub>10</sub> values were determined using linear interpolation. The model was not able to generate meaningful confidence intervals for either of these values and both are considered limited in terms of accuracy. Consequently, caution should be used when applying these values to a risk assessment framework.

CETIS Version 1.8 (Ives, 2013) was used to determine the NOEC/NOED, LOEC/LOED, LC/LD<sub>x</sub>, and EC/ED<sub>x</sub> values, where possible.

# **II. RESULTS AND DISCUSSION**

## **A. BIOLOGICAL RESULTS**

There were no significant differences in mortality between the test item treatments and the negative control. A summary of the mortality data is presented in Table 10.3.1.2/01-1 and a summary of endpoints is presented in Table 10.3.1.2/01-2.

There was a significant reduction in body weight among honeybees exposed to the 2.3 and 23 µg a.s./bee/day treatments. Due to a lack of clear dose response and the low inhibition observed at the reference dose rates (8.73 and 8.46%, respectively), this effect was not considered to be treatment related. A summary of the body weight data is presented in Table 10.3.1.2/01-1 and a summary of endpoints is presented in Table 10.3.1.2/01-2.

**Table 10.3.1.2/01-1: Mean mortality and body weight after 10 days exposure**

Calculated mean daily dose (µg a.s./bee/day)	% Mortality Mean ± SD	Body weight (g) Mean ± SD
Negative control	7 ± 6	0.0982 ± 0.0050
Solvent control	0 ± 0	0.0923 ± 0.0087
2.3	0 ± 0	0.0896* ± 0.0008
5.5	7 ± 12	0.0918 ± 0.0029
10	10 ± 10	0.1002 ± 0.0020
23	10 ± 10	0.0899* ± 0.0036
45	17 ± 15	0.0959 ± 0.0063

SD: Standard deviation

\* Significantly reduced compared to negative control, based on Dunnett's Multiple Comparison Test. Due to a lack of clear dose response and the low inhibition observed at the reference dose rates, this effect is not considered to be treatment related.

**Table 10.3.1.2/01-2: Summary of endpoints**

Endpoint	Measured diet concentration (µg a.s./mL)	Calculated mean daily dose (µg a.s./bee/day)
<b>Survival</b>		
NOEC/NOED	1900	45
LOEC/LOED	> 1900	> 45
LC <sub>10</sub> /LD <sub>10</sub> (95% CI)	1300 (n.d.)	31 (n.d.)
LC <sub>20</sub> /LD <sub>20</sub> (95% CI)	> 1900 (n.a.)	> 45 (n.a.)
LC <sub>50</sub> /LD <sub>50</sub> (95% CI)	> 1900 (n.a.)	> 45 (n.a.)
<b>Weight of surviving bees</b>		
NOEC/NOED	1900	45
LOEC/LOED	> 1900	> 45
EC <sub>10</sub> /ED <sub>10</sub> (95% CI)	> 1900 (n.a.)	> 45 (n.a.)
EC <sub>20</sub> /ED <sub>20</sub> (95% CI)	> 1900 (n.a.)	> 45 (n.a.)
EC <sub>50</sub> /ED <sub>50</sub> (95% CI)	> 1900 (n.a.)	> 45 (n.a.)

CI: Confidence intervals; n.a.: not applicable (LC/LD and EC/ED values were empirically estimated; therefore, 95% confidence intervals could not be determined). n.d.: not determined (no appropriate model tested generated meaningful confidence intervals. Caution should be used when applying these values to a risk assessment framework).

## B. FOOD CONSUMPTION

To more accurately reflect the exposure concentrations, the measured diet concentrations and actual amount of food consumed daily coupled with bee survival were used to calculate mean daily dose rates. The calculated mean daily dose rates and corresponding mean accumulated dose rates are presented in Table 10.3.1.2/01-3 below.

**Table 10.3.1.2/01-3: Food consumption, mean measured dietary concentrations, calculated mean daily dose and accumulated dose**

Nominal mean daily dose (µg a.s./bee/day)	Overall mean daily consumption of food solution (mg/bee/day)	Mean measured diet concentration (µg a.s./mL)	Calculated mean daily dose (µg a.s./bee/day) <sup>a)</sup>	Mean accumulated dose (µg a.s./bee)
Negative control	27	n.a.	n.a.	n.a.
Solvent control	28	n.a.	n.a.	n.a.
6.3	25	110	2.3	23
13	27	250	5.5	55
25	28	460	10	100
50	29	990	23	230
100	29	1900	45	450

n.a.: not applicable.

<sup>a)</sup> Adjusted dose (µg a.s./bee/day) = mean diet consumed (mg/bee/day) ÷ sucrose density (1232.02 mg/mL) × mean measured concentration (µg a.s./mL) ÷ 1000 mL/L. Daily honeybee diet consumption is corrected for average, daily evaporative loss.

### C. ANALYTICAL RESULTS

Analysis of the stock solution (100 mg a.s./mL) resulted in a measured concentration of 99% of nominal concentration. This result established that the appropriate amount of mandestrobin was added to the stock solution.

The results of the analysis of the sucrose solution diets for mandestrobin concentration are presented in Table 10.3.1.2/01-4. Measured diet concentrations maintained the expected concentration gradient over the 10-day period. Mean measured diet concentrations ranged from 36 to 40% of nominal concentrations.

**Table 10.3.1.2/01-4: Measured concentrations of mandestrobin in sucrose solution diets**

Nominal dose (µg a.s./bee/day)	Nominal diet concentration (µg a.s./mL)	Measured dietary concentration (µg a.s./mL)				% of nominal <sup>a)</sup>
		Day 3	Day 6	Day 8	Mean measured <sup>a)</sup>	
Negative control	0	< MDL	< MDL	< MDL	n.a.	n.a.
Solvent control	0	< MDL	< MDL	< MDL	n.a.	n.a.
6.3	320	64	140	140	110	36
13	630	160	300	300	250	40
25	1300	300	560	530	460	36
50	2500	610	1200	1200	990	40
100	5000	1200	2400	2200	1900	39

MDL: Method detection limit (20 µg a.s./mL); n.a.: not applicable.

<sup>a)</sup> Calculated based on the original raw data, not from the rounded values presented in this table.

### D. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (OECD 245, 2017) as detailed below:

- The average mortality across replicates for the untreated control and solvent control groups should be ≤ 15% at the end of the test. Mean mortality in the negative and solvent control groups was 7% and 0%, respectively.
- The average mortality in the reference substance treated group is ≥ 50% at the end of the test. Mortality in the reference item treatment was 100% at the end of the test.

### III. CONCLUSION

The chronic oral toxicity of mandestrobin to the honeybee (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test.

The 10-day NOED values for survival and body weight were both determined to be 45 µg a.s./bee/day. The 10-day NOEC for the same parameters was determined to be 1900 µg a.s./mL diet.

The 10-day LD<sub>10/20/50</sub> values for survival were determined to be 31, > 45 and > 45 µg a.s./bee/day, respectively. The 10-day ED<sub>10/20/50</sub> values for body weight were all determined to be > 45 µg a.s./bee/day.

The 10-day LC<sub>10/20/50</sub> values for survival were determined to be 1300, > 1900 and > 1900 µg a.s./mL diet, respectively. The 10-day EC<sub>10/20/50</sub> values for body weight were all determined to be > 1900 µg a.s./mL diet.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>LDD<sub>50</sub>: &gt; 45 µg a.s./bee/day</p> <p>NOEDD: 45 µg a.s./bee/day</p>
Comments of zRMS:	The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal.

#### A 2.3.1.2.2 Study 2

<b>Data point:</b>	KCP 10.3.1.2/02
<b>Report author:</b>	Noël, E.
<b>Report year:</b>	2016
<b>Report title:</b>	S-2200 25 SC - A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae).
<b>Report No.:</b>	036SRFR15C01
<b>Document No.:</b>	ROW-0099
<b>Guidelines followed in study:</b>	Draft OECD 245 (2016).
<b>Deviations from current test guideline:</b>	Compared to OECD 245 (2017): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic oral toxicity of S-2200 25SC to the honeybee (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test. The test item was administered in the diet at five nominal concentrations between 76.8 and 3000 mg product/kg sucrose solution. The test item was dispersed in sucrose solution and offered with feeders renewed each day. An untreated sucrose solution control was tested in parallel. Dimethoate was used as a toxic reference item at a single concentration of 1 mg a.s./L sucrose solution. Three replicates were set up per test item treatment and control, each containing ten bees (30 bees per treatment group). Observations for mortality and behavioural abnormalities were performed daily. Food consumption was determined by weighing feeders before and after they were offered to the bees.

There were no significant effects on mortality of bees in the test item treatments and no behavioural abnormalities were observed. The 10-day NOEDD was determined to be 65.8 µg product/bee/day (16.2 µg a.s./bee/day). The LDD<sub>10/20/50</sub> values were all determined to be > 65.8 µg product/bee/day (> 16.2 µg a.s./bee/day), based on actual consumption of the test solution.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** S-2200 25SC  
**Description:** White opaque liquid with traces of oil on the top  
**Lot/Batch:** C09-5L301  
**A.s. content:** 250 g/L (nominal); 24.63% w/w (analysed)
2. **Control:** Untreated 50% (w/v) sucrose solution
3. **Reference item:** Dimethoate (400 g/L)

### B. STUDY DESIGN AND METHODS

1. **Test organism:** Honeybee (*Apis mellifera* L.)  
**Age:** 2 day old emerged adult worker bees  
**Source:** In-house culture  
**Diet:** 50% (w/v) sucrose solution
2. **Test units:** 350 cm<sup>3</sup> stainless steel cage with a removable glass sheet as front side. A hole on the top of the cage allow the introduction of a syringe (sucrose feeder) containing the test solutions.
3. **Environmental conditions:**  
**Temperature:** 31.8 – 34.3°C  
**Relative humidity:** 52.2 – 69.8%  
**Photoperiod:** 24-hour darkness

#### 4. Animal assignment and treatment:

The chronic oral toxicity of S-2200 25SC to the honeybee (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test. The test item was administered in the diet at five nominal concentrations of 76.8, 192, 480, 1200 and 3000 mg product/kg sucrose solution. The test item was dispersed in sucrose solution and offered with feeders containing 2 mL test solution, renewed each day. An untreated sucrose solution control was tested in parallel. Dimethoate was used as a toxic reference item at a single concentration of 1 mg a.s./L sucrose solution. Three replicates were set up per test item treatment and control, each containing ten bees (30 bees per treatment group).

Bees were fed *ad libitum* with the treated sugar solutions. Feeders were weighed before and after they were offered, so that the dose consumed could be obtained by comparison of the weight of the remaining solution with the initial one. The individual daily consumption was corrected by the number of surviving bees.

#### 5. Dose preparation:

A stock solution was prepared with an initial concentration of 9.015 g a.s./L which was used to prepare the feeding solutions for each treatment level.

#### 6. Measurements and observations:

Observations for mortality and behavioural abnormalities were performed daily. Behavioural abnormalities were assessed according to the following categories:

- M: Moribund. Bees cannot walk and show only very feeble movements of legs and antennae; only weak response to stimulation, e.g. light.
- A: Affected. Bees still upright and attempting to walk but showing signs of reduced coordination, hyperactivity, aggressiveness, increased self-cleaning behaviour, rotations, shivering.
- C: Cramps. Bees contracting abdomen or entire body.
- AP: Apathy. Bees show only low or delayed reactions to stimulation e.g. light, bees are sitting motionless in the unit.
- V: Vomiting.

Feeders were weighed before and after they were offered, so that the dose consumed could be obtained by comparison of the weight of the remaining solution with the initial one. The individual daily consumption was corrected by the number of surviving bees.

The concentration of the test substance were confirmed by analytical verification of the lowest and highest concentrations of the final feeding solutions as well as the stock solution. This was done once during the experimental phase. Analysis was performed by liquid chromatography using a reverse phase column and a UV detector.

Environmental conditions (temperature and relative humidity) were recorded daily.

## **7. Statistics:**

Results were analysed using the non-parametric statistical test Kruskal-Wallis ( $p \leq 0.05$ ) plus Conover for comparison between the distilled water control and the test item.

# **II. RESULTS AND DISCUSSION**

## **A. BIOLOGICAL RESULTS**

There were no significant differences in mortality between the test item treatments and the negative control. A summary of the mortality data is presented in Table 10.3.1.2/02-1 along with a summary of endpoints and consumed doses.

Some bees were recorded as “affected” during the exposure; however, these were all in the reference treatment. No bees in the test item treatments showed any signs of behavioural abnormalities.

**Table 10.3.1.2/02-1: Mean consumed doses and mortality after 10 days exposure**

Assessment			Mortality (Day 10) (%) <sup>c)</sup>
Target concentrations (mg product/kg sucrose solution) <sup>a)</sup>	Consumed doses (µg product/bee/day) <sup>b)</sup>	Consumed doses (µg a.s./bee/day) <sup>b)</sup>	
Control	-	-	0.0
76.8	1.64	0.40	0.0 (n.s.)
192	3.84	0.95	3.33 (n.s.)
480	10.7	2.64	3.33 (n.s.)
1200	22.0	5.42	0.0 (n.s.)
3000	65.8	16.2	0.0 (n.s.)
Endpoints			
NOEC		3000 mg product/kg (738.9 mg a.s./kg)	
NOEDD		65.8 µg product/bee/day (16.2 µg a.s./bee/day)	
LOEC		> 3000 mg product/kg (> 738.9 mg a.s./kg)	
LOEDD		> 65.8 µg product/bee/day > 16.2 µg a.s./bee/day	
LC <sub>10/20/50</sub>		> 3000 mg product/kg (> 738.9 mg a.s./kg)	
LDD <sub>10/20/50</sub>		> 65.8 µg product/bee/day > 16.2 µg a.s./bee/day	

<sup>a)</sup> Based on a sucrose solution density of 1.22 g/mL.

<sup>b)</sup> Based on actual consumption of the test item solution and target solution concentration.

<sup>c)</sup> Based on the number of dead organisms. Since there was no control mortality, uncorrected values are presented.

n.s. not significantly different from the untreated sucrose solution control.

## B. ANALYTICAL RESULTS

The stock solution and the highest solution concentrations were within the required range of 80 –120% of the target concentrations. The lowest solution concentration was not within the required range of 80 – 120% of the target concentrations. Since there was no significance difference between the mortality at the highest concentration and the untreated control, there are no impacts of this deviation on the study results. The results of the analysis are presented in Table 10.3.1.2/02-2.

**Table 10.3.1.2/02-2: Summary of analytical results**

Specimen	Initial mandestrobin concentration in treated solution (g a.s./L)	Mean mandestrobin content in treated solution (g a.s./L)	Deviation from initial concentration (%)
Stock solution	9.015	8.845	-1.9
Highest nominal concentration	0.9015	0.8342	-7.5
Lowest nominal concentration	0.0231	0.0178	-22.9

## C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (OECD 245, 2017) as detailed below:

- The average mortality across replicates for the untreated control and solvent control groups should be ≤ 15% at the end of the test. Mean mortality in the control group was 0% at the end of the test.



- The average mortality in the reference substance treated group is  $\geq 50\%$  at the end of the test. Mortality in the reference item treatment was 100% at the end of the test.

### III. CONCLUSION

The chronic oral toxicity of S-2200 25SC to the honeybee (*Apis mellifera*) was determined in the laboratory during a 10-day feeding test.

There were no significant effects on mortality of bees in the test item treatments and no behavioural abnormalities were observed. The 10-day NOEDD was determined to be 65.8 µg product/bee/day (16.2 µg a.s./bee/day). The LDD<sub>10/20/50</sub> values were all determined to be > 65.8 µg product/bee/day (> 16.2 µg a.s./bee/day), based on actual consumption of the test solution.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>LDD<sub>10/20/50</sub>: &gt; 65.8 µg product/bee/day (&gt; 16.2 µg a.s./bee/day)</p> <p>NOEDD: 65.8 µg product/bee/day (16.2 µg a.s./bee/day)</p>
Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met:</p> <ul style="list-style-type: none"> <li>• average cumulative mean mortality was &lt; 15% in the untreated sucrose solution control; (actual value: 0.0%),</li> <li>• toxic reference cumulative mean mortality was &gt; 50 in the toxic reference, (actual value: 100%).</li> </ul> <p>The following endpoints were derived:</p> <ul style="list-style-type: none"> <li>• NOEC = 3000 mg f.p./kg (738.9 mg a.s./kg),</li> <li>• LOEC &gt;3000 mg f.p./kg (&gt;738.9 mg a.s./kg),</li> <li>• the LC<sub>10</sub>, LC<sub>20</sub> and LC<sub>50</sub> was estimated to be &gt;3000 mg f.p./kg (&gt;738.9 mg a.s./kg),</li> <li>• NOEDD = 65.8 µg f.p./bee/day (16.2 µg a.s./bee/day),</li> <li>• LOEDD &gt; 65.8 µg f.p./bee/day (16.2 µg a.s./bee/day),</li> </ul> <p>LDD<sub>10</sub>, LDD<sub>20</sub> and LDD<sub>50</sub> &gt; 65.8 µg f.p./bee/day (16.2 µg a.s./bee/day),</p>

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

#### A 2.3.1.3.1 Study 1

<b>Data point:</b>	KCP 10.3.1.3/01
<b>Report author:</b>	Picard, C.R.
<b>Report year:</b>	2018b
<b>Report title:</b>	S-2200 (Mandestrobin): Honey Bee ( <i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure
<b>Report No.:</b>	201800143 / 12709.6460
<b>Document No.:</b>	ROW-0100
<b>Guidelines followed in study:</b>	OECD Guideline 239 (2016)
<b>Deviations from current test guideline:</b>	Compared to OECD Guideline 239 (2021): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The chronic toxicity of mandestrobin to larvae of the honeybee (*Apis mellifera* L.) was determined in the laboratory over 22 days. The test item was administered in the diet at five nominal concentrations between 40 and 630 µg a.s./g diet, equivalent to dose rates between 6.3 and 100 µg a.s./larva. An untreated (negative) control and solvent (acetone) control were tested in parallel. Dimethoate was used as a toxic reference item at a single nominal cumulative dose of 7.4 µg dimethoate/larva. For each treatment group, 36 test organisms from three different hives were tested. A replicate was considered to be an individual larva/bee since they were reared in an individual cell. Larvae were observed daily for mortality and health. Survival of pupae was first checked on day 15. From this day, emergence of adults was recorded. Each bee was weighed individually at test termination.

The 8-day NOED for larval mortality was determined to be 100 µg a.s./larva. The LD<sub>10</sub> was determined to be 56 µg a.s./larva, and the LD<sub>20</sub> and LD<sub>50</sub> were both determined to be > 100 µg a.s./larva.

The 22-day NOED for pupal mortality was determined to be 100 µg a.s./larva. The LD<sub>10/20/50</sub> values were all determined to be > 100 µg a.s./larva.

The 22-day NOED for adult emergence was determined to be 100 µg a.s./larva. The LD<sub>10</sub> was determined to be 36 µg a.s./larva, and the LD<sub>20/50</sub> values were both determined to be > 100 µg a.s./larva.

For live weight of adults at emergence, the NOED was 50 µg a.s./larva. A significant reduction was observed at the highest treatment group. However, the percentage difference from the control was relatively low at 8.5% (PMSD for analysis was 8.1%), suggesting that the difference may be within the range of biological variability and not an ecologically relevant effect. The ED<sub>10/20/50</sub> were all determined to be > 100 µg a.s./larva.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** S-2200 Technical (mandestrobin)  
**Description:** White powder  
**Lot/Batch:** 21201  
**Purity:** 93.7% w/w

- 2. Controls:** Negative control: untreated diet  
Solvent control: diet containing acetone

- 3. Reference item:** Dimethoate (purity not reported)

## B. STUDY DESIGN AND METHODS

- 1. Test organism:** Honeybee (*Apis mellifera* L.)  
**Age:** ≤ 24-hour-old larvae at initiation of acclimation  
**Source:** Wood's Beekeeping Supply, Lincoln, Rhode Island, USA  
**Acclimation:** 2 days  
**Diet:** Refer to Table 10.3.1.3/01-1.
- 2. Test units:** The larval culture and test vessels were sterile, 48-well cell culture plates (1.6 mL/well) containing a plastic queen cup grafting cell in the 32 wells containing larva during acclimation and in 18 wells during exposure. The perimeter wells within each plate were partially filled with deionised water to assist in maintaining the relative humidity at > 90%.  
The pupation plates were sterile, 24-well cell culture plates (3.4 mL/well) each containing two layers of sterilised dust-free Kimwipes.
- 3. Environmental conditions:**  
**Temperature:** Larval phase (days 1 – 8): 34°C  
Pupal phase (days 9 – 22): 34 – 35°C  
**Relative humidity:** Larval phase (days 1 – 8): 95 – 99%  
Pupal phase (days 9 – 22): 58 – 81%  
**Photoperiod:** Darkness except during observations and feeding (approximately 30 minutes laboratory lighting per day).

## 4. Animal assignment and treatment:

The chronic toxicity of mandestrobin to larvae of the honey bee (*Apis mellifera* L.) after repeated feeding exposure was determined in the laboratory over 22 days. The study was conducted as a dose response test with a duration of 22 days from grafting on day 1 to the final assessment on day 22. It comprised of a negative control group, a solvent (acetone) control group and five test item groups with nominal dietary concentrations of 40, 83, 160, 310 and 630 µg a.s./g diet, corresponding to cumulative doses of 6.3, 13, 25, 50 and 100 µg a.s./larva. For each treatment group, 36 test organisms from three different hives were tested. A replicate was considered to be an individual larva/bee since they were reared in an individual cell.

Dimethoate was used as a reference toxicant at a single nominal cumulative dose of 7.4 µg a.s./larva. Dimethoate is known to be toxic to honeybee larvae; therefore, the reference test was terminated at the end of the larval phase (day 8).

The 22-day test was initiated by the exposure of third instar larvae to mandestrobin in a treated diet which results in both dermal and oral exposure until pupation (typically day 7). After pupation (non-feeding stage), test organisms were allowed to complete development to adult emergence.

Prior to test initiation, frames containing the isolated brood cells were removed from each hive, adult bees were removed from each frame, and the frames were brought into the laboratory. Frames containing newly hatched larvae were placed in a grafting chamber maintained at a temperature of 28°C. First instar (L1) synchronised larvae were removed from brood cells by placing via grafting.

The initial phase involved a 2-day acclimation period. Three untreated diets were prepared prior to test initiation and were stored frozen until use. Diets were prepared as shown in Table 10.3.1.3/01-1. Larvae were fed 20 µL of untreated diet A (untreated) on the day of transfer into the cell plates (day 1) and were

not fed on day 2. On feeding days, an aliquot of each diet was brought to test temperature by placing within the test incubator before being treated and added to the plate wells. The exposure phase was initiated on day 3, when the appropriate treated, solvent control, or control diet B was added to the larval cell plates.

On day 3, individual larvae in all plates were fed 20 µL of the appropriate treated diet B; on days 4, 5 and 6, respectively, all plates were fed 30, 40 and 50 µL of the appropriate treated diet C. Larvae that were observed to completely consume their diet on day 7 or 8 were transferred to the appropriate pupation plates until day 22.

**Table 10.3.1.3/01-1: Diet components**

Component	Diet A <sup>a)</sup>	Diet B <sup>b)</sup>	Diet C <sup>c)</sup>
Deionised water (mL)	398.7	860	480
D-glucose (g)	47.7	128	144
D-fructose (g)	47.7	128	144
Yeast extract (g) <sup>d)</sup>	8.1	26.0	32
Royal jelly (g)	398.7	860	800

<sup>a)</sup> Diet fed on day 1.

<sup>b)</sup> Used for treated diet fed on exposure day 3.

<sup>c)</sup> Used for treated diet fed on exposure days 4, 5 and 6.

<sup>d)</sup> Yeast extract is made from *Saccharomyces cerevisiae* species of yeast.

## 5. Dose preparation:

A 150 mg a.s./mL primary stock solution was prepared by bringing 8.0767 g of mandestrobin (7.5679 g as active ingredient) to a volume of 50 mL with acetone. The resulting primary stock solution was observed to be clear and slightly yellow in colour with no visible undissolved test substance following 30 seconds of sonication and 30 minutes of magnetic stirring. The primary stock was used to prepare the dosing stocks and was also used as a dosing stock to prepare the diet for the highest treatment level.

All dosing stock solutions were mixed by 15 seconds of sonication and one hour of magnetic stirring and were observed to be clear and colourless with no visible undissolved test substance following preparation. Treated diets were prepared individually.

All treated royal jelly diets appeared opaque and yellow in colour with no visible particulates following generally 30 seconds of sonication with a minimum of 45 minutes of mixing using a magnetic stir plate, sonication for approximately fifteen seconds followed by several manual shakes and inversions of the vessels. Diets were brought to test temperature before feeding. The solvent control diet contained an equivalent amount of untreated acetone (0.42%) as each treated diet. Untreated diet was used for the negative control.

## 6. Measurements and observations:

The health of the larvae was observed and recorded daily. Death of a larva was defined by lack of movement. Diet in the wells was observed on days 7 and 8, and larvae that had completely consumed their diet were transferred to pupal plates. The number of larvae transferred on days 7 and 8 to pupal plates was recorded. Survival of pupae was first checked on day 15 to avoid disturbing the fragile pre-pupal stage earlier. Larvae that failed to develop into pupae by day 15 were classified as dead.

Starting on day 15, the number of emerged adults each day was recorded. At the time of emergence, each adult bee was removed from the well plate and individually weighed.

The test was terminated on day 22. At test termination, after health observations and remaining individual bee weights were recorded, any remaining organisms were discarded appropriately. Pupae that had not emerged as adults by day 22 were considered dead.

Temperature and relative humidity were monitored continuously during the study.

On days 3, 4, 5, and 6 of the exposure, a sample of each prepared diet (diets B and C) as well as the negative control and solvent control were collected and analysed for mandestrobin concentrations. A sample of the stock solutions were also collected on day 3 and analysed for mandestrobin concentrations. Results of these analyses were used to confirm that the stock solutions and treated diets were correctly prepared and to quantify actual exposure concentrations. Analysis was performed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

## **7. Statistics:**

The solvent control and negative control data for each endpoint were compared using Fisher's Exact Test for percent survival/emergence and Equal Variance t-Test for adult weight data. No significant difference was observed between the controls; therefore the test item treatments were compared to the negative control.

The treatment data was tested for normality using either the Shapiro-Wilks' Test or Anderson-Darling's Test, and for homogeneity of variance using the Bartlett's Test or Levene's Test.

The Fisher's Exact Test with Bonferroni Holm's Adjustment was used to determine statistically significant differences in larval mortality (day 8) and pupal mortality (day 22) between the test item treatments and the negative control. Wilcoxon's Test with Bonferroni-Holm's Adjustment was used to determine statistically significant differences in adult weight of treatment bees compared to the negative control bees.

CETIS Version 1.8 (Ives, 2013) was used to determine the NOEC/NOED, LOEC/LOED, LC/LD<sub>x</sub>, and EC/ED<sub>x</sub> values, where possible.

## **II. RESULTS AND DISCUSSION**

### **A. BIOLOGICAL RESULTS**

Data for larval and pupal survival are shown in Table 10.3.1.3/01-2. There was no significant reduction in larval survival (day 8) or pupal survival (day 22) among honeybees exposed up to mandestrobin at any treatment level compared to the negative control. Endpoints are shown in Table 10.3.1.3/01-3.

Data on mean adult emergence (days 3 to 22) are shown in Table 10.3.1.3/01-2. There was no significant reduction in adult emergence among honeybees exposed to mandestrobin at any treatment level compared to the negative control. Endpoints are shown in Table 10.3.1.3/01-3.

Mean weight data for adults at emergence is shown in Table 10.3.1.3/01-2. There was a significant reduction in live weight for adults at emergence among honeybees exposed to the 100 µg a.s./larva treatment compared to the negative control. However, the percent difference from the negative control was relatively low at 8.5% (PMSD for analysis was 8.1%) suggesting that the difference may be within the range of biological variability and not an ecologically relevant effect. This should be taken into account when applying the effect values to a risk assessment framework. Endpoints are shown in Table 10.3.1.3/01-3.

**Table 10.3.1.3/01-2: Summary of the percent survival, adult emergence and adult weight at emergence**

Nominal diet concentration (µg a.s./g diet)	Nominal cumulative dose (µg a.s./larva)	8-Day Larval Survival (%) <sup>a)</sup>	22-Day Pupal Survival (%) <sup>b)</sup>	22-Day Adult Emergence (%) <sup>c)</sup>	Adult Weight at Emergence (g)
Negative control	0	97	91	89	0.1041
Solvent control	0	94	91	86	0.0993
40	6.3	89	81	72	0.1029
83	13	100	89	89	0.1046
160	25	94	91	86	0.0973
310	50	86	90	78	0.0984
630	100	81	93	75	0.0952*

<sup>a)</sup> Based on observations from days 3 to 8.

<sup>b)</sup> Based on observations from days 8 to 22 (termination).

<sup>c)</sup> Based on observations from days 3 to 22 (termination).

\* Significantly reduced compared to the negative control, based on Wilcoxon's Test with Bonferonni-Holm's Adjustment. While this was a statistically significant reduction, the percent difference from the control was relatively low at 8.5% (PMSD for analysis was 8.1%), suggesting that the difference may be within the range of biological variability and not an ecologically relevant effect.

**Table 10.3.1.3/01-3: Summary of endpoints**

Endpoint	Nominal cumulative dose (µg a.s./larva)				
	NOED	LOED	LD <sub>10</sub> /ED <sub>10</sub> (95% CI)	LD <sub>20</sub> /ED <sub>20</sub> (95% CI)	LD <sub>50</sub> /ED <sub>50</sub> (95% CI)
8-day larval survival	100	> 100	56 (26 – 89)	> 100 (n.d.)	> 100 (n.d.)
22-day pupal survival	100	> 100	> 100 (n.d.)	> 100 (n.d.)	> 100 (n.d.)
22-day adult emergence	100	> 100	36 (n.d.)	> 100 (n.d.)	> 100 (n.d.)
Adult weight at emergence	50*	100	> 100 (n.d.)	> 100 (n.d.)	> 100 (n.d.)

CI: Confidence interval; n.d.: not determined.

Note: point estimates with greater than values were empirically estimated as no treatment group resulted in a reduction of ≥ 10, 20 or 50% for the specified endpoint or the dose response was not appropriate for the calculation of point estimates.

\* A significant reduction was observed at the highest treatment group. While this was a statistically significant reduction, the percent difference from the control was relatively low at 8.5% (PMSD for analysis was 8.1%), suggesting that the difference may be within the range of biological variability and not an ecologically relevant effect.

## B. ANALYTICAL RESULTS

Analysis of the stock solutions (9.5, 20, 38, 74, and 150 mg a.s./mL) resulted in measured concentrations ranging from 75 to 120% of nominal concentrations. These results established that the appropriate amount of mandestrobin was added to each stock solution.

The results of the analysis of the royal jelly diets for mandestrobin concentration on day 3, 4, 5 and 6 are presented in Table 10.3.1.3/01-4. Measured diet concentrations were generally consistent over time and maintained the expected concentration gradient over this 4-day period. Mean measured diet concentrations ranged from 76 to 110% of nominal concentrations. All biological endpoints were based on both nominal diet concentrations and dose.

**Table 10.3.1.3/01-4: Measured concentrations of mandestrobin in royal jelly diets**

Nominal cumulative dose (µg a.s./larva)	Nominal cumulative diet concentration (µg a.s./g diet)	Measured dietary concentration (µg a.s./g diet)					% of nominal <sup>a)</sup>
		Day 3 <sup>b)</sup>	Day 4 <sup>c)</sup>	Day 5 <sup>c)</sup>	Day 6 <sup>c)</sup>	Mean measured <sup>a)</sup>	
Negative control	0	< MDL	< MDL	< MDL	< MDL	n.a.	n.a.
Solvent control	0	< MDL	< MDL	< MDL	< MDL	n.a.	n.a.
6.3	40	45	45	42	42	44	110
13	83	66	58	64	63	63	76
25	160	160	180	170	160	170	110
50	310	340	290	330	310	320	100
100	630	690	690	660	630	670	110

MDL: Method detection limit (13 µg a.s./g diet); n.a.: not applicable.

<sup>a)</sup> Calculated based on the original raw data, not from the rounded values presented in this table.

<sup>b)</sup> Diet B was fed on day 3.

<sup>c)</sup> Diet C was fed on days 4, 5 and 6.

## C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guidelines (OECD Guidance Document 239, 2021) as detailed below:

- In the control plate(s), cumulative larval mortality from day 3 to day 8 should be ≤ 15% across all replicates. Larval mortality in the control and solvent control was 3 and 6%, respectively.
- In the control plate(s), the adult emergence rate on day 22 should be ≥ 70% across all replicates. Emergence in the control and solvent control was 89% and 86%, respectively.
- Positive control: if dimethoate is used, larval mortality should be ≥ 50% on day 8 across all replicates. Larval mortality in the 7.4 µg a.s. dimethoate/larva treatment was 89%.

## III. CONCLUSION

The chronic toxicity of mandestrobin to larvae of the honeybee (*Apis mellifera* L.) was determined in the laboratory during a 22-day study.

The 8-day NOED for larval mortality was determined to be 100 µg a.s./larva. The LD<sub>10</sub> was determined to be 56 µg a.s./larva, and the LD<sub>20</sub> and LD<sub>50</sub> were both determined to be > 100 µg a.s./larva.

The 22-day NOED for pupal mortality was determined to be 100 µg a.s./larva. The LD<sub>10/20/50</sub> values were all determined to be > 100 µg a.s./larva.

The 22-day NOED for adult emergence was determined to be 100 µg a.s./larva. The LD<sub>10</sub> was determined to be 36 µg a.s./larva, and the LD<sub>20/50</sub> values were both determined to be > 100 µg a.s./larva.

For live weight of adults at emergence, the NOED was 50 µg a.s./larva. A significant reduction was observed at the highest treatment group. However, the percentage difference from the control was relatively low at 8.5% (PMSD for analysis was 8.1%), suggesting that the difference may be within the range of biological variability and not an ecologically relevant effect. The ED<sub>10/20/50</sub> were all determined to be > 100 µg a.s./larva.

Assessment and Conclusion by Applicant:	The study has been performed to current standards and is considered to be fully valid.
---	--

	<p>The relevant endpoints derived from the study are:</p> <p>NOED (8-day larval mortality): 100 µg a.s./larva/dev. period  NOED (22-day pupal mortality): 100 µg a.s./larva/dev. period  NOED (22-day adult emergence): 100 µg a.s./larva/dev. period</p> <p>An NOED (adult weight) of 50 µg a.s./larva/dev. period was also reported. Although there was a statistically significant difference in body weight of bees at 100 µg a.s./larva/dev period when compared to the negative control, the percentage difference from the control was relatively low (8.5%), suggesting that the difference may be within the range of biological variability and not ecologically relevant. Moreover, this endpoint for adult weight is not a required endpoint in OECD GD 239 and is not relevant to the risk assessment scheme in the EU.</p> <p><b>Therefore, the most relevant endpoint recommended for use in the risk assessment is:</b>  <b>100 µg a.s./larva/dev. period, the NOED for all other measured parameters.</b></p>
--	--

Comments of zRMS:	The study was not evaluated as it was performed with active substance. The study should be submitted and evaluated at the EU level during active substance renewal
-------------------	--

#### A 2.3.1.3.2 Study 2

<b>Data point:</b>	KCP 10.3.1.3/02
<b>Report author:</b>	Aguilar-Alberola, J.A.
<b>Report year:</b>	2019
<b>Report title:</b>	S-2200 (Mandestrobin) 25SC: Honey Bee ( <i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions
<b>Report No.:</b>	S18-05345
<b>Document No.:</b>	ROW-0101
<b>Guidelines followed in study:</b>	OECD Guidance Document 239 (2016)
<b>Deviations from current test guideline:</b>	<p>Compared to OECD Guidance Document 239 (2021):</p> <ul style="list-style-type: none"> <li>- The temperature during days 15 to 22 was below the minimum value established in the test guidance (34°C) several times reaching a minimum of 33.6°C. This minor deviation is not considered to have impacted the validity or reliability of the study, as the validity criteria were fulfilled.</li> <li>- The records of relative humidity outside the established intervals in the test guidance were given when the desiccators were opened and in no case exceeded 30 minutes. Therefore, these incidents are not considered deviations.</li> </ul>
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes



## Executive Summary

The chronic toxicity of S-2200 25SC to larvae of the honeybee (*Apis mellifera* L.) was determined in the laboratory over 22 days. The test item was administered in the diet at five nominal concentrations between 166.27 and 2660.57 mg product/kg diet (40.58 to 649.35 mg a.s./kg diet), equivalent to 25.61, 51.22, 102.43, 204.86 and 409.73 mg test item/larva (6.25, 12.5, 25, 50 and 100 µg a.s./larva). An untreated control was tested in parallel. Dimethoate was used as a toxic reference item at a single nominal concentration of 48.0 mg a.s./kg diet (7.39 µg dimethoate/larva). Each treatment group comprised of 48 larvae from three different colonies (each colony representing one replicate). Larvae were observed for mortality on days 4, 5, 6, 7 and 8. Survival of pupae was assessed on day 15 and emergence was recorded on day 22. Each bee was weighed individually at test termination.

The 8, 15 and 22-day NOED for mortality and adult emergence were determined to be 409.73 µg product/larva (equivalent to 100 µg a.s./larva). The ED<sub>10/20/50</sub> were empirically estimated to be > 409.73 µg product/larva (> 100 µg a.s./larva).

## I. MATERIALS AND METHODS

### A. MATERIALS

- 1. Test material:** S-2200 25SC  
**Description:** Liquid with traces of oil on the top  
**Lot/Batch:** C09-5F102G  
**A.s. content:** 25.70% w/v
- 2. Control:** Untreated diet
- 3. Reference item:** BAS 152 I (dimethoate, 99.7%)

### B. STUDY DESIGN AND METHODS

- 1. Test organism:** Honeybee (*Apis mellifera* L.)  
**Age:** 1<sup>st</sup> instar (L1), ≤ 30 hours old  
**Source:** Commercial beehives from an in-house test facility stock  
**Acclimation:** 2 days  
**Diet:** Refer to Table 10.3.1.3/02-1.
- 2. Test units:** Sterilised crystal polystyrene grafting cells with 9 mm of diameter. Each cell was placed into a well of a sterile 48-well cell culture plate placed in a Plexiglas desiccator containing a dish with saturated K<sub>2</sub>SO<sub>4</sub>. On day 8, the plates were transferred to another Plexiglas desiccator containing a dish with saturated NaCl. On day 15, each plate was transferred into an emergence box in an incubator.
- 3. Environmental conditions:**  
**Temperature:** Larval phase (days 1 - 8): 34.0 – 34.9°C  
Pupal phase (days 8 - 22): 32.0 – 34.5°C  
**Relative humidity:** Larval phase (days 1 - 8): 53.9 - 100%  
Pupal phase (days 8 - 22): 43.9 – 85.0%  
**Photoperiod:** Constant darkness except during feeding and assessments.
- 4. Animal assignment and treatment:**

The chronic toxicity of S-2200 25SC to larvae of the honey bee (*Apis mellifera* L.) after repeated feeding exposure was determined in the laboratory over 22 days. The study was conducted as a dose response test with a duration of 22 days from grafting on day 1 to the final assessment on day 22. It comprised of a negative control group, and five test item groups with nominal dietary concentrations of 166.27, 332.58, 665.15, 1330.30 and 2660.57 µg product/kg diet (40.58, 81.17, 162.34, 324.68 and 649.35 µg a.s./kg diet), corresponding to cumulative doses of 25.61, 51.22, 102.43, 204.86 and 409.73 µg product/larva (6.25,

12.50, 25.50, 50.00 and 100.00 µg a.s./larva). Each treatment group comprised of 48 larvae from three different colonies (each colony representing one replicate, thus 16 larvae from each replicate were used).

Dimethoate was used as a reference toxicant at a single nominal cumulative dose of 7.39 µg a.s./larva (equivalent to 48.0 mg a.s./kg diet).

At day -3, the queen from at least three colonies was isolated for 1 day to provide known-aged eggs and subsequent larvae. At day -2, maximum 30 hours after isolation, the queens were released. Frames containing eggs were left in the excluder cages until hatching on day 1. Three frames from different hives, containing the highest number of synchronised larvae, were selected for grafting in the laboratory. On day 1, 20 µL of diet A was dropped into each grafting cell before larvae were transferred from the comb to the surface of the diet using a grafting tool.

From day 3 until day 6, five different concentrations of S-2200 25SC were applied to the larvae of the test item groups and one single concentration of the reference item was applied to the larvae of the reference item group.

Diets were prepared as shown in Table 10.3.1.3/02-1. Both test and reference item groups were supplied with diet B and C during 4 days of the larval treatment phase (days 3, 4, 5 and 6). The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1.1 g/mL) were considered in the calculation of the cumulative doses per larva.

**Table 10.3.1.3/02-1: Diet components**

Component	Diet A <sup>a)</sup>	Diet B <sup>b)</sup>	Diet C <sup>c)</sup>
50% royal jelly + 50% aqueous solution containing:			
Yeast extract (% weight)	2	3	4
Glucose (% weight)	12	15	18
Fructose (% weight)	12	15	18

<sup>a)</sup> Diet fed on day 1 (volume administered: 20 µL/larva).

<sup>b)</sup> Used for treated diet fed on exposure day 3 (volume administered: 20 µL/larva).

<sup>c)</sup> Used for treated diet fed on exposure days 4, 5 and 6 (volume administered: 30, 40, 50 µL/larva, respectively).

## 5. Dose preparation:

For the preparation of the test item stock solution and the test item dilutions (application solutions), deionised water was used as a solvent.

For the preparation of the reference item stock solution and the preparation of the application solution of the reference item, deionised water was used as a solvent. A unique stock solution of the reference item was prepared on day 3 and stored in a refrigerator until day 6. The stock solution of the test item (St) contained 0.2927 g test item and up to 10 mL deionised water was added. The stock solution of the reference item (Rs) contained 0.0267 g test item and up to 5 mL deionised water. The preparation of the application solutions from day 3 to day 6 is shown in Table 10.3.1.3/02-2 below.

**Table 10.3.1.3/02-2: Preparation of application solutions from day 3 to day 6**

Treatment	Concentration (mg product/L diet)	Solution taken for dilution	Volume of solution taken for dilution (mL)	Volume of deionised water added (mL)	Application solution obtained
T5	2926.63	St	--	--	TAs5
T4	1463.32	TAs5	5	5	TAs4
T3	731.66	TAs4	5	5	TAs3
T2	365.83	TAs3	5	5	TAs2
T1	182.91	TAs2	5	5	TAs1

**Table 10.3.1.3/02-3: Preparation of diet B and C from day 3 to day 6**

Treatment	Concentration (mg product/L diet)	Application solution taken for dilution	Volume of application solution added to diet (mL)	Final volume of diet (mL)
<b>Untreated control</b>				
C	--	--	--	10
<b>Test item: S-2200 25SC</b>				
T5	2926.63	TAs5	1	10
T4	1463.32	TAs4	1	10
T3	731.66	TAs3	1	10
T2	365.83	TAs2	1	10
T1	182.91	TAs1	1	10
<b>Reference item: dimethoate</b>				
R	52.8 a)	Rs	0.05	5

a) mg dimethoate/L diet.

## 6. Measurements and observations:

Assessment of larval mortality was conducted on day 4, 5, 6, 7 and 8. Using a stereo microscope, larvae were recorded as dead if no respiration (movement of spiracles) was observed. On day 8, during the assessment of mortality, the presence of uneaten food was qualitatively recorded. Assessment of mortality during the pupation phase was evaluated on day 15 and assessment of emergence on day 22. Individual weight of emerged bees was measured on day 22. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group.

Air temperature and relative humidity were recorded at intervals of 15 minutes with calibrated data loggers placed into each desiccator from day 1 to day 22.

Samples of the stock solution and control diet on day 6 and samples of the highest and lowest concentrations of the test item treated diet from day 3 to day 6 were taken directly after preparation and frozen for analysis of mandestrobin concentrations. Analysis was performed using LC-MS/MS detection.

## 7. Statistics:

As no individuals with uneaten diet were observed at day 8, no individuals were discarded from the statistical analysis.

In order to determine the NOEC, a Chi<sup>2</sup> 2x2 table with Bonferroni correction (one-sided greater,  $\alpha = 0.05$ ) was used. EC<sub>x</sub> values could not be calculated since no statistically significant concentration/response was obtained. The values were empirically estimated from the results.

Statistical calculations were performed using MS Excel 2010 v.14.0 and the statistical program ToxRatPro Version 3.2.1.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL RESULTS

On day 8, all individuals consumed their diet and no individuals were observed with effects (e.g. small). At the end of the test on day 22, no emerged bees were recorded as being affected (e.g. malformations).

No statistically significant differences were found in the mortality of any of the test item groups compared to the control on day 8, 15 or 22. Since no statistically significant dose response was obtained, the EC<sub>x</sub> values could not be calculated, but were empirically estimated from the data. Mortality and emergence observed during the study are shown in Table 10.3.1.3/02-4 and endpoints are shown in Table 10.3.1.3/02-5.

**Table 10.3.1.3/02-4: Summary of mortality, adult emergence and bee weight**

Nominal diet concentration (mg product/kg diet)	Nominal dose (µg a.s./larva)	% Mortality Day 8 (M <sub>corr</sub> %) <sup>a)</sup>	% Mortality Day 15 (M <sub>corr</sub> %) <sup>a)</sup>	% Mortality Day 22 (M <sub>corr</sub> %) <sup>a)</sup>	22-Day Adult Emergence (%)	Mean bee weight at emergence (mg) <sup>b)</sup>
<b>Untreated control</b>						
0	0	0.00 (n.a.)	2.08 (n.a.)	8.33 (n.a.)	91.67	102.63
<b>Test item (S-2200 25SC)</b>						
166.29	6.25	2.08 (n.a.)	4.17 (2.13)	8.33 (0.00)	91.67	100.97
332.57	12.50	6.25 (n.a.)	10.42 (8.51)	10.42 (2.27)	89.58	100.09
665.14	25.00	0.00 (n.a.)	8.33 (6.38)	8.33 (0.00)	91.67	103.07
1330.29	50.00	2.08 (n.a.)	12.50 (10.64)	14.58 (6.82)	85.42	102.99
2660.57	100.00	2.08 (n.a.)	10.42 (8.51)	12.50 (4.55)	87.50	102.81
<b>Reference item (dimethoate)</b>						
48.00	7.39	79.17 (n.a.)	93.75 (93.62)	95.83 (95.45)	4.17	98.60

n.a.: not applicable.

<sup>a)</sup> M<sub>corr</sub>: corrected for control mortality according to Abbott modified by Schneider-Orelli.

<sup>b)</sup> Mean values calculated based on individual weights of surviving bees presented in original study report.

**Table 10.3.1.3/02-5: Summary of endpoints**

Endpoint	Period	Concentration <sup>a)</sup>		Dose <sup>b)</sup>	
		mg product/kg diet	mg a.s./kg diet	µg product/larva	µg a.s./larva
NOEC / NOED	Day 3 – 8	2660.57	649.35	409.73	100.00
	Day 3 – 15	2660.57	649.35	409.73	100.00
	Day 3 – 22	2660.57	649.35	409.73	100.00
EC <sub>10/20/50</sub> / ED <sub>10/20/50</sub> (95% CI)	Day 3 – 22	> 2660.57 (n.d.)	> 649.35 (n.d.)	> 409.73 (n.d.)	> 100.00 (n.d.)

CI: Confidence intervals.

n.d.: not determined.

<sup>a)</sup> Based on the density of the diet (1.1 g/mL).

<sup>b)</sup> Based on the cumulative feeding from day 3 until day 6 of 140 µL diet/larva.

## B. ANALYTICAL RESULTS

The results of the analysis of the royal jelly diets for S-2200 concentration on day 3, 4, 5 and 6 are presented in Table 10.3.1.3/02-6. The measured diet concentrations were within 20% of nominal, except for the diet sample of treatment T5 on day 6 (recoveries of 69% and 65% of nominal for main and retain samples, respectively). These recoveries may be due to small errors in the preparation/handling of the samples since in the stock solution and in the diet sample of the T1 treatment of the same day the recoveries are correct. Thus, the concentrations of the test item were confirmed, and the endpoints are based on nominal concentrations.

**Table 10.3.1.3/02-6: Summary of analytical results**

Sample description	Nominal mandestrobin concentration (mg a.s./kg diet)	Analysed mandestrobin concentration (mg a.s./kg diet)	Recovery (%)
Stock solution on D6 (S)	7170	7240	101
Untreated diet from control group (S)	40.58	< LOD	-
Treated diet from T1 on day 3 (S)	40.58	35.5	87
Treated diet from T1 on day 4 (S)	40.58	37.0	91
Treated diet from T1 on day 5 (S)	40.58	33.4	82
Treated diet from T1 on day 6 (S)	40.58	32.3	80
Treated diet from T5 on day 3 (S)	649.35	578	89
Treated diet from T5 on day 4 (S)	649.35	642	99
Treated diet from T5 on day 5 (S)	649.35	605	93
Treated diet from T5 on day 6 (S)	649.35	447	69
Treated diet from T5 on day 6 (R)	649.35	423	65

S: Main sample; R: Retain sample; LOD: Limit of detection (0.3 mg S-2200/kg diet).

T1: Lowest treatment level (nominal 166.27 mg product/kg diet or 40.58 mg a.s./kg diet)

T5: Highest treatment level (nominal 2660.57 mg product/kg diet or 649.35 mg a.s./kg diet).

--: not applicable.

## C. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guidelines (OECD Guidance Document 239, 2021) as detailed below:

- In the control plate(s), cumulative larval mortality from day 3 to day 8 should be  $\leq 15\%$  across all replicates. Larval mortality in the control was 0% on day 8.
- In the control plate(s), the adult emergence rate on day 22 should be  $\geq 70\%$  across all replicates. Emergence in the control was 91.67% on day 22.
- Positive control: if dimethoate is used, larval mortality should be  $\geq 50\%$  on day 8 across all replicates. Larval mortality in the dimethoate treatment was 79.17% on day 8.

### III. CONCLUSION

The chronic toxicity of S-2200 25SC to larvae of the honeybee (*Apis mellifera* L.) was determined in the laboratory during a 22-day study.

The 8, 15 and 22-day NOED for mortality and adult emergence were determined to be 409.73 µg product/larva (equivalent to 100 µg a.s./larva). The ED<sub>10/20/50</sub> were empirically estimated to be > 409.73 µg product/larva.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>NOED (8-day larval mortality): 409.73 µg product/larva/dev. period (100 µg a.s./larva/dev. period)</p> <p>NOED (22-day pupal mortality): 409.73 µg product/larva/dev. period (100 µg a.s./larva/dev. period)</p> <p>NOED (22-day adult emergence): 409.73 µg product/larva/dev. period (100 µg a.s./larva/dev. period)</p>
Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>Some deviations were noted, but they do not have any effect on final results.</p> <p>The following endpoints were derived:</p> <ul style="list-style-type: none"> <li>• NOED/NOEC = 2660.57 mg test item/kg diet</li> <li>• EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> &gt; 2660.57 mg test item/kg diet</li> <li>• NOED/NOEC = 409.73 mg test item/larva/d (100 µg a.s./larva/d,</li> <li>• ED<sub>10</sub>, ED<sub>20</sub> and ED<sub>50</sub> &gt; 409.73 mg test item/larva/d (100 µg a.s./larva/d.</li> </ul>

**A 2.3.1.4            KCP 10.3.1.4            Sub-lethal effects**

**A 2.3.1.5            KCP 10.3.1.5            Cage and tunnel tests**

**A 2.3.1.6            KCP 10.3.1.6            Field tests with honeybees**

## A 2.4 KCP 10.3.2 Effects on non-target arthropods other than bees

### A 2.4.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

#### A 2.4.1.1 Study 1

<b>Data point:</b>	KCP 10.3.2.1/01
<b>Report author:</b>	Leopold, J.
<b>Report year:</b>	2023a
<b>Report title:</b>	Mandestrobin 40 SC: Effects on the Parasitoid, <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) in the Laboratory (A Dose Response Test on Glass Plates)
<b>Report No.:</b>	172881001
<b>Document No.:</b>	ROW-0152
<b>Guidelines followed in study:</b>	Mead-Briggs <i>et al.</i> , 2000.
<b>Deviations from current test guideline:</b>	Compared to Mead-Briggs <i>et al.</i> (2000): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The effects of Mandestrobin 40SC on the aphid parasitoid *Aphidius rhopalosiphi* were investigated on glass plates under laboratory conditions at five application rates between 155 and 2480 mL product/ha (between 62.5 and 1000 g a.s./ha). An untreated control was tested in parallel, and Danadim Progress (400 g dimethoate/L) was used as a reference item at 0.3 mL test item/ha. Four replicates were prepared per treatment group, each containing ten wasps (three males and seven females). All applications were made at a volume of 200 L water/ha.

Survival of the parasitoids was assessed after 2, 24 and 48 hours. After 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 11 to 12 days before the numbers of aphid mummies that had developed were assessed.

There were no statistically significant adverse effects on mortality up to and including the highest tested rate of 2480 mL product/ha (1000 g a.s./ha). Reproduction was not statistically significantly reduced compared to the control at all treatment rates except at 1240 mL product/ha (500 g a.s./ha). However, considering the overall high level of reproduction in the test item treatments with mean values of more than 50 mummies per female and since no clear dose response relationship was found, it was considered unlikely that the significance at 500 g a.s./ha was caused by a test item related effect.

Therefore, the NOER for reproduction is considered to be 2480 mL product/ha (1000 g a.s./ha), and the LR<sub>50</sub> and ER<sub>50</sub> were both determined to be > 2480 mL product/ha (> 1000 g a.s./ha).

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Mandestrobin 40SC  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L (nominal), 403.3 g/L (analysed)
2. **Control:** Untreated deionised water only
3. **Reference item:** Danadim Progress (dimethoate, 413 g/L analysed)

### B. STUDY DESIGN AND METHODS

1. **Test organism:** Parasitic wasp (*Aphidius rhopalosiphi*)  
**Age:** Adults < 48 hours old  
**Source:** Katz Biotech AG, Baruth, Germany  
**Diet:** A solution of fructose (10%). During acclimatisation food was provided on a cotton wool pad, *ad libitum*. During the exposure period, food was provided in small test tubes (approximately 1 cm in diameter) which were connected to the exposure units at the beginning of the experiment, *ad libitum*.
2. **Test units:**  
Hatching chambers: glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening.  
Exposure units: comprised of two treated glass plates, (13 cm x 13 cm), held apart by an untreated aluminium 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 (approximately 1 cm in diameter) covered with a cloth. The 4<sup>th</sup> side of the frame had 1 small hole (approximately 1 cm in diameter) for inserting and feeding the test organisms.  
Post exposure units (parasitisation and post-parasitisation period): This test unit comprised of untreated pots (13 cm in diameter) with barley seedlings, infested with 100 - 200 host aphids of all developmental stages, were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). In order to improve ventilation, the cylinder had two holes (70 x 195 mm) which were closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.
3. **Environmental conditions:**  
**Temperature:** 19 – 21°C  
**Relative humidity:** 74 – 75% (acclimatisation and exposure period)  
73 - 83% (post-exposure period; within the test units)  
  
**Photoperiod:** 16 hours light: 8 hours darkness (600 – 1580 lux during acclimatisation, exposure and parasitisation period, and 16050 - 17370 lux during post-parasitisation period).
4. **Animal assignment and treatment:**

The effects of Mandestrobin 40SC on the aphid parasitoid *Aphidius rhopalosiphi* were investigated on glass plates under laboratory conditions at five application rates of 155, 310, 620, 1240 and 2480 mL product/ha (62.5, 125, 250, 500 and 1000 g a.s./ha). An untreated control was tested in parallel, and Danadim Progress (dimethoate, 413 g/L) was used as a reference item at 0.3 mL test item/ha. Four replicates were used per treatment group, each containing ten wasps (three males, seven females). All applications were made at a volume of 200 L water/ha.



The parasitoids were introduced approximately 20 to 45 minutes after the application of the test items. For the assessment of the parasitic capacity, aphids (*Rhopalosiphum padi*) were prepared. A pot containing 13 – 22 barley seedlings (10 days old) infested with > 100 aphids were enclosed within a clear polyacrylic cylinder. Female parasitoids were introduced impartially using an aspirator.

After 48 hours exposure to the treated glass plates, the surviving test organisms were removed with an aspirator. Up to 20 of the survived and unaffected females per treatment were released individually to parasitise aphids for a period of 24 hours.

## 5. Dose preparation:

The spray solutions were prepared with deionised water. The required quantities of the test item and reference item were weighed and filled up with deionised water. Application was performed with a laboratory-spraying equipment. Preparation of the spray solutions is shown in Table 10.3.2.1/01-1 below.

**Table 10.3.2.1/01-1: Preparation of spray solutions for application**

Active substance concentration (g a.s./ha)	Test item target concentration (mL product/ha)	Test item (mg) <sup>a)</sup>	Deionised water (mL)	Concentration of the spray dilution (g test item/L)	Applied amount (mL test item/ha)	Applied amount (g a.s./ha)
Mandestrobin 40SC						
62.5	155	210.2	250	0.841	155.3	62.7
125	310	418.9	250	1.676	309.6	124.9
250	620	839.0	250	3.356	620.0	250.1
500	1240	1677.9	250	6.712	1240.0	500.1
1000	2480	3355.1	250	13.420	2479.5	1000.0
Danadim Progress						
Reference item 9.0 mL test item/ha		(µL)	(mL)	(µL/L)	(mL test item/ha)	
		0.75	500	1.5	0.3	

<sup>a)</sup> Amount of test item weighed in.

## 6. Measurements and observations:

Observations of mortality were recorded approximately 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead. Parasitoids were classed as either:

- Live: alive and apparently unaffected.
- Affected: still upright and attempting to walk but showing signs of reduced coordination; or generally inactive with respect to the insects in the control treatment.
- Moribund: on their back or side, still twitching, but generally unable to right themselves.
- Dead: not moving.

For the reproduction assessments, the number of aphid mummies was counted 11 to 12 days after the 24-hour parasitisation period in all replicates where the females were alive after the 24-hour parasitisation period (n = 19 - 20). The number of live, dead or not found females after the 24-hour parasitisation period was documented. The reproduction assessment was performed for the control and for those test item groups where the corrected mortality was ≤ 50%. No reproduction testing was performed with the reference item.

Temperature and humidity were recorded continuously during the test.

## 7. Statistics:

Bonferroni-Holm Fisher's Exact Binomial Test ( $\alpha = 0.05$ , one-sided greater) was used to detect statistically significant differences between mortality data of each test item treatment group and the control group. 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 and homogeneity of variance and the presence of linear trends using Shapiro-Wilk's Test and 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 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.

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

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL RESULTS

No statistically significant effects on mortality were observed at any treatment level of Mandestrobin 40SC. Mortality in the reference item treatment was 100%. Mortality and other effects are summarised in Table 10.3.2.1/01-2 below.

**Table 10.3.2.1/01-2: Mortality of *Aphidius rhopalosiphi* adults after 48 hours**

Insects	Control	Mandestrobin 40SC (g a.s./ha)					Reference item
		62.5	125.0	250.0	500.0	1000.0	
# alive	40	40	40	40	40	40	0
# affected	0	0	0	0	0	0	0
# moribund	0	0	0	0	0	0	0
# dead	0	0	0	0	0	0	40
M (%)	0.0	0.0	0.0	0.0	0.0	0.0	100.0*

M: Mortality, calculated as the mean value of four replicates of each treatment, based on the number of dead and moribund organisms.

\* Statistically significant effects compared to the control (Fisher's Exact Binomial Test, one-sided greater;  $\alpha = 0.05$ ).

Reproduction was not statistically significantly reduced compared to the control at all test item application rates with the exception of 1240 mL product/ha (500 g a.s./ha). However, considering the overall high level of reproduction in the test item treatments with mean values of more than 50 mummies per female and since no clear dose response relationship was found, it is unlikely that the statistical significance at 500 g a.s./ha was caused by a test item related effect. Therefore, it was concluded that the NOER for reproduction is 2480 mL product/ha (1000 g a.s./ha), see Table 10.3.2.1/01-3 below.

**Table 10.3.2.1/01-3: Reproduction of *Aphidius rhopalosiphi***

Parameter	Control	Mandestrobin 40SC (g a.s./ha)				
		62.5	125	250	500	1000
# females	20	19	19	19	20	19
Mean no. mummies per female $\pm$ SD	68.0 $\pm$ 21.5	56.4 $\pm$ 19.4	55.7 $\pm$ 17.7	56.9 $\pm$ 18.7	50.6* $\pm$ 22.0	61.1 $\pm$ 15.8
Reduction of reproduction rate (%)	-	17.0	18.0	16.3	25.7	10.1

SD: Standard deviation.

\* Statistically significant effects compared to the control (Dunnett's t-Test; one-sided smaller;  $\alpha = 0.05$ ).

**Table 10.3.2.1/01-4: Summary of endpoints**

Endpoint	mL product/ha	g a.s./ha
LR <sub>50</sub>	> 1000	> 2480
NOER (mortality)	1000	2480
LOER (mortality)	> 1000	> 2480
ER <sub>50</sub> (reproduction)	> 1000	> 2480
NOER (reproduction)	1000	2480
LOER (reproduction)	n.d.	n.d.

n.d.: not determined.

## B. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (Mead-Briggs *et al.* 2000) as detailed below:

- Mortality in the control treatment should not exceed 5 out of 40 wasps (13%). In this study, 0% mortality was observed in the control after 48 hours.
- The mortality in the toxic reference treatment should be  $\geq 50\%$  corrected mortality. Mortality in the reference item group was 100% after 48 hours.
- For the reproduction assessments, wasps in the control treatment should produce a minimum of 5 mummies per female. A mean value of 68.0 mummies per female was observed in the control.
- In the control treatment, there should be no more than two wasps producing zero mummies. No females produced zero mummies in the control.

## III. CONCLUSION

The effects of Mandestrobin 40SC on the aphid parasitoid *Aphidius rhopalosiphi* were investigated on glass plates under laboratory conditions.

There were no statistically significant adverse effects on mortality up to and including the highest tested rate of 2480 mL product/ha (1000 g a.s./ha). Reproduction was not statistically significantly reduced compared to the control at all treatment rates except at 1240 mL product/ha (500 g a.s./ha). However, considering the overall high level of reproduction in the test item treatments with mean values of more than 50 mummies per female and since no clear dose response relationship was found, it was considered unlikely that the significance at 500 g a.s./ha was caused by a test item related effect.

Therefore, the NOER for reproduction is considered to be 2480 mL product/ha (1000 g a.s./ha), and the LR<sub>50</sub> and ER<sub>50</sub> were both determined to be > 2480 mL product/ha (> 1000 g a.s./ha).

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>LR<sub>50</sub> and ER<sub>50</sub>: &gt; 2480 mL product/ha (&gt; 1000 g a.s./ha)</p> <p>NOER (mortality and reproduction): 2480 mL product/ha (1000 g a.s./ha)</p>
Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>No study deviations were noted.</p> <p>The following endpoints were derived:</p> <ul style="list-style-type: none"> <li>• mortality</li> </ul> <p>LR<sub>50</sub> : &gt; 2480 mL product/ha (&gt; 1000 g a.s./ha)</p> <p>NOER/LOER &gt;2480 mL product/ha (1000 g a.s./ha)</p> <ul style="list-style-type: none"> <li>• reproduction</li> </ul>

	ER <sub>50</sub> : > 2480 mL product/ha (> 1000 g a.s./ha) NOER = 2480 mL product/ha (1000 g a.s./ha)
--	--

#### A 2.4.1.2 Study 2

<b>Data point:</b>	KCP 10.3.2.1/02
<b>Report author:</b>	Leopold, J.
<b>Report year:</b>	2023b
<b>Report title:</b>	Mandestrobin 40 SC: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates
<b>Report No.:</b>	172881063
<b>Document No.:</b>	ROW-0153
<b>Guidelines followed in study:</b>	Blümel <i>et al.</i> (2000)
<b>Deviations from current test guideline:</b>	Compared to Blümel <i>et al.</i> (2000): None
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

#### Executive Summary

The effects of Mandestrobin 40SC on the predatory mite, *Typhlodromus pyri* were evaluated over 14 days under laboratory test conditions on glass plates at five application rates between 155 and 2480 mL product/ha (between 62.5 and 1000 g a.s./ha). An untreated control was tested in parallel, and Danadim Progress (400 g dimethoate/L), was used as a toxic reference item at a single rate of 9.0 mL product/ha. Three replicates were prepared per treatment, each containing 20 mites. Mortality was assessed on day 3 and 7. Reproduction assessments were carried out at 7, 10, 13 and 14 days after treatment.

No significant effects on mortality were observed at any test item rate, and no significant effects on reproduction were observed up to and including 500 g a.s./ha. At 1000 g a.s./ha, a significant reduction in reproduction was observed, which was less than 50% (34.2%). The LR<sub>50</sub> and ER<sub>50</sub> (reproduction) were both determined to be > 2480 mL product/ha (> 1000 g a.s./ha).

The NOER for mortality was determined to be 2480 mL product/ha (1000 g a.s./ha), and the NOER for reproduction was determined to be 1240 mL product/ha (500 g a.s./ha).

### I. MATERIALS AND METHODS

#### A. MATERIALS

- Test material:** Mandestrobin 40SC  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L (nominal), 403.3 g/L (analysed)
- Control:** Untreated deionised water only
- Reference item:** Danadim Progress (dimethoate, 413 g/L)

#### B. STUDY DESIGN AND METHODS

- Test organism:** Predatory mite (*Typhlodromus pyri*)  
**Age:** Protonymphs < 24 hours old

**Source:** Katz Biotech AG, Baruth, Germany  
**Diet:** A mixture of pine (*Pinus* sp.) and birch (*Betula* sp) pollen (3:1)

2. **Test units:** Two glass cover slides (24 mm × 60 mm) fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side-ends. The glue barrier was placed on the test unit to keep the mites on test arena.

3. **Environmental conditions:**

**Temperature:** 24 – 26.0°C

**Relative humidity:** 64 – 68%

**Photoperiod:** 16 hours light: 8 hours darkness (340 - 400 lux)

4. **Animal assignment and treatment:**

The effects of Mandestrobin 40SC on the predatory mite, *Typhlodromus pyri* were evaluated over 14 days under laboratory test conditions at five application rates of 155, 310, 620, 240 and 2480 mL product/ha (62.5, 125, 250, 500 and 1000 g a.s./ha). An untreated control was tested in parallel, and Danadim Progress (413 g/L dimethoate) was used as a toxic reference item at a single rate of 9.0 mL product/ha. Three replicates were prepared per treatment, each containing 20 mites. All applications were made at a volume of 200 L water/ha.

After drying of the test units (25 to 40 minutes after application) impartially selected mites were introduced with a fine brush. Mites were exposed for 14 days in the control and all test item groups where reproduction was assessed after 7 days.

5. **Dose preparation:**

The spray solutions were prepared with deionised water. The required quantities of the test item Mandestrobin 40SC and the reference item Danadim Progress were weighed in glass bottles and filled up with deionised water. Application was performed with a laboratory spraying equipment. Preparation of the spray solutions is shown in Table 10.3.2.1/02-1 below.

**Table 10.3.2.1/02-1: Preparation of spray solutions for application**

Test item target concentration (g a.s./ha)	Test item target concentration (mL product/ha)	Test item (mg) <sup>a)</sup>	Deionised water (mL)	Concentration of the spray dilution (g product/L)	Applied amount (mL product /ha)	Applied amount (g a.s./ha)
Mandestrobin 40SC						
62.5	155	210.2	250	0.841	155.3	62.7
125.0	310	418.9	250	1.676	309.6	124.9
250.0	620	839.0	250	3.356	620.0	250.1
500.0	1240	1677.9	250	6.712	1240.0	500.1
1000.0	2480	3355.1	250	13.420	2479.5	1000.0
Danadim Progress						
Reference item 9.0 mL test item /ha		(µL)	(mL)	(µL/L)	(mL product/ha)	
		11.3	250	45.2	9.04	

<sup>a)</sup> Amount of test item weighed in.

## 6. Measurements and observations:

The number of living, dead and escaped mites was counted on day 3 and day 7 after test initiation. Dead mites were removed, escaped mites were considered as dead for the assessment of mortality.

The sex-ratio for reproduction testing was 1 male : 5 females at a minimum on day 7. Since the sex-ratio was less than 1 male : 5 females in rate 4 (500 g a.s./ha), one male was transferred from replicate 3 to replicate 1 and two males were transferred from replicate 2 to replicate 1. Additionally, three males were transferred from replicate 1 to replicate 3 of rate 5 (1000 g a.s./ha).

Number of eggs laid and number of live and dead juvenile stages per female was counted and removed afterwards on 3 assessment days from day 7 onwards with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted.

The reproduction assessment was performed for the control and for those test item groups where the corrected mortality was  $\leq 50\%$ . No reproduction assessment was performed for the reference item.

Temperature and humidity were recorded continuously.

## 7. Statistics:

The 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.

Mortality data obtained from the control and test item treatments were analysed for significance using the Chi<sup>2</sup> 2x2 Table Test with Bonferroni Correction (one-sided greater,  $\alpha = 0.05$ ). A qualitative trend analysis by contrasts ( $\alpha = 0.05$ ) was carried out previously to check for the presence of linear or quadratic trends.

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 a linear trend was revealed, the Williams 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.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL RESULTS

Mortality was not statistically significantly increased when compared to the control group up to and including the highest tested rate of 1000 g a.s./ha. The mean mortality observed in the control group was 10%. Mortality in the reference item treatment was 100% (corrected mortality: 100%) after 7 days.

Mortality and number of escapees are summarised in Table 10.3.2.1/02-2 below.

**Table 10.3.2.1/02-2: Mortality and escape of *Typhlodromus pyri***

Rate (g a.s./ha)	Mortality (%) <sup>a)</sup>	M <sub>corr</sub> (%) <sup>a)</sup>	Escapees (%) <sup>b)</sup>
<b>Control</b>			
0	10.0 ± 5.0	-	0.0 ± 0.0
<b>Mandestrobin 40SC</b>			
62.5	13.3 ± 7.6	3.7	1.7 ± 2.9
125.0	3.3 ± 2.9	-7.4	0.0 ± 0.0
250.0	15.0 ± 5.0	5.6	3.3 ± 2.9
500.0	3.3 ± 5.8	-7.4	0.0 ± 0.0
1000.0	8.3 ± 7.6	-1.9	0.0 ± 0.0
<b>Danadim Progress (mL test item/ha)</b>			
9.0	100.0 ± 0.0*	100.0	11.7 ± 7.6

<sup>a)</sup> Negative values indicate better survivorship compared to control.

<sup>b)</sup> Percentage values represent means and standard deviation from 3 replicates each with 20 mites.

M<sub>corr</sub>: Corrected mortality according to Schneider-Orelli (1947).

\* Statistically significant increase compared to the control (Fisher's Exact Binomial Test, one-sided greater;  $\alpha = 0.05$ ).

Reproduction was statistically significantly reduced compared to the control at 1000 g a.s./ha (see Table 10.3.2.1/02-3 below).

**Table 10.3.2.1/02-3: Reproduction rate of *Typhlodromus pyri***

Parameter	Control	Mandestrobin 40SC (g a.s./ha)				
		62.5	125.0	250.0	500.0	1000.0
Mean no. eggs per female ± SD	7.3 ± 0.9	6.6 ± 0.5	6.8 ± 1.7	5.7 ± 0.9	6.1 ± 1.4	4.8 ± 1.1
Effect on reproduction (%)	-	10.2	6.7	21.5	16.4	34.2

\*Statistically significant difference Williams t-Test; one-sided smaller;  $\alpha = 0.05$ .

<sup>a)</sup> from day 7 to day 14 after test start; values represent means and standard deviation from 3 replicates .

SD: Standard deviation.

**Table 10.3.2.1/02-4: Summary of endpoints**

Endpoint	mL product/ha	g a.s./ha
LR <sub>50</sub>	> 2480	> 1000
NOER (mortality)	2480	1000
LOER (mortality)	> 2480	> 1000
ER <sub>50</sub> (reproduction)	> 2480	> 2000
NOER (reproduction)	1240	500
LOER (reproduction)	2480	1000

## B. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (Blümel *et al.* 2000) as detailed below:

- Mortality in the control treatment should not exceed 20% on day 7 after treatment application. During the study, 10% mortality was recorded in the control treatment.
- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the toxic reference item should range between 50 and 100%. In this study, 100% corrected mortality was observed in the reference item treatment.
- The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be at least 4 eggs/female. Mean number of eggs per female in the control was 7.3.

## III. CONCLUSION

The effects of Mandestrobin 40SC on the predatory mite, *Typhlodromus pyri* were evaluated over 14 days under laboratory test conditions on glass plates.

No significant effects on mortality were observed at any test item rate, and no significant effects on reproduction were observed up to and including 500 g a.s./ha. At 1000 g a.s./ha, a significant reduction in reproduction was observed, which was less than 50% (34.2%). The LR<sub>50</sub> and ER<sub>50</sub> (reproduction) were both determined to be > 2480 mL product/ha (> 1000 g a.s./ha).

The NOER for mortality was determined to be 2480 mL product/ha (1000 g a.s./ha), and the NOER for reproduction was determined to be 1240 mL product/ha (500 g a.s./ha).

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoints derived from the study are:</p> <p>LR and ER<sub>50</sub>: &gt; 2480 mL product/ha (&gt; 1000 g a.s./ha)</p>
Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>No study deviations were noted.</p> <p>The following endpoints for mortality were derived:</p> <ul style="list-style-type: none"> <li>• NOER = 1000 g a.s./ha, equivalent to 2480 mL product/ha</li> <li>• LOER &gt; 1000 g a.s./ha, equivalent to &gt; 2480 mL product/ha</li> <li>• LR<sub>50</sub> &gt; 1000 g a.s./ha, equivalent to &gt; 2480 mL product/ha</li> </ul>



## A 2.5 KCP 10.4 Effects on non-target soil meso- and macrofauna

### A 2.5.1 KCP 10.4.1 Earthworms

#### A 2.5.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

##### A 2.5.1.1.1 Study 1

<b>Data point:</b>	KCP 10.4.1.1/01
<b>Report author:</b>	Straube, D.
<b>Report year:</b>	2023
<b>Report title:</b>	Mandestrobin 40 SC: Effect on Reproduction and Growth of Earthworm <i>Eisenia andrei</i> in Artificial Soil
<b>Report No.:</b>	172881022
<b>Document No.:</b>	ROW-0156
<b>Guidelines followed in study:</b>	OECD 222 (2016)
<b>Deviations from current test guideline:</b>	Compared to OECD 222 (2016): None.
<b>Previous evaluation:</b>	No, not previously submitted
<b>GLP/Officially recognised testing facilities:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The chronic toxicity of Mandestrobin 40SC to the earthworm (*Eisenia andrei*) was determined in a laboratory study over 56 days. Adult earthworms were exposed to eight nominal test item concentrations between 2.94 and 180 mg product/kg soil dry weight (dw) corresponding to an active substance concentration range between 1.10 and 67.10 mg a.s./kg soil dw. An untreated control was tested in parallel. Four replicates were set up per test item treatment and eight replicates for the control. Each replicate contained ten adult earthworms. Assessments of adult earthworm mortality, behavioural effects and body weight change were made after 28 days, and assessment of reproduction (number of juveniles) was performed after 56 days.

The NOEC values for survival, body weight change and reproduction were determined to be 180 mg product/kg soil dw (67.10 mg a.s./kg soil dw), 55.6 mg product/kg soil dw (20.7 mg a.s./kg soil dw) and 30.9 mg product/kg soil dw (11.5 mg a.s./kg soil dw), respectively. The EC<sub>10/20/50</sub> values for reproduction were determined to be 42.2, 53.4 and 83.6 mg product/kg soil dw (15.7, 19.9 and 31.1 mg a.s./kg soil dw), respectively.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** Mandestrobin 40SC  
**Description:** Off-white liquid  
**Lot/Batch:** AE20-2F2102  
**A.s. content:** 400 g/L (nominal), 403.3 g/L (analysed)
- Control:** Untreated soil (moistened with deionised water)
- Reference item:** Carbendazim, tested in a separate study

## B. STUDY DESIGN AND METHODS

- 1. Test organism:** Earthworm (*Eisenia andrei*)  
**Age:** Adult worms, with clitellum (9 months old)  
**Weight:** 314 – 599 mg  
**Source:** Bred under standardised conditions in test facility  
**Acclimation:** 24 hours  
**Diet:** Finely ground cattle manure
- 2. Test medium:** Artificial soil containing 10% sphagnum peat, 20% kaolin clay, 69.6% quartz (silica) sand, 0.46% calcium carbonate to adjust pH.  
**Water holding capacity:** 49% (WHC<sub>max</sub>)
- 3. Test units:** Plastic boxes (18.3 cm x 13.6 cm x 6 cm, tapered towards the bottom, with a soil surface of approximately 16.5 cm x 11.5 cm = 189.75 cm<sup>2</sup>) with perforated transparent lids to enable exchange of air, to minimise evaporation from the artificial soil, and to prevent the earthworms from escaping. Each container was filled with 625.4 g of the prepared soil (500 g dry weight plus deionised water). The height of the soil layer in the containers was approximately 5 cm.
- 4. Environmental conditions:**  
**Temperature:** 18 – 22°C  
**pH of soil:** 5.9 – 6.1  
**Soil water content:** 25.6 – 28.1% (mean values)  
**Photoperiod:** 16 hours light: 8 hours dark (400 – 800 lux)

### 5. Animal assignment and treatment:

The chronic toxicity of Mandestrobin 40SC to the earthworm (*Eisenia andrei*) was determined in a laboratory study over 56 days. Adult earthworms were exposed to eight nominal test item concentrations of 2.94, 5.29, 9.53, 17.2, 30.9, 55.6, 100 and 180 mg product/kg soil dw corresponding to 1.10, 1.97, 3.55, 6.39, 11.5, 20.7, 37.3 and 67.10 mg a.s./kg soil dw. An untreated control was tested in parallel. Four replicates were set up per test item treatment and eight replicates for the control. Each replicate contained ten adult earthworms.

Before test initiation, all earthworms were washed with tap water and dried with dry paper towels and weighed individually before being randomly assigned to batches of ten earthworms. The different batches were sorted into four classes on the basis of the total weight and one batch of each weight class was assigned to each treatment group (two batches for the control) to ensure weights were homogeneous.

The earthworms were placed on the surface of the artificial soil after application. 5 g/container of finely grounded cattle manure was scattered on the soil surface at day 1 after application and was moistened with 5 g deionised water, and 5 g/container (moistened with 2 g deionised water) was added each week for the first 28 days of the experiment. Four weeks after application, the food was mixed into the substrate following removal of the adult earthworms.

After 28 days, the artificial soil was transferred to a tray and adult earthworms were counted, removed and weighed per replicate. Juveniles were removed by placing the test units in a water bath at 50 - 60°C for about 30 minutes and counting all emerging earthworms.

### 6. Dose preparation:

A stock solution was prepared by weighing 870.2 mg of test item (Mandestrobin 40SC) and deionised water was added to reach a final net weight of 596.0 g. The resulting suspension contained a concentration of 1.4601 mg test item/g. A magnetic stirrer was used to obtain a homogenous dispersion of the test item. For

the lowest test concentration, a dilution was prepared. The prepared amounts of this stock solution or corresponding dilution were added to 2100 g dry artificial soil to prepare the target nominal concentrations of the test item in the artificial soil. The control was left untreated. While mixing the artificial soil in a laboratory mixer for approximately 5 min the soil of each treatment group (including the control) was moistened with deionised water. Each group was treated in one batch (two in the control) which was then split into the replicates.

## **7. Measurements and observations:**

Survival and behavioural and morphological abnormalities were assessed 28 days after treatment. Body weights were determined at test start (day 0) and 28 days after application. Cumulative amount of food added to each test container during the test period was also assessed.

On day 56, the vessels were placed in a water bath set between 50 and 60°C. The vessels were then left for approximately 30 minutes, and all emerging earthworms were counted. In addition, the soil of each container was emptied out onto a tray and checked visually for any remaining juvenile earthworms, and the total number of juveniles per vessel determined.

Water content of the soil was checked at the start and end of the test and once per week during the test by weighing each container and evaporated water was replenished. pH was determined at the start and end of the test for each treatment group. Temperature was monitored throughout the test. Light intensity was measured.

## **8. Statistics:**

Mortality data was statistically analysed using the Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction,  $\alpha = 0.05$ , one-sided greater).

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

Since the reproduction data were normally distributed and heterogeneous the Welsh-t-test After Bonferroni-Holm (multiple comparison,  $\alpha = 0.05$ , one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values and their 95% confidence limits for reproduction were calculated by applying 3-param. Normal CDF.

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

# **II. RESULTS AND DISCUSSION**

## **A. MORTALITY**

After 28 days, adult earthworm mortality of up to 12.5% was found in the test item treated groups, which was not statistically significantly different compared to the control, where 2.5% of the earthworms died (see Table 10.4.1.1/01-1).

No behavioural abnormalities were observed in any of the treatment groups and earthworms burrowed into the soil within 15 minutes after introduction.

## B. BODY WEIGHT AND FEEDING ACTIVITY

The body weight changes of the earthworms after 28 days exposure to Mandestrobin 40SC were not statistically significantly different compared to the control up to and including the test concentration of 55.6 mg product/kg soil dw, equivalent to 20.7 mg a.s./kg soil dw. At the test concentration of 100 mg product/kg soil dw, (37.3 mg a.s./kg soil dw) and above, body weight was statistically significantly reduced compared to the control (see Table 10.4.1.1/01-1).

The feeding activity in all the treated groups was comparable to the control.

## C. REPRODUCTION

At 55.6 mg product/kg soil dw, (20.7 mg a.s./kg soil dw) and above, reproduction was statistically significantly reduced compared to the control (see Table 10.4.1.1/01-1). There were no other observations noted.

**Table 10.4.1.1/01-1: Summary of effects on mortality, body weight change and reproduction**

Test item concentration (mg product/kg)	Active substance conc. (mg a.s./kg)	Mean mortality after 28 d (%)	Mean body weight change ± SD after 28 d (%)	Mean reproduction rate ± SD after 56 d	
				(juveniles/test vessel)	(% of control)
Control					
Control	0	2.5	18.5 ± 4.6	105 ± 9	-
Test item (Mandestrobins 40SC)					
2.94	1.10	0.0	16.7 ± 5.7	84 ± 19	80.0
5.29	1.97	0.0	22.1 ± 3.0	88 ± 12	83.6
9.53	3.55	2.5	21.4 ± 3.0	96 ± 9	91.0
17.2	6.39	0.0	21.0 ± 6.1	82 ± 31	77.9
30.9	11.5	0.0	24.3 ± 4.0	103 ± 15	97.6
55.6	20.7	2.5	20.8 ± 2.8	65 ± 4*	61.8
100	37.7	0.0	12.3 ± 7.0*	38 ± 10*	36.3
180	67.10	12.5	13.7 ± 5.2*	4 ± 2*	4.0
Test endpoints after 28 and 56 days					
			mg product/kg soil dw	mg a.s./kg soil dw	
28 d NOEC (mortality)			180	67.10	
28 d LOEC (mortality)			>180	> 67.10	
28 d LC50			>180	> 67.10	
28 d NOEC (body weight)			55.6	20.7	
28 d LOEC (body weight)			100	37.3	
56 d NOEC (reproduction)			30.9	11.5	
56 d LOEC (reproduction)			55.6	20.7	
EC50 (reproduction)			83.6	31.1	
EC20 (reproduction)			53.4	19.9	
EC10 (reproduction)			42.2	15.7	

SD: Standard deviation; -: not applicable.

\*Significantly different compared to the control.

The reference item carbendazim was tested in a separate study. The EC<sub>50</sub> value was calculated to be 1.10 mg/kg soil dw, which was within the given toxicity range of 1 to 5 mg/kg soil dw, indicating that the worms responded as expected in the test system.

## D. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent version of the EU test guideline (OECD 222, 2016) as detailed below:

- In the control, each replicate (containing 10 adults) should have produced  $\geq 30$  juveniles by the end of the test. During the test, the number of juveniles per vessel was 94 – 124 in the control.
- In the control, the coefficient of variation of reproduction should be  $\leq 30\%$ . The coefficient of variation of the reproductive output was 8.9% in the control.
- In the control, adult mortality over the initial 4 weeks of the test should be  $\leq 10\%$ . Mortality was 2.5% in the control.

## III. CONCLUSION

The chronic toxicity of Mandestrobin 40SC to the earthworm (*Eisenia andrei*) was determined in a laboratory study over 56 days.

The NOEC values for survival, body weight change and reproduction were determined to be 180 mg product/kg soil dw (67.10 mg a.s./kg soil dw), 55.6 mg product/kg soil dw (20.7 mg a.s./kg soil dw) and 30.9 mg product/kg soil dw (11.5 mg a.s./kg soil dw), respectively. The EC<sub>10/20/50</sub> values for reproduction were determined to be 42.2, 53.4 and 83.6 mg product/kg soil dw (15.7, 19.9 and 31.1 mg a.s./kg soil dw), respectively.

Assessment and Conclusion by Applicant:	<p>The study has been performed to current standards and is considered to be fully valid.</p> <p>The relevant endpoint derived from the study is:</p> <p>NOEC for reproduction: 30.9 mg product/kg soil dw (11.5 mg a.s./kg soil dw)</p>																						
Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>No study deviations were noted.</p> <p>The following endpoints were derived:</p> <table><tr><th>Endpoint</th><th>mg test item/ kg soil dw</th></tr><tr><td>NOEC (day 28 mortality)</td><td>≥180</td></tr><tr><td>LOEC (day 28 mortality)</td><td>&gt;180</td></tr><tr><td>LC50 4)</td><td>&gt;180</td></tr><tr><td>NOEC (day 28 weight changes)</td><td>55.6</td></tr><tr><td>LOEC (day 28 weight changes)</td><td>100</td></tr><tr><td>NOEC (day 56 reproduction)</td><td>30.9</td></tr><tr><td>LOEC (day 56 reproduction)</td><td>55.6</td></tr><tr><td>EC<sub>10</sub> (reproduction)</td><td>42.2</td></tr><tr><td>EC<sub>20</sub> (reproduction)</td><td>53.4</td></tr><tr><td>EC<sub>50</sub> (reproduction)</td><td>83.6</td></tr></table>	Endpoint	mg test item/ kg soil dw	NOEC (day 28 mortality)	≥180	LOEC (day 28 mortality)	>180	LC50 4)	>180	NOEC (day 28 weight changes)	55.6	LOEC (day 28 weight changes)	100	NOEC (day 56 reproduction)	30.9	LOEC (day 56 reproduction)	55.6	EC <sub>10</sub> (reproduction)	42.2	EC <sub>20</sub> (reproduction)	53.4	EC <sub>50</sub> (reproduction)	83.6
Endpoint	mg test item/ kg soil dw																						
NOEC (day 28 mortality)	≥180																						
LOEC (day 28 mortality)	>180																						
LC50 4)	>180																						
NOEC (day 28 weight changes)	55.6																						
LOEC (day 28 weight changes)	100																						
NOEC (day 56 reproduction)	30.9																						
LOEC (day 56 reproduction)	55.6																						
EC <sub>10</sub> (reproduction)	42.2																						
EC <sub>20</sub> (reproduction)	53.4																						
EC <sub>50</sub> (reproduction)	83.6																						

**A 2.5.1.2 KCP 10.4.1.2 Earthworms - field studies**

**A 2.5.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)**

**A 2.5.2.1 KCP 10.4.2.1 Species level testing**

**A 2.5.2.2 KCP 10.4.2.2 Higher tier testing**

**A 2.6 KCP 10.5 Effects on soil nitrogen transformation**

**A 2.7 KCP 10.6 Effects on terrestrial non-target higher plants**

**A 2.7.1 KCP 10.6.1 Summary of screening data**

**A 2.7.2 KCP 10.6.2 Testing on non-target plants**

**A 2.7.3 KCP 10.6.3 Extended laboratory studies on non-target plants**

**A 2.8 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)**

<b>Data point:</b>	KCP 10.7/01
<b>Report author:</b>	Soma, M.
<b>Report year:</b>	2017
<b>Report title:</b>	Evaluation of Herbicidal Activity of DX-CA-S-2200 and S-2200
<b>Report No.:</b>	ROG-0007
<b>Document No.:</b>	ROG-0007
<b>Guidelines followed in study:</b>	Not applicable
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously evaluated
<b>GLP/Officially recognised testing facilities:</b>	No, not conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

**Executive Summary**

The effects of DX-CA-S-2200 and S-2200 (mandestrobin) on seven species of terrestrial plants were assessed in a laboratory study. Each test item was applied to the plants pre-emergence at a rate of 400 g a.s./ha. The results of the study indicated that none of the test items showed any herbicidal activity to the plants tested.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

- Test material:** DX-CA-S-2200 (metabolite of mandestrobin)

**Lot/Batch:** 089-160803-1

**Purity:** 99.9%

**Test material:** S-2200 (mandestrobin)

**Lot/Batch:** No060912-1

**Purity:** 99.7%

## B. STUDY DESIGN AND METHODS

1. **Test plants:**
  - Blackgrass (*Alopecurus myosuroides*)
  - Italian ryegrass (*Lolium multiflorum*)
  - Barnyardgrass (*Echinochloa crus-galli*)
  - Giant foxtail (*Setaria faberi*)
  - Cleavers/catchweed bedstraw (*Galium aparine*)
  - Velvetleaf (*Abutilon theophrasti*)
  - Wheat (*Triticum aestivum*)

2. **Experimental method:**

DX-CA-S-2200 and S-2200 (mandestrobin) were formulated as 8% w/w formulation using *N,N*-dimethylformamide containing 2% w/w of Tween20. The test plants were sown in a plastic pot (8.8 cm in diameter) and cultivated in a greenhouse at 25°C (day) and 20°C (night). Numbers of crop and weed seeds were 5 and 20 per pot, respectively. Crops and weeds were sown in a separate pot. Soil type was sandy clay (organic matter content, 1.7%; pH 5.2). The trial was carried out with two replications.

The 8% w/w SL formulation of each test chemical was applied with a compressed-air sprayer at 0.75 kg/cm<sup>2</sup> at the dose rate of 400 g a.s./ha (500 L/ha) to the test plants in post-emergence application on 12<sup>th</sup> January 2017, and herbicidal activity of each chemical was assessed on 31<sup>st</sup> January 2017. Criteria of assessment were stunting, yellowing of plants and inhibition of germination.

## II. RESULTS AND DISCUSSION

Neither of the test compounds showed any herbicidal activity on blackgrass, Italian ryegrass, barnyardgrass, giant foxtail, cleavers/catchweed bedstraw, velvetleaf and wheat at 400 g a.s./ha. Also, none of them showed crop injury on wheat.

## III. CONCLUSION

The results of this study indicate that DX-CA-S-2200 and S-2200 (mandestrobin) did not show any herbicidal activity to the plants tested.

Assessment and Conclusion by Applicant:	The results of this study indicate that DX-CA-S-2200 and S-2200 (mandestrobin) did not show any herbicidal activity to the plants tested.
Comments of zRMS:	The study was not evaluated as it was performed with metabolite of active substance. The study should be submitted and evaluated at the EU level during active substance renewal.

<b>Data point:</b>	KCP 10.7/02
<b>Report author:</b>	Kamezaki, M.
<b>Report year:</b>	2017
<b>Report title:</b>	Evaluation of Insecticidal Activity of DX-CA-S-2200 and S-2200
<b>Report No.:</b>	ROG-0008
<b>Document No.:</b>	ROG-0008
<b>Guidelines followed in study:</b>	Not applicable
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously evaluated
<b>GLP/Officially recognised testing facilities:</b>	No, not conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The effects of DX-CA-2200 and S-2200 (mandestrobin) on five species of arthropod were assessed in a laboratory study. Each test item was applied to the plants at a rate of 200 ppm a.s.. The results of the study indicated that none of the test items showed any insecticidal activity to the insects tested.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** DX-CA-S-2200 (metabolite of mandestrobin)  
**Lot/Batch:** 089-160803-1  
**Purity:** 99.9%  
  
**Test material:** S-2200 (mandestrobin)  
**Lot/Batch:** No060912-1  
**Purity:** 99.7%

### B. STUDY DESIGN AND METHODS

1. **Test species:** Diamondback moth (*Plutella xylostella*)  
Cotton aphid (*Aphis gossypii*)  
Tobacco whitefly (*Bemisia tabaci*)  
Brown planthopper (*Nilaparvata lugens*)  
Two-spotted spider mite (*Tetranychus urticae*)

2. **Experimental method:**

12 mg of crystals of each chemical along with 7 mg of solid spreader adjuvant (polyoxyethylene alkyl ether fulfate ammonium salt) was crushed by an exclusively designated homogenizer. This homogenizing procedure was done with adding 60 mL of water in order to avoid generation of heat (200 ppm a.s.).

#### Diamondback moth (*Plutella xylostella*)

The test chemical solution prepared as described above was sprayed to a cabbage seedling in 5 leaf stage. Then 3<sup>rd</sup>-instar larvae of diamondback moth were released onto the seedling one hour after the spraying.



Mortality of the larvae was determined 4 days after release of the larvae. The results were expressed in numbers 0, 1, 2, 3 and 4 in accordance with the following criteria:

- 0: Mortality is 0 or < 30%
- 1: Mortality is  $\geq 30\%$  and < 60%
- 2: Mortality is  $\geq 60\%$  and < 80%
- 3: Mortality is  $\geq 80\%$  and < 100%
- 4: 100% mortality

#### **Cotton aphid (*Aphis gossypii*)**

The test chemical solution prepared as described above was sprayed to a cucumber seedling in 1 leaf stage, which was infested with approximately 30 aphids. Mortality was determined 6 days after treatment. The results were expressed in numbers 0, 1, 2, 3 and 4 in accordance with the following criteria:

- 0: Mortality is 0 or < 30%
- 1: Mortality is  $\geq 30\%$  and < 60%
- 2: Mortality is  $\geq 60\%$  and < 90%
- 3: Mortality is  $\geq 90\%$  and < 100%
- 4: 100% mortality

#### **Tobacco whitefly (*Bemisia tabaci*)**

The test chemical solution prepared as described above was sprayed to a tomato seedling in 2-3 leaf stage, which was infested with approximately 100 nymphae. Efficacy was determined 7 days after treatment and results expressed in accordance with the same criteria described above for the cotton aphid (*Aphis gossypii*).

#### **Brown planthopper (*Nilaparvata lugens*)**

The test chemical solution prepared as described above was sprayed to rice seedlings (2 leaf stage) grown in a plastic cup. Twenty nymphae of brown planthopper were released onto the seedling. Mortality was determined 6 days after releasing of nymphae. The results were expressed in in accordance with the same criteria described above for the cotton aphid (*Aphis gossypii*).

#### **Two-spotted spider mite (*Tetranychus urticae*)**

The test chemical solution prepared as described above was sprayed to a seedling of kidney bean, which had a pair of primary leaves and was infested with 40 individuals of *T. urticae*. The efficacy was determined 7 days after spraying, based on damage on leaves. The results were expressed in numbers 0, 1, 2, 3 and 4 in accordance with the following criteria

- 0: Damage is  $\geq 61\%$  and <100%
- 1: Damage is  $\geq 41\%$  and <60%
- 2: Damage is  $\geq 11\%$  and <40%
- 3: Damage is  $\geq 1\%$  and <10%
- 4: 0% damage

## **II. RESULTS AND DISCUSSION**

Neither DX-CA-S-2200 nor S-2200 (mandestrobin) demonstrated any insecticidal activity on any of the species tested at a concentration of 200 ppm a.s. (i.e. in all cases, for both test items and the untreated controls, mortality and damage was rated as 0).

## **III. CONCLUSION**

The results of this study indicate that DX-CA-S-2200 and S-2200 (mandestrobin) did not show any insecticidal activity to the insects tested.

Assessment and Conclusion by Applicant:	The results of this study indicate that DX-CA-S-2200 and S-2200 (mandestrobin) did not show any insecticidal activity to the insects tested.
---	--

Comments of zRMS:	The study was not evaluated as it was performed with metabolite of active substance. The study should be submitted and evaluated at the EU level during active substance renewal.
-------------------	---

<b>Data point:</b>	KCP 10.7/03
<b>Report author:</b>	Suemoto, H.
<b>Report year:</b>	2017
<b>Report title:</b>	Comparison of Fungicidal Activity of DX-CA-S-2200 with S-2200
<b>Report No.:</b>	ROG-0006
<b>Document No.:</b>	ROG-0006
<b>Guidelines followed in study:</b>	Not applicable
<b>Deviations from current test guideline:</b>	Not applicable
<b>Previous evaluation:</b>	No, not previously evaluated
<b>GLP/Officially recognised testing facilities:</b>	No, not conducted under GLP/Officially recognised testing facilities
<b>Acceptability/Reliability:</b>	Yes

### Executive Summary

The effects of DX-CA-S-2200 on a strain of white mould (*Sclerotinia sclerotiorum*) compared to S-2200 (mandestrobin) were assessed in a laboratory study. The test concentrations were 0.008, 0.04, 0.2, 1.0 and 5.0 ppm. The results of the study indicated that DX-CA-S-2200 did not show any fungicidal activity to the test strain. This result indicates that DX-CA-S-2200 is not a relevant metabolite of S-2200 (mandestrobin).

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** DX-CA-S-2200 (metabolite of mandestrobin)  
**Lot/Batch:** 089-160803-1  
**Purity:** 99.9%  
  
**Test material:** S-2200 (mandestrobin)  
**Lot/Batch:** No060912-1  
**Purity:** 99.7%

### B. STUDY DESIGN AND METHODS

- Test species:** White mould (*Sclerotinia sclerotiorum*)  
 Strain JPU: susceptible to S-2200

#### 2. Experimental method:

The mycelia of the test strain, *Sclerotinia sclerotiorum*, was inoculated onto potato dextrose agar containing both 100 ppm of salicylhydroxamic acid (SHAM) and the predetermined concentration of the test chemicals (0.008, 0.04, 0.2, 1.0 and 5.0 ppm) on 8<sup>th</sup> February 2017. An untreated control (UTC) containing the test strain inoculated onto potato dextrose agar containing 100 ppm of SHAM was also incubated. The radius of the mycelial growth was measured after incubation for one day at 23°C on 9<sup>th</sup> February 2017.

## II. RESULTS AND DISCUSSION

The results from the study are summarised in the following table (Table 10.7/03-1). DX-CA-S-2200 did not show any fungicidal activity to the test strain (i.e.  $EC_{50} > 5$  ppm). However, the  $EC_{50}$  for S-2200 (mandestrobin) was 0.020 ppm.

**Table 10.7/03-1: Effects of DX-CA-S-2200 and S-2200 (mandestrobin) on *Sclerotinia sclerotiorum***

Test chemicals	Concentration (ppm)	Radius (mm)			Growth Inhibition (%)
		Rep. 1	Rep. 2	Mean	
DX-CA-S-2200	0.008	20	20	20	0
	0.04	21	19	20	0
	0.2	20	20	20	0
	1.0	21	20	20.5	0
	5.0	20	20	20	0
$EC_{50} > 5.0$ ppm					
S-2200 (mandestrobin)	0.008	12	13	12.5	37.5
	0.04	8	8	8	60
	0.2	4	4	4	80
	1.0	0	0	0	100
	5.0	0	0	0	100
$EC_{50} = 0.020$ ppm					
UTC containing 100 ppm of SHAM	0.0	20	20	20	-

## III. CONCLUSION

The results of this study indicate that DX-CA-S-2200 did not show any fungicidal activity to the test strain. This result indicates that DX-CA-S-2200 is not a relevant metabolite of S-2200 (mandestrobin).

Assessment and Conclusion by Applicant:	The results of this study indicate that DX-CA-S-2200 did not show any fungicidal activity to the test strain. This result indicates that DX-CA-S-2200 is not a relevant metabolite of S-2200 (mandestrobin) for fungicidal activity.
Comments of zRMS:	The study was not evaluated as it was performed with metabolite of active substance. The study should be submitted and evaluated at the EU level during active substance renewal.

### A 2.9 KCP 10.8 Monitoring data