FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: PP-113H

Product name(s): BARILOCHE / BARILOCHE 100

Chemical active substance:

Clopyralid 100 g/L (10% w/v) SL

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: PROPLAN Plant Protection Company, SL

Submission date: December 2021

MS Finalisation date: **August 2022; November 2022**

New version: April 2023 corrected I 04.2023; corrected II 06.2023 January 2024

Version history

When	What
February 2019	Initial version
December 2021	Version 2, Update for the renewal.
August 2022	Assessment dRR art.43
October 2022	Version 3, applicant's update after assessment by zRMS
April 2023	Revision based on comments received
June 2023	The final version of RR after II commenting period.
January 2024	The final version of RR after 3rd round of commenting period

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9 Ecotoxicology (KCP 10)

The product BARILOCHE (Clopyralid 10% w/v SL), is currently registered in Italy (16096), Spain (ES-00493), UK (Re. No. 17577), Poland (Reg. No. R-26/2018wu), Germany (Reg. No. 008865-00), Czech Republic (Reg. No. 5583-0) and Romania (Reg. No. 466PC) in Sugar beet.

This new dossier has been carried out to support the renewal of the approval of the active substance Clopyralid.

All the changes that have been made in this section, with respect to the original dossier, have been highlighted in yellow. It must be taken into account that the format of the dossier has changed.

9.1 Critical GAP and overall conclusions

Table 9-1: Critical use pattern of the formulated product

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmen-tal stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	C. EU (CZ, DE, PL, RO)	Sugar beet	F	CIRAR and COMPOSITAE	Tractor boom sprayer	BBCH 10-39	1	1.2	120	80-400	-	Do not use between the 31st August and 1st March#

* 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 applicatio

Explanation for column 15 “Conclusion”

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

* 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

The risk assessment was calculated for 125 g/ha as worst case except risk assessment for *A.rhopalosiphi* and non-target plants. In this case, the risk assessment was calculated for 120 g/ha.

9.1.1 Overall conclusions

zRMS comments:

This report was prepared following renewal of the active substance clopyralid. As a result all authorisations of plant protection products containing clopyralid have to be renewed in order to comply with the new list of endpoints EFSA Journal 2018;16(7):5389. The dRR was prepared by Applicant. All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

9.1.1.1 Effects on birds (KCP 10.1.1)

Clopyralid passed at the screening stage for both dietary and reproductive assessments. The Log Pow of Clopyralid are both below 3, thus the risks from secondary poisoning to birds does not require assessment. No specific calculations of exposure for birds through drinking water for the puddle scenario were necessary.

9.1.1.2 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

Clopyralid passed the acute dietary assessment. The reproductive assessment found that clopyralid passed at the screening stage. No specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. The Log P_{ow} of Clopyralid meant that the risks from secondary poisoning to mammals does not require assessment.

9.1.1.3 Since the Log Pow of Clopyralid (pH dependent and ranging between -1.81 & -2.55) is < 3 the risks from secondary poisoning to mammals does not require assessment.

ZRMS comments:

The risk assessment at screening step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of clopyralid for mammals were confirmed based on TERA and TERLT above the trigger values of 10 and 5, respectively. Based on the intended use on for **BARILOCHE** no unacceptable risk for mammals is expected from acute or long-term exposure.

The risk assessment was calculated for 125 g/ha were accepted as worst case.

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

There is currently no agreed guidance for the effects on reptiles and amphibians.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

Clopyralid poses an acceptable risk at STEPS 1&2. The formulation did not show an unacceptable risk..

9.1.1.6 Effects on bees (KCP 10.3.1)

HQ values on an acute timescale for the product, clopyralid were below the trigger value and thus do not pose an unacceptable risk to bees.

9.1.1.7 Effects on arthropods other than bees (KCP 10.3.2)

Low risk was not demonstrated at first tier testing for the product, PP-113 H so higher tier testing was conducted. Tier 2 level testing showed that the product poses a low risk to non-target arthropods.

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

Clopyralid on an acute timescale and PP-113 H on a chronic timescale were found not to pose an unacceptable risk to non-target soil meso- and macrofauna.

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

PP-113 H had an observed effect at the GAP application rate. A quantitative risk assessment was required and found that the most sensitive species passed at tier 2 meaning no mitigation was needed.

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

The effect of PP-113 H on the nitrogen transformation in soil is considered negligible. It is concluded that PP-113 H has no long-term effects on the nitrogen transformation activity of soil microflora.

9.1.2 Grouping of intended uses for risk assessment

No grouping or risk envelope was applied.

9.1.3 Consideration of metabolites

There are no metabolites of concern for either active ingredient of PP-113 H.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with clopyralid. The risk assessment for birds was carried out following the latest Guidance of EFSA Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12):1438. European Food Safety Authority), hereafter cited as EFSA (2009).

Table 9-2 holds the endpoints used in assessing the ecotoxicological risk to birds from clopyralid. This information was sourced from Appendix A to the conclusion on the peer review of the pesticide risk assessment of the active substance clopyralid. EFSA Journal 2018 and hereafter referred to as EFSA (2018).

Table 9-2: Endpoints and effect values relevant for the clopyralid risk assessment for birds

Species	Substance	Exposure	Results	Endpoint used
Mallard duck *(Anas platyrhynchos)*	a.s.	Acute	LD_{50} 1465 mg a.s./kg b.w.	LD_{50} 1465 mg a.s./kg b.w.
		Long-term	NOEL 118 mg a.s./kg b.w/d	NOEL 118 mg a.s./kg b.w/d

9.2.2 Risk assessment for spray applications

9.2.2.1 Screening assessment (indicator species)

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

Table 9-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of clopyralid in Sugar Beet (BBCH 10-39 at 125 g a.s./ha)

Intended use	Sugar Beet				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 × 125				
Acute toxicity (mg/kg bw)	1465				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
BBCH 10-39	Small omnivorous bird	158.8	1	19.85	73.8
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-39	Small omnivorous bird	64.8	0.53	4.29	27.5

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.

The above TER_a and TER_{lt} values exceed their respective thresholds at the screening level and, as such, higher tiered assessment is not required for clopyralid.

ZRMS comments:
The risk assessment at screening step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of clopyralid for birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for **BARILOCHE** no unacceptable risk for birds is expected from acute or long-term exposure.
The risk assessment was calculated for 125 g/ha were accepted as worst case.

9.2.2.2 Drinking water exposure

Given the proposed use of PP-113 H, only the puddle drinking water scenario is considered relevant for birds.

Puddle scenario

EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./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} > 500 L/kg).

The K_{OC} for Clopyralid is ~~4.9~~ 1.41 L/kg and as it is < 500 L/kg so the trigger of 50 is relevant. The application rate of 125 g a.s/ha results in ratios of 0.085, using the acute endpoint of 1465 mg a.s/kg b.w, and 1.06 using the NOAEL reproductive endpoint of 118 mg a.s/kg b.w. Again here, these results indicate that no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary. In conclusion, the acute and reproductive risk to birds from contaminated drinking water is acceptable.

ZRMS comments: Due to the ratio of the total annual application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the relevant critical value for at least one use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

9.2.2.3 Effects of secondary poisoning

Since the Log P_{ow} of Clopyralid (pH dependent and ranging between -1.81 & -2.55) is < 3 the risks from secondary poisoning to birds does not require assessment.

ZRMS comments: The risk assessment for earthworm-eating birds via secondary poisoning is not required as active sub-stance has log Pow<3.

9.2.3 Overall conclusions

With the acute dietary and reproductive assessments for birds for, clopyralid is found to pass at the screening stage.

Since the Log Pow of Clopyralid (ranging between -1.81 & -2.55) is below 3, the risks from secondary poisoning to birds does not require assessment.

Given the proposed use of PP-113 H, only the puddle drinking water scenario is considered relevant for birds. EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./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} > 500 L/kg).

The K_{OC} for Clopyralid is ~~4.9~~ 1.41 L/kg and as it is < 500 L/kg so the trigger of 50 is relevant. The application rate of 125 g a.s/ha results in ratios of 0.085, using the acute endpoint of 1465 mg a.s/kg b.w, and 1.06 using the NOAEL reproductive endpoint of 118 mg a.s/kg b.w. Again here, these results indicate that no specific calculations of exposure for birds through drinking water for the puddle scenario are neces-

sary. In conclusion, the acute and reproductive risk to birds from contaminated drinking water is acceptable.

Again here, these results indicate that no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary. In conclusion, the acute and reproductive risk to birds from contaminated drinking water is acceptable.

ZRMS comments:
The acute and chronic risks of **BARILOCHE** to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active substances, and maximum residues occurring on food items. For active substance all TER values exceed the relevant triggers indicating that **BARILOCHE** does not pose an unacceptable risk to birds following applications according to recommended use pattern. Evalua-tion of exposing to mammals through the drinking water demonstrated the acceptable risk.
The risk to earthworm - and fish-eating animals from secondary poisoning is low.
The risk assessment was calculated for 125 g/ha were accepted as worst case.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with clopyralid. The risk assessment for mammals was carried out following the latest EFSA guidance for the risk assessment for birds and mammals (EFSA, 2009).

Table 9- holds the endpoints used in assessing the ecotoxicological risk to mammals from clopyralid. This information was sourced from the Appendix A to the Conclusion on the peer review of the pesticide risk assessment of the active substance clopyralid (EFSA, 2018).

Table 9-4: Endpoints and effect values relevant for the clopyralid risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	a.s.	Acute	LD_{50} >5000 mg a.s./kg b.w.	LD_{50} >5000 mg a.s./kg b.w.
Rabbit		Long-term	NOAEL 50 mg a.s./kg b.w/d	NOAEL 50 mg a.s./kg b.w/d

9.3.2 Risk assessment for spray applications

9.3.2.1 Screening assessment

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

Table 9-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of clopyralid in Sugar Beet (BBCH 10-39 at 125 g a.s./ha)

Intended use	Sugar beet				
Active substance/product	clopyralid				
Application rate (g/ha)	1 × 125				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
BBCH 10-39	Small herbivorous mammal	118.4	1	14.8	337.8
Reprod. toxicity (mg/kg bw/d)	50				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-39	Small herbivorous mammal	48.3	0.53	3.20	15.63

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.

The above TER_a and TER_{lt} values both exceed their respective thresholds and, as such, higher tiered assessment is not required for clopyralid.

ZRMS comments:

The risk assessment at screening step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of clopyralid for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for **BARILOCHE** no unacceptable risk for mammals is expected from acute or long-term exposure.

The risk assessment was calculated for 125 g/ha were accepted as worst case.

9.3.2.2 Drinking water exposure

Given the proposed use of PP-113 H, only the puddle drinking water scenario is considered relevant for mammals.

Puddle scenario

The K_{OC} for Clopyralid is ~~4.9~~ 1.41 L/kg and as it is < 500 L/kg so the trigger of 50 is relevant. The application rate of 125 g a.s/ha results in ratios of 0.085, using the acute endpoint of 1465 mg a.s/kg b.w, and 1.06 using the NOAEL reproductive endpoint of 118 mg a.s/kg b.w. Again here, these results indicate that no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary. In conclusion, the acute and reproductive risk to birds from contaminated drinking water is acceptable.

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EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./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} > 500 L/kg).

The K_{OC} for Clopyralid is ~~4.9~~ 1.41 L/kg and as it is < 500 L/kg so the trigger of 50 is relevant. The application rate of 125 g a.s/ha results in ratios of 0.025, using the acute endpoint of 5000 mg a.s/kg b.w, and 12.5, using the NOAEL reproductive endpoint of 50 mg a.s/kg b.w. Again here, these results indicate that no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. In conclusion, the acute and reproductive risk to mammals from contaminated drinking water is acceptable.

ZRMS comments: Due to the ratio of the total annual application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the relevant critical value for at least one use scenario, a quantitative risk assessment (calculation of TER values) is not necessary

9.3.2.3 Effects of secondary poisoning

Since the Log P_{ow} of Clopyralid (pH dependent and ranging between -1.81 & -2.55) is < 3 the risks from secondary poisoning to mammals does not require assessment.

ZRMS comments: The risk assessment for earthworm - eating mammals via secondary poisoning is not required as active substance has log Pow<3.

9.3.3 Overall conclusions

For the mammalian acute dietary and reproductive risk assessments, it was found that both clopyralid passed the acute dietary assessment with TER value of 337.8. For the reproductive risk assessment, clopyralid passes the screening stage with a TER of 15.63.

EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./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} > 500 L/kg).

The K_{OC} for Clopyralid is ~~4.9~~ 1.41 L/kg and as it is < 500 L/kg so the trigger of 50 is relevant. The application rate of 125 g a.s/ha results in ratios of 0.025, using the acute endpoint of 5000 mg a.s/kg b.w, and 12.5, using the NOAEL reproductive endpoint of 50 mg a.s/kg b.w. Again here, these results indicate that no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. In conclusion, the acute and reproductive risk to mammals from contaminated drinking water is acceptable.

Since the Log P_{ow} of Clopyralid (pH dependent and ranging between -1.81 & -2.55) is < 3 the risks from secondary poisoning to mammals does not require assessment.

ZRMS comments:
The risk assessment at screening step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of clopyralid for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for **BARILOCHE** no unacceptable risk for mammals is expected

from acute or long-term exposure.

The risk assessment was calculated for 125 g/ha were accepted as worst case.

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

There is currently no agreed guidance on performing risk assessment for effects on reptiles and amphibians and so completing this section is not required.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with, clopyralid, and the formulation (PP-113 H). Full details of these studies are provided in the respective list of endpoint documentation.

Clopyralid

The endpoints stated in the table below were used for the aquatic risk assessment carried out with clopyralid (EFSA, 2018).

Table 9-6: Endpoints and effect values relevant for the risk assessment for aquatic organisms – clopyralid (EFSA 2018). Lowest RAC is denoted in bold font

TER for Most Sensitive Organism - Clopyralid					
Active Substance / Metabolite & Species	**Time Scale / TER Trigger**	**Endpoint**	**Toxicity (ug a.s. /L)**	**RAC**	**Source**
Clopyralid					
O. mykiss (fish)	Acute / 100	LC_{50}	> 99900	999	
P. promelas (fish)	Chronic / 10	NOEC	10800	1080	
D. magna (aquatic invertebrates)	Acute / 100	EC_{50}	> 99000	990	EFSA (2018)
D. magna (aquatic invertebrates)	Chronic / 10	NOEC	17000	1700	
C. riparius (sediment dweller)	Chronic / 10	NOEC	50000	5000	
S. capricornutum (algae)	Chronic / 10	E_rC_{50}	30000	3000	
M. spicatum (higher plant)	Chronic / 10	E_rC_{50}	3000	300	

Formulation PP-113 H (clopyralid 10%)

The endpoints stated in the table below were carried out using the PP-113 H formulation.

Table 9-7: Formulation endpoints and effect values relevant for the risk assessment for aquatic organisms – PP-113 H (Clopyralid 10%). Lowest RAC is denoted in bold font

TER for Most Sensitive Organism – PP-113 H					
Active Substance / Metabolite & Species	**Time Scale / TER Trigger**	**Endpoint**	**Toxicity (ug prod./L)**	**Toxicity (ug a.s./L)**	**Source**
PP-113 H					
D. magna (aquatic invertebrates)	Acute / 100	EC_{50}	100000	9500	Teodosi (2011)
P. subcapitata (algae)	Chronic / 10	E_rC_{50}	100000	9500	Teodosi (2011)
Lemna gibba (higher plant)	Chronic / 10	E_rC_{50}	673100	63400	Juckeland (2012)

In order to answer the requirement from the zRMS an study for *Myriophyllum spicatum* has been included. The study was carried out with the product Faworyt 300 SLthat is considered worse case to PP-113 H. Comparation between formulation has been included in the Part C.

Species	Substance	Exposure System	**Results**	Reference
Myriophyllum spicatum	Faworyt 300 SL	14 d,s	**ErC_{50} = 10.038 mg/L** $_{mm}$ ErC_{50} = 2.68 mg a.s./L $_{mm}$ (average spcific growth rate for dry weight) NOEC = 9.53 mg /L	Faworyt 300 SL Water-sediment *Myriophyllum spictum* toxicity test according to OECD 239; A. Kamińska; 2019; study code: 0016/0061/E

9.5.1.1. Justification for new endpoints

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

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" (EFSA Journal, 2013;11(7):3290, 268 pp.) and hereafter referred to as EFSA (2013).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern are presented in the tables below. Additionally, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group. Values in excess of 1 would be considered to not pose an acceptable risk.

Table 9-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Clopyralide for each organism group based on FOCUS Steps 1 & 2 calculations for the use of PP-113 H in sugar beet

Group		Fish acute	Fish prolonged	Invert. acute	Invert. prolonged	Algae	Sed. dwell. prolonged	Higher-tier information
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***S. capricornutum***	***C. riparius***	***M. spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	E_rC_{50}
(µg/L)		99900	10800	99000	17000	30000	50000	3000
AF		100	10	100	10	10	10	10
RAC (µg/L)		999	1080	990	1700	3000	5000	300
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	42.7381	0.04	0.04	0.04	0.02	0.01	0.01	0.014
Step 2								
N-Europe	5.6355	0.01	0.01	0.01	0.01	0.00	0.00	0.03
S-Europe	10.1061	0.01	0.01	0.01	0.00	0.00	0.00	0.02
Step 3								
D3/ditch	0.6652	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D4/pond	0.04972	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D4/stream	0.5602	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R1/pond	0.02700	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R1/stream	0.6150	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R3/stream	4.470	0.01	0.01	0.01	0.01	0.00	0.00	0.01

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

With respect to clopyralid for the proposed GAP application and timing, it can be seen that the ratio of PEC/RAC does not exceed the threshold of 1 for any species

under STEPS 1 or 2 for any scenario. Thus, clopyralid is noted to pose an acceptable risk to the aquatic compartment and its associated species. Escalation to STEP 3 was not necessary since PECsw values at STEP 2 do not exceed the lowest RAC for clopyralid (*M. spicatum* at 300 ug/L). This being said, STEP 3 was conducted. There are no scenarios where clopyralid exceeds its threshold at STEP 3.

Table 9-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for formulation for each organism group based on FOCUS Steps 1 & 2 calculations for the use of PP-113 H in sugar beet

Group		**Invert. acute**	**Algae**	**Higher-tier information**	**Higher-tier information**
Test species		***D. magna***	***P. subcapitata***	***L. gibba***	***M. spicatum***
Endpoint		EC_{50}	E_rC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		9500	9500	63400	2680
AF		100	10	10	10
RAC (µg/L)		95	950	6340	268
FOCUS Scenario	**PEC_{max} (µg/L)**				
STEP1					
	42.7381	0.45	0.045	0.06	0.16

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

With respect to PP-113 H for the proposed GAP application and timing, it can be seen that the ratio of PEC/RAC does not exceed the threshold of 1 for any of the tested species or taxa based on the formulated product in surface water. Since the PEC/RAC ratios do not exceed the value of 1, it can be determined that the formulation PP-113 H does not pose an unacceptable risk to the aquatic compartment or to the species which it contains.

9.5.3 Overall conclusions

With respect to clopyralid for the proposed GAP application and timing, it can be seen that the ratio of PEC/RAC does not exceed the threshold of 1 for any species under STEPS 1, 2 an STEP 3 for any scenario. Thus, clopyralid is noted to pose an acceptable risk to the aquatic compartment and its associated species. Since clopyralid fell below the lowest RAC (*M. spicatum* at 300 ug/L) at STEPS 1, 2 an STEP 3, higher tier testing was not required.

ZRMS comments: The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290). The ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for a.s.- clopyralid and for product **BARILOCHE** based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organism. The risk assessment was calculated for 125 g/ha were accepted as worst case.

However, as aquatic plants are the most sensitive group of aquatic organisms, further studies should be provided at Member State level. The study with *Myriophyllum* should be conducted in accordance with OECD 239 and the root weight and the shoot weight should be measured separately. A final conclusion on the risk to the aquatic environment from the formulation **BARILOCHE** can only be drawn after the studies with the formulation and aquatic plants are made available. This should be addressed during product authorisation at Member State level.

DATA GAP:
In case formulation BARILOCHE:
1. Risk assessment for aquatic plants (*M. spicatum*) has been not performed (insufficient data set - data gap).
2. The new study the product **BARILOCHE** and *M.spicatum* should be performed.

Data requirement

In order to answer the requirement from the zRMS a study for *Myriophyllum spicatum* has been included. The study was carried out with the product Faworyt 300 SL that is considered worse case to **BARILOCHE**. (The comparison of BARILOCHE and Faworyt 300 SL is detailed in Section C).
The zRMS agrees that FAWORYT 300 SL can be considered as a worse case than BARILOCHE for the ecotoxicology studies. Comparation between formulation has been included in the attached Part C. The new risk assessment based on the study with the product Faworyt 300 SL was accepted by zRMS. The ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for for product **BARILOCHE** and *M.spicatum* based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organism.
Further action is not needed.

Final decision should be taken into account at MSs level.

9.6 Effects on bees (KCP 10.3.1)

Since this registration report is being submitted as a zonal dossier, the bee risk assessment is thus based on the EPPO bee guidance from 2010 (EPPO Standard PP3/10 (3) Environmental risk assessment scheme

for plant protection products. Chapter 10: Honeybees), as updated from the EPPO 2001 guidance, which is referred to in the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002); hereafter referred to as EC (2002).

9.6.1 Toxicity data

Application of PP-113 H can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food. The following tables are the EU agreed endpoints used for the bee risk assessment.

Clopyralid

Table 9-2: Endpoints for clopyralid: Ecotoxicological endpoints for bees (EFSA 2018)

Test substance	Endpoints	Species
Clopyralid	Oral LD_{50} >100 µg a.s/bee	*Apis Mellifera L.*
	Contact LD_{50} >98.1 µg a.s/bee	*Apis Mellifera L.*

PP-113 H Formulation

Table 9-3: Endpoints for formulation PP-121 H: Ecotoxicological endpoints for bees

Test substance	Endpoints	Species	Source
PP-121 H	Oral LD_{50} >100 µg FP*/bee	*Apis Mellifera L.*	Barcarotti R.,(2001). BT 102/11
	Contact LD_{50} >100 µg FP*/bee		
	10 day feeding Chronic Oral LDD_{50} > 54.20 µg a.i/bee/day LC_{50} > 4000 mg a.i/L diet		Ansaloni T. (2020) Study Code: S19-03760
	22 day NOEC 622.28 mg a.i/Kg diet		Ansaloni T. (2020) Study Code: S19-03761

* Note that FP stands for formulated product.

9.6.2 Risk assessment

9.6.2.1 Hazard quotients for bees (Acute)

Table 9-12: First-tier assessment of the acute risk for bees due to the use of clopyralid in sugar beet (125 g a.s./ha)

Intended use	sugar beet
Active substance	clopyralid
Application rate (g/ha)	1 × 125

Test design	LD_{50} (lab.) (µg/bee)	HQ	Trigger
Oral toxicity	>100	1.25	50
Contact toxicity	>98.1	1.27	

Table 9-4: First-tier assessment of the acute risk for bees due to the use of the PP-113 H formulation in sugar beet (125 g a.s./ha)

Product	PP-113 H formulation
Active substances	10 % clopyralid
Application rate (g/ha)	1.25 L/ha eq 1314.5 gr/ha based on an application rate of 1250 L/ha and a formulation density of 1.0516 g/ml

Test design	LD_{50} (lab.) (µg/bee)	HQ	Trigger
Oral toxicity	> 100	13.1	<50
Contact toxicity	> 100	13.1	

HQ values calculated for clopyralid and formulation are below the trigger value of 50 as stipulated under SANCO/10329/2002 guidelines meaning the they do not pose an unacceptable risk to bees.

ZRMS comments:

The risk assessment was provided and assessed during first registration of the product **BARILOCHE** in 2013. According to recommendation given in "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). Based on the acute risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values for adult bees from exposure of **BARILOCHE** are < 50, indicating un acceptable risk to adult bees. The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of **BARILOCHE**. In addition, the chronic studies for bees were submitted by the applicant. The risk assessment based on these studies should be considered when GD for Bees, 2013 is implemented at EU level. **Final decision should be taken into account at MSs level.**

The risk assessment was calculated for 125 g/ha were accepted as worst case.

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

Higher tier testing was not required as the risk to bees was addressed with laboratory testing.

9.6.3 Hazard Quotients for Bees (Chronic)

Risk assessment of chronic effects on honeybees is not required under SANCO/10329/2002.

9.6.4 Effects on bumble bees

Risk assessment of effects on bumble bees is not required under SANCO/10329/2002.

9.6.5 Effects on solitary bees

Risk assessment of effects on solitary bees is not required under SANCO/10329/2002.

9.6.6 Overall conclusions

HQ values on an acute time-scale were calculated for the formulated product and clopyralid and are all below the trigger value of 50 as stipulated under SANCO/10329/2002 guidelines meaning that the use of PP-113 H in line with the proposed GAP does not pose an unacceptable risk to bees.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with clopyralid and the PP-113 H formulation. Full details of these studies are provided in the respective list of endpoints documentation as previously mentioned (clopyralid from EFSA 2018). The relevant are presented in the following tables.

Table 9-15: Endpoints and effect values relevant for the risk assessment for non-target arthropods from clopyralid (EFSA, 2018)

Species	Stage	Application (g a.s. / ha)	Effects	Reference
Aphidius rhopalosiphi Tier 1	Adult	>200	LR_{50}	EFSA (2018)
Typhlodromus pyri Tier 1	Protonymphs	>200	LR_{50}	

Table 9-14: Endpoints and effect values relevant for the risk assessment for non-target arthropods from PP-113 H

Species	Stage	Endpoint (g f.p. / ha)	Effects	Reference
Aphidius rhopalosiphi Tier 2 - Extended Laboratory (3-D)	Adult	>3154	LR_{50}	Corbolli M. (2011) BT098/11
Typhlodromus pyri	Protonymphs	3154	LR_{50}	Corbolli M. (2011)

Species	Stage	Endpoint (g f.p. / ha)	Effects	Reference
Tier 2 - Extended Laboratory (2-D)				BT098/11
O. laegivatus Tier 2 - Extended lab study (2-D)	Adult	>2.5 l pf/ha	LR_{50}	Luna (2020) Study Code: S19-03762
C. septempunctata L Tier 2 - Extended lab study (2-D)	Larvae	>2.5 l pf/ha	LR_{50}	Luna (2020) Study Code: S19-03763

The density of the formulation (FP) is Density: 1.0516 g/mL and was used to calculate the application rate in g FP/ha from that of L FP/ha.

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 referred to hereafter as EC 2002, and in consideration of the recommendations of the guidance document ESCORT 2 and ESCORT 3 (SETAC, 2002 & 2012).

9.7.2.1 Risk assessment for in-field exposure

Non-target arthropods living in the crop can be exposed to residues from PP-113H by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. PP-113H is applied at a maximum rate of 1.25 L formulation/ha. The maximum in-field exposure (Predicted Environmental Rate, PER) to foliar-dwelling or soil-dwelling organisms is therefore 1.25 L formulation/ha.

The in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{in-field} = Application\ rate\ (g\ ai/ha) \times MAF$$

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values following four applications are given in the ESCORT 2 Guidance Document. Since PP-113H is applied only once a season, the multiple application factor MAF, can be omitted.

The maximum predicted environmental residues (PER) occurring within the field after application of PP-113H at the maximum application rate is 1250 mL f.p/ha or 125 g as/ha.

Table 9-15: Risk assessment of the in-field risk for non-target arthropods due to the use of PP-113 H in sugar beet (1.25 L/ha)

Intended use	sugar beet
Active substance/product	PP-113 H
Application rate (g/ha)	1 x 1.25 L formulation /ha (density of 1.0516 g/mL)
MAF	1

Test species Higher-tier	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
A. rhopalosiphi (Tier II)	>3154 993.22*	1314 1261	0.4 **1.26**
T. pyri (Tier II)	3154	1314	0.4
P. cupreus (Tier II)	>2.5 l f.p/ha	1.25 l f.p/ha	<0.50
C. septempunctata (Tier II)	>2.5 l f.p/ha	1.25 l f.p/ha	<0.50

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
*ER_{50} based on effect on reproduction for 1 x 1.20 L formulation/ha (density of 1.0516 g/mL)

Testing was performed with Tier 2 extended laboratory studies on *T. pyri, A. rhopalosiphi*, *P.cupreus* and *C. septempunctata* with an application equal or higher than that proposed for PP-113 H. No effect on mortality or reproduction reached 50% for any species and therefore the in-field risk to non-target arthropods from the use of PP-121 H is acceptable.

ZRMS comments:
The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2**.** The calculations of the risk assessment for in – field for 4 indicator species - *T. pyri, A. rhopalosiphi*, *P.cupreus* and *C. septempunctata* based on extended laboratory studies were accepted by zRMS as HQ values were below 1 for these species. No effect on mortality or reproduction reached 50% for any species and therefore the in-field risk to non-target arthropods from the use of **BARILOCHE** is acceptable. The risk assessment was calculated for 125 g/ha were accepted as worst case.

The risk assessment was calculated for 125 g/ha were accepted as worst case.

ZRMS comments: The calculations of the risk assessment for in – field for *Aphidius rhopalosiphi* based on reproduction effect for extended laboratory studies was performed by zRMS. zRMS used the lowest toxicity endpoints from study based on reproduction parameter (the worst case – ER_{50} = 993.22 g/ha). HQ values is slightly above 1 for this species – **1.26** (and $PER_{in\text{-}field}$ is slightly above rate with ≤ 50 % effect on reproduction), indicating further refinement. However - in this case - ZRMS proposes to accept the risk. (The hazard ratio is only slightly above the threshold 1 (being 1.26), no significant effects for mortality was observed for the highest tested dose as 3154 g formulation/ha (2.5x$PER_{in\text{-}field}$), the calculations of the risk assessment for in–field for 3 others species - *T. pyri,, P.cupreus and C. septempunctata* based on extended laboratory were accepted by zRMS as HQ values were below 1 for these species. No effect on mortality or reproduction reached 50% for *T. pyri, P.cupreus and C. septempunctata.*

The risk assessment for *A.rhopalosiphi* was calculated for 120 g/ha. The risk assessment for *T. pyri,*

P.cupreus and C. septempunctata was calculated for 125 g/ha were accepted as worst case. Finally - in-field risk assessment to non-target arthropods from the use of **BARILOCHE** is acceptable.

Additionally, the new risk assessment based on the study FAWORYT 300 SL was performed by RMS. FAWORYT 300 SL can be considered as a worse case than BARILOCHE for the ecotoxicology studies. Comparation between formulation has been included in the attached Part C.

Faworyt 300 SL

Aphidius rhopalosiphi	Formulation: Faworyt 300 SL	Extended study,barley plants	LR_{50}/ER_{50} > 400 ml prod-uct/ha (121 g a.s./ha)	Moll M., 2019, Study No. 140601002

In-field risk assessment for *Aphidius rhopalosiphi* based on study with Faworyt 300 SL

Test species Tier II (Extended lab testing)	**ER_{50}**	**$PER_{in\text{-}field}$**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	> 0.4 L/ha, equivalent to 121 g a.s./ha	1.2 L/ha, equivalent to 120 g a.s./ha	Yes; (< 1.0)

$PER_{in\text{-}field}$ is below rate with ≤ 50 % effect on reproduction for *Aphidius rhopalosiphi* based on study with Faworyt 300 SL. In opinion RMS, in this case in-field risk assessment for *A. rhopalosiphi* is accepted indicating no further action is needed (FAWORYT 300 SL can be considered as a worse case than BARILOCHE).

Final decision for in-field risk assessment for *Aphidius rhopalosiphi* for BARILOCHE should be taken into account at MSs level.

9.7.2.2 Risk assessment for off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to PP-113H will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the *BBA (2000)*[1] as shown in the following equation:

$$\text{Off - field foliar PER} = \frac{\text{Maximum in - field foliar PER x (\% drift/100)}}{\text{vegetation distributi on factor}}$$

[1] 90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The drift value at 1 m distance is 2.77% of the application rate (90th percentile drift). The drift factor (% drift/100) is therefore 2.77/100 = 0.0277.

The resulting PERoff-field values are

Table 9-16: Off-field foliar Predicted Environmental Rates (PER)

Study type	Maximum in-field foliar PER (g pf/ha)	drift factor (% drift/100)	Vegetation distribution factor and 5 (2D)**, n.a. (3D)***	Off-field foliar PER g formulation/ha)
	1314	2.77	10	3.64 7.28*

**According to Working document on Risk Assessment of Plant Protection Products in the Central Zone (CZSC, May 2021) VDF of 5 should be used for all the tiers of the assessment as an interim solution until the revision of the current risk assessment scheme.
*** not applicable

Table 9-6: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of PP-121 H in sugar beet (1.25 L/ha)

Intended use	sugar beet
Active substance/product	PP-113 H
Application rate (g/ha)	1 x 1.25 L formulation /ha (density of 1.0516 g/mL)
MAF	1

Test species Higher-tier	LR_{50} (lab.) (g/ha)	$PER_{in\text{-}field}$ (g/ha)	$HQ_{in\text{-}field}$ criterion: HQ ≤ 1
A. rhopalosiphi (Tier II) (3-D)	>3154** 993.22*	3.64	0.001 0.00366
A. rhopalosiphi (Tier II) (3-D)	>3154	3.64	0.001
T. pyri (Tier II) (2-D)	3154	3.64 7.28	0.001 0.0023
P. cupreus (Tier II) (2-D)	>2.5 l f.p/ha	0.0034 l f.p/ha 0.0069 l f.p/ha	0.001 0.00276
C. septempunctata (Tier II) (2-D)	>2.5 l f.p/ha	0.0034 l f.p/ha 0.0069 l f.p/ha	0.001 0.00276

*ER_{50} based on effect on reproduction

**LR_{50} based on mortality parameter

Testing was performed with Tier 2 extended laboratory studies on *T. pyri, A. rhopalosiphi, P.cupreus* and *C. septempunctata* with an application equal or higher than that proposed for PP-113 H. No effect on mortality or reproduction reached 50% for any species and therefore the off-field risk to non-target arthropods from the use of PP-121 H is acceptable.

ZRMS comments:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2. The calculations of the risk assessment for off – field for 4 indicator species - *T. pyri, A. rhopalosiphi, P. cupreus* and *C. septempunctata* based on extended laboratory studies were accepted by zRMS as HQ values were below 1 for these species. In addition, based on the results from extended laboratory tests for 4 indicator species - *T. pyri, A. rhopalosiphi, P. cupreus* and *C. septempunctata* the $PER_{in\text{-}field}$ of **BARILOCHE** the risk off -field for these species is considered acceptable as PEROff-field was below rate with ≤50 % effect. Finally, the risk off-field for NTA is considered acceptable.
The risk assessment was calculated for 125 g/ha were accepted as worst case.

The calculations of the risk assessment for off–field for *Aphidius rhopalosiphi* based on reproduction effect for extended laboratory studies was performed by zRMS. zRMS used the lowest toxicity endpoints from study based on reproduction parameter (the worst case – ER_{50} = 993.22 g/ha). HQ values is below 1 and $PER_{off\text{-}field}$ is below rate with ≤ 50% effect on reproduction, indicating no further refinement is needed. Finally-off-field risk assessment to non-target arthropods from the use of **BARILOCHE** is acceptable. No further action is required.

Final decision for off-field risk assessment for *Aphidius rhopalosiphi* for **BARILOCHE** should be taken into account at MSs level.

9.7.2.3 Risk mitigation measures

No risk mitigation is needed.

9.7.3 Overall conclusions

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 SETAC (Society of Environmental Toxicology and Chemistry) 2001 "ESCORT 2" and 2012 ESCORT 3 guidance documents.

Studies on the toxicity to non-target arthropods were carried out with clopyralid and the PP-113 H formulation. Full details of these studies are provided in the respective list of end-points documentation as previously mentioned (EC 2008, EFSA 2018).

Low risk was not demonstrated of PP-113 H for *T. pyri, A. rhopalosiphi, P.cupreus* and *C. septempunctata*. As such, PP-122 H can be seen to pose an acceptable risk to non-target arthropods.

ZRMS comments:

Accepted.

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

9.8.1 Toxicity data

Studies on the toxicity of earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with clopyralid and the PP-121 H formulation. Full details of these studies are provided in the respective list of endpoints documentation as previously mentioned (EFSA 2018).

Table 9-177: 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
Eisenia fetida	Clopyralid	Overspray / 10 % peat content	28 d NOEC = 1.97 mg/kg sdw	EFSA 2018
Folsomia candida	GF-1374 (Clopyralid representative formulation)	Mixed into soil	NOEC = 50 mg/kg sdw	EFSA 2018
Hypoaspis aculeifer	GF-1374 (Clopyralid representative formulation)	Mixed into soil	NOEC = 100 mg/kg sdw	EFSA 2018
Eisenia fetida	*PP-121 H*	*Acute*	*LC50 > 1000 mg f.p./kg*	*Tediosi E. (2011) DR-CH60511*
Eisenia fetida	~~PP-121 H~~ PP-113 H	56-day exposure Mixed into soil / 10 % peat content	NOEC = 91.59 mg f.p /kg dw, equivalent to 8.92 mg a.s./kg dw	Anton.B (2020) Study code: S20-02714
Folsomia candida	~~PP-121 H~~ PP-113 H	28-day exposure Mixed into soil 5% sphagnum peat	NOEC = 308.64 mg f.p./kg dw, equivalent to 30.07 mg a.s./kg dw	Anton.B (2020) Study code: S20-02712
Hypoaspis aculeifer	PP-113 H	Mixed into soil 14 d, chronic 5 % peat content	NOEC = >2250 mg f.p /kg dw, equivalent to = > 219.20 mg s.a./kg dw	Lozano.J (2020) Study Code: S120-02713

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 Eco-toxicology” (EC 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate),. According to the assessment of environmental-fate data, multi-annual accumulation in soil for clopyralid was calculated to be 0.133 mg a.s./kg, respectively.

To achieve a concise risk assessment, the risk assessment was conducted for the formulation PP-113 H Here, the assessment for the use of PP-113 H to sugar beet with a (BBCH 10-39) rate of 1.25 L formulation/ha covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna).
The PECmax for the for the use of PP-113 H to sugar beet was 1.4016 mg f.p/kg

Table 9-18: 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 PP-113 H in sugar beet (1.25 L/ha at BBCH 10-39)

Intended use	Sugar beet		
Acute effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 10)**
~~PP-113 H~~	~~1000~~	~~1.4016~~	~~713~~
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
PP-113 H	91.59	~~1.4016~~ 1.345	~~65.34~~ 68.09
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
PP-113 H	308.64	~~1.4016~~ 1.345	~~220~~ 229.47
PP-113 H	2250	~~1.4016~~ 1.345	~~1589~~ 1672.86

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

~~The acute risk assessment for earthworms has shown that for clopyralid, their TER values far exceed the threshold value of 10, thus indicating that the active ingredients do not pose an unacceptable risk to earthworms.~~ On a chronic timescale, the product is tested against *Eisenia, F. candida and H. aculeifer*. The TER calculations evidence values that far exceed the threshold value of 5. As such, the product PP-113 H can be seen not to pose an unacceptable risk to non-target soil meso- and macrofauna.

ZRMS comments:

The relevant PECsoil for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). The intended is are covered by the presented PEC_{soil} calculations, however zRMS performed new calculations PEC_{soil} (mg/kg) for proposed use in GAP 120 g/ha. The TER_{LT} values for active substance and for product are above trigger value of 5, indicating an acceptable risk for earthworm and soil macroorganism for proposed use of the product **BARILOCHE.** The assessment acute toxicity study on earthworms was not finally considered because acute data are no more appropriate for the risk evaluation.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on the effects to soil microorganisms have been carried out with clopyralid and the PP-121 H formulation. Full details of these studies are provided in the respective list of endpoints documentation as previously mentioned (EC 2008, EFSA 2018).

Table 9-19: Endpoints and effect values relevant for the risk assessment for soil microorganisms for clopyralid, and the formulation PP-121 H

Endpoint	Substance	Exposure System	Endpoint	Reference
N-mineralisation	clopyralid	56 d, aerobic soil type	209 mg/kg soil dw	EFSA (2018)
	SP-1274 (clopyralid representative formulation)	28 d, aerobic soil type	12.9 mg/kg soil dw	
N-mineralisation	PP-113 H	28 d, aerobic soil type	4.6 and 23.2µL/kg No effects	Dottorini, F.(2011). BT 154/11

9.9.2 Risk assessment

To achieve a concise risk assessment, the risk assessment was conducted for the formulation PP-113 H. Here, the assessment for the use of PP-113 H has been applied at low dose of 3l/ha and a high dose of 15 l/ha without effects.

Table 9-20: Assessment of the risk for effects on soil micro-organisms due to the use of PP-113 H in sugar beet (BBCH 10-39, 1.25 L formulation/ha)

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**effects**	**Risk acceptable?**
PP-121 H	3l f.p/ha 15l f.p/ha	No effects No effects	yes

In order to answer the requirement from the zRMS an se study effects on the nitrogen transformations has been included.
The study was carried out with the product Faworyt 300 SLthat is considered worse case to PP-113 H.
Comparation between formulation has been included in the Part C.

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Faworyt 300 SL	28 d, aerobic	No negative effect > 25% at 28 d at 3.7152 mg product/kg dws	Woźniak A., 2021 Study code: 0016/0138/E

Intended use	sugar beet		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Faworyt 300 SL * equivalent to mg s.a./kg dw	3.7152 1.11456*	1.345 0.133*	YES YES

9.9.3 Overall conclusions

The effect of PP-113 H on the nitrogen transformation in soil is considered negligible. It is concluded that PP-121 H has no long-term effects on the nitrogen transformation activity of soil microflora.

ZRMS comments:
The risk assessment for soil micro-organism after exposure of ppp **BARILOCHE** couldn't be performed by the zRMS-PL. The study effects on the nitrogen transformations are not accepted. RMS pointed out that The Applicant should provide the following data in this study:
☑ calculation of soil nitrate-N transformation rate.

DATA GAP:

1. Calculation of soil nitrate-N transformation rate in the effect of **BARILOCHE** on the nitrogen transformation in soil study should be provided. After providing complementary information to this study, the study will be reassessed by RMS. The risk assessment for microorganism will be performed after supplementing provided by the Applicant.
2. Risk assessment for soil microorganisms has been not performed (insufficient data set - data gap).

Data requirement:
In order to answer the requirement from the zRMS a study effect on the nitrogen transformation has been included. The study was carried out with the product Faworyt 300 SL that is considered worse case to Bariloche. (The comparison of BARILOCHE and Faworyt 300 SL is detailed in Section C).

N-mineralisation

Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC_{soil} (mg/kg dw)	Risk acceptable?
Faworyt 300 SL	3.7152	1.345	YES

The new risk assessment based on the study with the product Faworyt 300 SL was accepted by zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of **BARILOCHE**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **BARILOCHE.**
Further action is not needed.

Final decision should be taken into account at MSs level.

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

9.10.1 Toxicity data

Studies on the effects to non-target terrestrial plants have been carried out with, clopyralid and the PP-113 H formulation. Full details of these studies are provided in the respective list of endpoints documentation as previously mentioned (EFSA 2018).

Consideration of toxicity for the active substances is not considered to be necessary. The clopyralid endpoints are derived from representative formulations at Annex I. Therefore, consideration of the formulation toxicity to non-target plants is considered to be sufficient.

Table 9-21: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Lactuca sativa [d]	Clopyralid			EFSA 2018
	~~CF 1374 (clopyralid representative formulation)~~ Clopyralid	Vegetative Vigour	ER_{50} = 33.78 g a.s./ha	
Glycine max [d]	Clopyralid	Vegetative Vigour	ER_{50} = 21.47 g a.s./ha	

Species	Substance	Exposure System	Results	Reference
Brassica napus $_d$ *Sinapis alba* $_d$ *Daucus carota* $_d$ *Medicago sativa* $_d$ *Glycine max* $_d$ *Solanum lycopersicum* $_d$ *Hordeum vulgare* $_m$ *Triticum aestivum* $_m$ *Sorghum halepense* $_m$ *Zea mays* $_m$	PP-113 H	Seedling emergence	Lowest ER_{50} = 0.19 l prod/ha (*Medicago sativa)*	Corboli M, 2012 Study Code: BT 101/11
		Vegetative vigour	Lowest ER_{50} = 0.59 l prod/ha (*Glycine max*)	Corboli M, 2012 Study Code: BT 100/11

m: monocotyledonous; *d*: dicotyledonous

In order to answer the requirement from the zRMS an se study effects on vegetative vigor has been included.
The study was carried out with the product Faworyt 300 SLthat is considered worse case to PP-113 H.
Comparation between formulation has been included in the Part C.

Species	Substance	Exposure System	Results	Reference
Cucumber $_d$ Carrot $_d$ Broccoli $_d$ Mung bean $_d$ Oat $_m$ Corn $_m$	Faworyt 300 SL	21 d Vegetative vigour	[1)] ER_{50} survival > 0.4 L product/ha (all tested species) [2)] ER_{50} plant fresh weight = 0.211 L product/ha (carrot) [3)] ER_{50} plant dry weight = 0.151 L product/ha (Mung bean) [3)] ER_{50} plant height > 0.4 L product/ha (all species) [4)] ER_{50} phytotoxicity = 0.170 L product/ha (carrot)	Kamińska A., 2019 with amendment by Woźniak A., 2021 Study code: 0016/0060/E

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

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

The proposed application rate for PP-113 H is 1.25 L/ha, 1250 ml prod./ha. Since >50% effects were observed at 190 ml prod./ha, a quantitative risk assessment is required.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology" (EC 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9-22: Assessment of the risk for non-target plants due to the use of PP-113 H sugar beetl (BBCH 10-39 – 1.25 L product /ha)

Intended use	Sugar beet			
Active substance/product	PP-113 H			
Application rate (g/ha)	1 × 1.25 L product/ha			
MAF	1			
Test species	**ER_{50} (ml prod./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ ml/ha)**	**TER criterion: TER ≥ 5**
Medicago sativa	190	2.77	34.6	5.5

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

As the TER for *Glycine max* is above the threshold of 5, it is considered to have passed the risk assessment at Tier 2 and is thus seen as not posing an unacceptable risk to non-target plants.

Intended use	Sugar beet			
Active substance/product	clopyralid/PP113H			
Application rate	1 × 1.25 L product/ha (1× 125 g ai/ha)			
MAF	n.a.			
Test species	**ER_{50}**	**Drift rate**	**$PER_{off\text{-}field}$**	**TER criterion: TER ≥ 5**
Cucumber *d* Carrot *d* Broccoli *d* Mung bean *d* Oat *m* Corn m	ER_{50} plant fresh weight = 0.151 L product/ha Equivalent to 45.3 g s.a./ha	2.77%	0.0346 L product/ha Equivalent to 3.4625 g s.a./ha	~~**4.36**~~ 13

~~**Risk assessment for non-target terrestrial plants due to the use of PP113H in sugar beet considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)**~~

~~**Intended use**~~	~~Sugar beet~~

Active substance/product		clopyralid/PP113H			
Application rate		1 × 1.25 L product/ha (1× 125 g ai/ha)			
MAF		n.a.			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (L product/ha)**	**$PER_{off-field}$ 50 % drift red. (L product/ha)**	**$PER_{off-field}$ 75 % drift red. (L product/ha)**	**$PER_{off-field}$ 90 % drift red. (L product/ha)**
1	2.77	0.0346	0.0173	-	-
5	0.57	0.0071	-	-	-
Toxicity value		**TER**			
ER_{50} = 0.151 (L product/ha)		**criterion: TER ≥ 5**			
1		4.4	8.7	-	-
5		21.3	-	-	-

ZRMS comments:
zRMS comment: The risk assessment for non-target plants after exposure of ppp **BARILOCHE** couldn't be performed by the zRMS-PL. The study to determine a potential phytotoxic effect of the product **BARILOCHE** for non-target plant species in terms of vegetative vigour are not accepted. Although the validity criteria are met, the study cannot be accepted by RMS. Due to an inadequately selected dose range, in this case, ER_{50} based on phytotoxicity effect cannot be determined. Even at the lowest tested concentration at 1 L **BARILOCHE**/ha, the phytotoxicity effect was above 75% (chlorosis). *All phytotoxicity endpoint should be considered in the risk assessment, in line with EFSA Technical Report (2019), i.e. all effects and endpoints will be reported in the study summary and the lowest endpoint should be used by the zRMS ensuring a harmonized risk assessment at zonal level.* Therefore, the *new study to determine a potential phytotoxic effect of the product **BARILOCHE** for non-target plant species in terms of vegetative vigour should be performed.*

Peer review of the pesticide risk assessment of the active substance clopyralid in 2018 also confirmed that a data gap was identified for a new study with non-target plants for the formulation which should be addressed at Member States level.

DATA GAP:
1. The new study to determine a potential phytotoxic effect of the product **BARILOCHE** for non-target plant species in terms of vegetative vigour should be performed including phytotoxicity effect.
2. Risk assessment for non-target plants has been not performed (insufficient data set - data gap).

The risk assessment for non-target plants will be performed after supplementing provided by the Applicant.

Data requirement:
In order to answer the requirement from the zRMS a study effect on vegetative vigour has been included. The study was carried out with the product Faworyt 300 SL that is considered worse case to Bariloche.

Risk assessment for non-target terrestrial plants due to the use of BARILOCHE in sugar beet considering risk mitigation (in field no spray buffer zones, and drift reducing nozzles)

~~Intended use~~		~~Sugar beet~~			
~~Active substance/product~~		~~clopyralid/BARILOCHE~~			
~~Application rate~~		~~1 × 1.25 L product/ha (1× 125 g ai/ha)~~			
~~MAF~~		~~n.a.~~			
~~Buffer strip (m)~~	~~Drift rate (%)~~	~~$PER_{off\ field}$ (L product/ha)~~	~~$PER_{off\ field}$ 50 % drift red. (L product/ha)~~	~~$PER_{off\ field}$ 75 % drift red. (L product/ha)~~	~~$PER_{off\ field}$ 90 % drift red. (L product/ha)~~
~~1~~	~~2.77~~	~~0.0346~~	~~0.0173~~	~~-~~	~~-~~
~~5~~	~~0.57~~	~~0.0071~~	~~-~~	~~-~~	~~-~~
~~Toxicity value~~		~~TER~~			
~~ER_{50} = 0.151 (L product/ha)~~		~~criterion: TER ≥ 5~~			
~~1~~		~~4.4~~	~~8.7~~	~~-~~	~~-~~
~~5~~		~~21.3~~	~~-~~	~~-~~	~~-~~

~~The new risk assessment based on the study with the product **Faworyt 300 SL** was accepted by zRMS.~~

~~The risk based on the ER_{50} = 0.151 L formulation/ha value *(Mung bean)* from vegetative vigour test and $PER_{off\ field}$, indicated needs for further refinement.~~
~~The risk following mitigation measures are proposed: **BARILOCHE** achieve the acceptability criteria TER ≥ 5 with applying:~~
- ~~5 m buffer zone without drift reducing nozzles~~
- ~~1 m and use of 50% drift reducing nozzles~~

~~Final decision should be taken into account at MSs level.~~

Updated June 2023
For the risk assessment of NTP, the new study with formulation Faworyt 300 SL was used (is considered worse case to Bariloche). Therefore, for the risk assessment the endpoint from this study should be expressed as amount of active substance/ha and not as amount of amount PPP/ha. In particular because the formulations are not identical and therefore the application rate for Bariloche and the endpoint for Faworyt 300 SL cannot be used in the evaluation. (The comparison of BARILOCHE and Faworyt 300 SL is detailed in Section C).

Intended use	Sugar beet
Active substance/product	clopyralid/PP113H
Application rate	1 × 1.25 L product/ha (1× 125 g ai/ha)
MAF	n.a.

Test species	**ER_{50}**	**Drift rate**	**$PER_{off\text{-}field}$**	**TER criterion: TER ≥ 5**
Cucumber Carrot Broccoli Mung bean Oat Corn	ER_{50} plant fresh weight = 0.151 L product/ha Equivalent to 45.3 g s.a./ha	2.77%	0.0346 L product/ha Equivalent to 3.4625 g s.a./ha	13

The risk based on the ER_{50} = 0.151 L formulation/ha value *(Mung bean)* from vegetative vigour test and $PER_{off\text{-}field}$, not posing an unacceptable risk. The refinement risk assessment is not needed.

The final version of RR after 3rd round of commenting period

The risk assessment is finally performed based on a new study data. The study was carried out with the product Faworyt 300 SL that is considered worse case to Bariloche.

Problem description (DE comment): Currently, it is assumed that the formulation Faworyt 300 SL is worst case in comparison to PP-113 H and, thus, can be taken for the risk assessment of the later. This makes sense looking at the formulation data. With respect to the NTTP data, however, more considerations might be needed. Although, the study with PP-113 H was not accepted by the zRMS, this is not due to a missing validity (the study was fully valid), but the fact that phytotoxic effects were observed even in the lowest doses. Therefore, the effect value for phytotoxic effects should be below lowest ER_{50} of that test, which is currently 0.19 L product/ha (for *Medicago sativa*). In case the test with Faworyt 300 SL is worst case for PP-113 H the lowest ER_{50} of the test with Faworyt 300 SL should be lower. This is the case for the unit expressed in L product/ha (the ER_{50} is 0.151 L product/ha). However, a comparison based with respect to the active substance leads to the fact that the formulation is not worst case for PP-113 H. The recalculated endpoints would be equivalent to 45.3 g a.s./ha (Faworyt 300 SL) vs. 19.5 g a.s./ha (PP-113 H). The endpoint expressed in g a.s./ha would be 2.3 times lower for the actual formulation and, in addition, according to the test this isn't even based on the most sensitive endpoint (because high phytotoxicity is already occurring at the lowest concentration). Even though we are late in the authorization procedure we would advise reconsidering if an extrapolated test which is not worst case should be the sole base for risk assessment.

zRMS comments: There are no methods for quantitative analysis of the phytotoxicity parameter. The toxicity endpoint based on the phytotoxicity parameter may be subject to uncertainty as it is estimated based on the expert judgment of a laboratory test rather than on quantitative chemical analysis. Let us anticipate that in the future there will be a refined method for measuring this parameter and this will be taken into account in the current methodology for evaluation of OECD 227 study. In this case, the method of estimating this parameter raises great uncertainty. Additionally, after converting the toxicity endpoint from L formulation/ha to g a.s.a.L, it can be seen that the use in the evaluation of the study for FAWORYT 300 SL, although the composition of the agent appears to be the worst case, in practice this is not in this case. The endpoint expressed in g a.s./ha would be 2.3 times lower for the actual formulation and, in addition, according to the test this isn't even based on the most sensitive endpoint (because high phytotoxicity is already occurring at the lowest concentration). Even though we are late in the authorization procedure we consider adding an additional uncertainty factor to address the additional uncertainty in this situation (uncertainties = extrapolation with not worst case formulation as well as phytotoxic effects even in the lowest concentration with the actual formulation). We propose used the recalculated endpoints 45.3 g a.s./ha (Faworyt 300 SL) divided by 10 for risk assessemnt. ER_{50} = 4.53 g a.s.ha with risk mittigation to protect non-target plant.

Risk assessment for non-target terrestrial plants due to the use of BARILOCHE in sugar beet considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use	**Sugar beet**
Active substance/product	**clopyralid/BARILOCHE**
Application rate	**(1× 120 g ai/ha)**
MAF	**n.a.**

Buffer strip (m)	Drift rate (%)	$PER_{off-field}$ (L product/ha)	$PER_{off-field}$ 50 % drift red. (L product/ha)	$PER_{off-field}$ 75 % drift red. (L product/ha)	$PER_{off-field}$ 90 % drift red. (L product/ha)
1	2.77	3.324	1.662	0.831	-
5	0.57	0.684	-	-	-
Toxicity value		TER			
ER_{50} = 4.53 g a.s./ha		criterion: TER ≥ 5			
1		1.3	2.73	5.45	-
5		6.36	-	-	-

The new risk assessment based on the study with the product **Faworyt 300 SL** was accepted by zRMS.

The risk based on the ER_{50} = 4.53 g s.a./ha *(Mung bean)* from vegetative vigour test and $PER_{off-field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: **BARILOCHE** achieve the acceptability criteria TER ≥ 5 with applying:
- 5 m buffer zone without drift-reducing nozzles
- 1 m and use of 75% drift reducing nozzles

On the other hand the lowest toxicity endpoint for Faworyt 300 SL and non-target plants is ER_{50} = 0.031 L product/ha (mung bean) (seedling emergence test based on plant fresh weight), equivalent to 9.3 g a.s./ha. Based on this toxicity endpoint the Faworyt 300 SL is the worst case compared on formulation Bariloche.

Final decision should be taken into account at MSs level.

9.10.2.3 Risk mitigation measures

~~No risk mitigation needed.~~

The risk based on the ER_{50} = 4.53 g s.a./ha *(Mung bean)* from vegetative vigour test and $PER_{off-field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: **BARILOCHE** achieve the acceptability criteria TER ≥ 5 with applying:
- 5 m buffer zone without drift-reducing nozzles
- 1 m and use of 75% drift reducing nozzles

9.10.3 Overall conclusions

~~Consideration of toxicity for the active substances is not considered to be necessary. For non-target plants and the clopyralid endpoints are derived from representative formulations at Annex I. Therefore, consideration of the formulation toxicity to non-target plants is considered to be sufficient. As the proposed application rate for PP 113 H is 1.25 L/ha, 1250 ml/ha; since >50% effects were observed at 190 ml/ha, a quantitative risk assessment was required.~~

~~The tier 2 results for the most sensitive species, *Medicago sativa*, was found to pass with a TER value of 5.5, which stemmed from a drift value of 0.0277 (1 m) and an ER_{50} of 190 ml/ha.~~

The risk assessment is finally performed based on a new study data. The study was carried out with the product Faworyt 300 SL that is considered worse case to Bariloche.(The comparison of BARILOCHE and Faworyt 300 SL is detailed in Section C).

TER_{LT} for all use patterns of BARILOCHE are above the trigger of 5 when the either 75% drift reduction or a 5 m unsprayed buffer zone is applied to non-crop land.

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

No information

9.12 Monitoring data (KCP 10.8)

No information

9.13 Classification and Labelling

Use of PP-113 H at the proposed label rates, with a minimum buffer according to different MS practises for all proposed crops when applied adjacent to water and according to good agricultural practice, poses low risk to all non-target species.

10 References

EC (2002) European Commission Services Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2 (final)

EFSA (2009) Guidance of EFSA - Risk Assessment for Birds and Mammals. EFSA Journal;7(12):1438

EFSA (2013) EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

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

FOCUS (2001) FOCUS groundwater scenarios in the EU review of active substances. Sanco/321/2000 rev. 2 Version 2.2.

SETAC (2002) ESCORT 2 – Linking Non-target arthropod testing and risk assessment with protection goals.

SETAC (2012) ESCORT 3 – Linking Non-target arthropod testing and risk assessment with protection goals: Hotel Zuiderduin, Egmond Aan Zee, the Netherlands, 8-11 March 2010

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Data protection claimed Y/N	Owner
KCP 10.2.1	Tediosi E., Garagna D.	2011	PP-113H (clopyralid 10 % w/v sl): acute toxicity to Daphnia magna in a 48-hour immobilization test under static exposure Chemservice S-L.R. Report CH-602-2011 GLP Unpublished	N	Y	PROPLAN
KCP 10.2.1	Tediosi E., Dini R.	2011	PP-113H (CLOPYRALID 10 % w/v SL):toxicity to green algae Pseudokirchneriella subcapitata determined in a growth inhibition study Chemservice S-L.R. Report CH603-2011 GLP :yes Unpublished	N	Y	PROPLAN

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.2.1	Juckeland D.	2012	Effects of PP-113H (Clopyralid 10% w/v SL) on Lemna minor in a growth inhibition test under static test conditions Bio Chem Agrar Report 12 10 48 004 w GLP: yes Unplubished	N	Y	PROPLAN
KCP 10.2.1	Kamińska A.	2019	Water-sediment Myriophyllum spicatum toxicity test according to OECD 239 Sorbolab Research Labolatory LLC 0016/0061/E GLP Unpublished	N	CIECH Sarzyna S.A.	Kamińska A.
KCP 10.3.1	Barcarotti M.	2011	EFFECTS, ACUTE ORAL AND ACUTE CONTACT TOXICITY, OF PP-113H (Clopyralid 10% w/v SL) ON THE HONEYBEE APIS MELLIFERA L. IN THE LABORATORY (LIMIT TEST) Biotecnologie BT Document No: BT102/11 GLP YES Unpublished	N	Y	PROPLAN

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	Data protection claimed Y/N	Owner
KCP 10.3.1	Ansaloni T.	2020	PP-113H (Clopyralid 100 g/L SL): Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee, Apis mellifera L. under Laboratory Conditions Trialcamp S-L.U Report Nº: S19-03760 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.3.1	Ansaloni T.	2020	PP-113H (Clopyralid 100 g/L SL): Honey Bee (Apis mellifera L.) Larval Toxicity Test Following Repeated Exposure Under Laboratory Conditions Trialcamp S-L.U Report Nº: S19-03761 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.3.2	Colli, M.	2011	Effects of the product PP-113H (CLOPYRALID 10% w/v SL) on the aphid parasitoid Aphidius rhopalosiphi De Stefani-Perez (Hymenoptera: Braconidae) under Extended Laboratory Conditions (Rate Response Test) Biotecnologie BT Document No: BT098/11 GLP yes Unpublished	N	Y	PROPLAN

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.3.2	Colli, M.	2011	Effects of the product PP-113H (CLOPYRALID 10% w/v SL)on the predatory mite, Typhlodromus pyri Scheuten (Acari: Phytoseiidae) under Extended Laboratory Conditions (Rate Response Test) Biotecnologie BT Document No: BT099/11 GLP yes Unpublished	N	Y	PROPLAN
KCP 10.3.2	Luna F.	2020	PP-113H (Clopyralid 100 g/L SL): Toxicity to the Predatory Bug, Orius laevigatus Fieber (Heteroptera, Anthocoridae) Using an Extended Laboratory Test with Freshly Applied Spray DepositsTrialcamp S-L.U Report Nº: S19-03762 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.3.2	Luna F.	2020	PP-113H (Clopyralid 100 g/L SL): Toxicity to the Ladybird, Coccinella septempunctata L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits Trialcamp S-L.U Report Nº: S19-03763 GLP YES Unpublished	N	Y	PROPLAN

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.4	Tediosi E.,Dini R.,	2011	PP-113H (clopyralid 10 % w/v sl):acute toxicity to earthworm determined in an artificial soil study Chemservice S-L.R. Document No: DR-CH60511 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.4	Anton B.	2020	PP-113H (Clopyralid 100 g/L SL): Sublethal Toxicity to the Earthworm Eisenia fetida (Oligochaeta, Lumbricidae) in Artificial Soil with 10 % Peat Trialcamp S-L.U Report Nº: S20-02714 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.4	Anton B.	2020	PP-113H (Clopyralid 100 g/L SL): Effects on the Reproductive Output of the Springtail Folsomia candida Willem (Collembola, Isotomidae) in Artificial Soil Trialcamp S-L.U Report Nº: S20-02712 GLP YES Unpublished	N	Y	PROPLAN

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.4	Lozano J.	2020	PP-113H (Clopyralid 100 g/L SL): Effects on the Reproductive Output of the Predatory Soil Mite Hypoaspis (Geolaelaps) aculeifer Canestrini (Acari: Laelapidae) in Artificial Soil Trialcamp S-L.U Report Nº: S20-02713 GLP YES Unpublished	N	Y	PROPLAN
KCP 10.5	Dottorini, F.	2011	Assessment of the effects of PP-113H (CLOPIRALIDE 10% w/v SL) on soil microorganism respiration and nitrification Biotecnologie BT Document No: BT154/11 GLP Yes Unpublished	N	Y	PROPLAN
KCP 10.5	Woźniak A.	2021	Study of impact of test item Faworyt 300 SL on soil microorganisms - nitrogen transformation test according to guideline OECD 216 SORBOLAB Research Laboratory LLC Study code: 0016/0138/E GLP Unpublished	N	CIECH Sarzyna S.A.	Woźniak A.

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.6.2	Corbolli M,	2011	Vegetative vigour rate response test for non-target plants following application of the product PP-113H (Clopyralid 10% w/v SL) Biotecnologie BT Document No: BT 100/11 GLP yes Unplubished	N	Y	PROPLAN
KCP 10.6.2	Kamińska A.	2019	Vegetative Vigour Test according to OECD 227 SORBOLAB Research Laboratory LLC Study code: 0016/0060/E GLP Unpublished	N	CIECH Sarzyna S.A.	KCP 10.6_02
10.6.2a	Woźniak A.	2021	Annex No. 1 to the Final report: Vegetative Vigour Test according to OECD 227 SORBOLAB Research Laboratory LLC Study code: 0016/0060/E GLP Unpublished	N	CIECH Sarzyna S.A.	10.6_02a

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**	**Data protection claimed Y/N**	**Owner**
KCP 10.6.1	Corboli, M.	2012	"Seedling emergence rate response test for non-target plants following application of the product PP-113H (Clopyralid 10% w/v SL)" Biotecnologie BT Document Nº: BT 101/2011 GLP: yes Unplubished	N	Y	PROPLAN

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 – 10.6	EFSA (European Food Safety Authority)	2018	Conclusion on the peer review of the pesticide risk assessment of the active substance clopyralid. EFSA Journal 2018;16(7):5389, 28 pp. doi:10.2903/j.efsa.2018.5389 EFSA (European Food Safety Authority) GLP Published	Y	EFSA (European Food Safety Authority)

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

List of data relied on not submitted by the applicant but necessary for evaluation

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 Study 1: Acute toxicity to daphnia

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. **Agreed endpoints:** The obtained experimental results allowed calculating the IC_0, IC_{100} and the IC_{50} at 24 and 48 hours. The results, in terms of test item nominal concentrations, were as follows:

Time (h)	EC_0 (mg/L)	EC_{100} (mg/L)	EC_{50} (mg/L)
24	≥ 100.0	> 100.0	> 100.0
48	≥ 100.0	> 100.0	> 100.0

	The results, in terms of nominal active ingredient clopyralid concentrations, were as follows:			
	Time (h)	EC_0 (mg a.i. /L)	EC_{100} (mg a.i. /L)	EC_{50} (mg a.i. /L)
	24	≥ 9.50	> 9.50	> 9.50
	48	≥ 9.50	> 9.50	> 9.50

Reference	KCP 10.2.1/01
Report:	Tediosi E.,Garagna D.,2011 PP-113H (clopyralid 10 % w/v slow): acute toxicity to D*aphnia magna* in a 48-hour immobilization test under static exposure Report Nº CH-602-2011
Guidelines	OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The acute toxicity of the test item PP-113H (Clopyralid 10 % w/v SL) to *Daphnia magna* was determined in a 48-hour static test according to the OECD Guideline for Testing of Chemicals, No. 202 (2004) and to Council Regulation EC 440/2008 (C.2).

For this purpose, juvenile daphnias' (< 24 hours old at test initiation) were exposed to an aqueous test medium containing the test item at five different concentrations, namely 4.3, 9.4, 20.7, 45.5 and 100.0 mg/L, corresponding to 0.41, 0.89, 1.97, 4.32, and 9.50 mg/L as active ingredient Clopyralid (corrected for its purity, 9.5 % w/w).

The exposed organisms were checked for immobilization 24 and 48 hours after test initiation.

The pH of the test media after preparation was in the range 6.77 – 7.35 for test item solutions and 6.98 for negative control.
The incubation temperature was in the range 19.3 – 22.7 °C, thus slightly over the OECD recommended range (20 ± 2 °C). This did not affect the results of the test, because it happened only for a very short period of time.
Light intensity during the 48 hours of test period was in the range 825 - 832 Lux, thus within the provided range 700-1000 Lux.

The actual test concentrations of active ingredient, Clopyralid, were analytically measured at the beginning and at the end of the test period.

In the fresh solutions the average analytical recovery of the a.i. concentrations was 103.8 % of the nominal values (range: 98.7 % - 105.8 %); after 48 hours of exposure the a.i. concentration was stable, being the average analytical recovery 102.5 % (range: 96.3 % - 104.6 %).

As recommended by the OECD Guidance Document on testing of difficult substances Nr 23 (2000), the biological results refer to the nominal concentrations of the active ingredient, since all the a.i. analytical recoveries during the test period were in the range 80 % – 120 %.
Moreover the toxic endpoints were calculated in terms of nominal test item concentrations.

At the end of test period, in the negative control 0 % of immobilization was observed and no daphnia was found to be trapped on the test water surface. These values comply with the validity criterion of the test, that provide a maximum immobilization and/or a maximum number of daphnias' trapped into water surface of 10% in the negative control medium at the end of the test.

The obtained experimental results allowed calculating the IC_0, IC_{100} and the IC_{50} at 24 and 48 hours.
The results, in terms of test item nominal concentrations, were as follows:

Time (h)	IC_0 (mg/L)	IC_{100} (mg/L)	IC_{50} (mg/L)
24	≥ 100.0	> 100.0	> 100.0
48	≥ 100.0	> 100.0	> 100.0

The results, in terms of nominal active ingredient Clopyralid concentrations, were as follows:

Time (h)	IC_0 (mg a.i. /L)	IC_{100} (mg a.i. /L)	IC_{50} (mg a.i. /L)
24	≥ 9.50	> 9.50	> 9.50
48	≥ 9.50	> 9.50	> 9.50

The IC_{50} values were determined from the Raw Data by the statistical analysis in comparison with the negative control (linear interpolation analysis), while IC_0 and IC_{100} values at each observation time were directly extracted from the Raw Data.

Data requirement
zRMS updated dRR B9 in terms of tabular information on the dose response, e.g. a table listing dose and effect data, and recovery to the dRR for the sake of transparency:

Analytical recovery of the measured test concentrations to the nominal ones

Time (hours)	Nominal test item concentration [mg/L]	Nominal a.i. Clopyralid concentration [mg a.i./L]	Actual a.i. Clopyralid concentration [mg a.i./L]	analytical recovery [%]
0	4.3	0.41	0.40	98.7
0	9.4	0.89	0.93	104.2
0	20.7	1.97	2.06	104.7
0	45.5	4.32	4.57	105.6
0	100.0	9.50	10.05	105.8
48	4.3	0.41	0.39	96.3
48	9.4	0.89	0.92	103.3
48	20.7	1.97	2.05	104.4
48	45.5	4.32	4.52	104.6
48	100.0	9.50	9.86	103.8

Immobilization effect of PP-113H (Clopyralid 10 % w/v SL) to *Daphnia magna*

Nominal test item concentration [mg/L]	No. of *Daphnia* tested	No. of immobilized *Daphnia*		% of immobilized *Daphnia*	
		24 h	48 h	24 h	48 h
0.0 (negative control)	20	0	0	0	0
4.3	20	0	0	0	0
9.4	20	0	0	0	0
20.7	20	0	0	0	0
45.5	20	0	0	0	0
100.0	20	0	1	0	5

A 2.2.1.2 Study 3: Toxicity to algae

Comments of zRMS:	The study is considered as acceptable . All validity criteria were met. In the negative control the cell density increased on average by a factor of 227. This value complies with the validity criterion of the test, according to the mentioned guideline. The negative control also met the other validity criterion, with a coefficient of variation of daily growth rates at 72 hours of 34.4 % and a coefficient of variation of average growth in replicates negative control cultures during the test period of 2.6 %. **Agreed endpoints:**

Endpoint	72 h EC_{10} (mg/L)	72 h EC_{20} (mg/L)	72 h EC_{50} (mg/L)
Growth rate	> 100.0	> 100.0	> 100.0
Yield	81.3 (74.6 – 86.6)	> 100.0	> 100.0

Reference:	KCP 10.2.1/02
Report	Tediosi E., Dini R., 2011 PP-113H (CLOPYRALID 10 % w/v SL):toxicity to green algae Pseudo-kirchneriel-la subcapitata determined in a growth inhibition study.
Guideline(s):	OECD 201
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

The influence of the test item PP-113H (Clopyralid 10 % w/v SL) on the growth of the green algal species *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*, was investigated in a 72-hour test according to the OECD Guideline No. 201, 2006.

For this purpose, exponentially growing test algae were exposed for 72 hours to an aqueous test medium containing the test item at five concentrations in a geometric series, namely 6.3, 12.5, 25.0, 50.0 and 100.0 mg/L, under defined conditions, corresponding to 0.60, 1.19, 2.38, 4.75 and 9.50 mg/mL as active ingredient Clopyralid (corrected for its purity, 9.5 % w/w).

Besides the five concentrations, a negative control without the test item was also prepared to check the acceptability of the algal inoculum.

The actual test concentrations of the active ingredient Clopyralid were analytically measured at the beginning and at the end of the test.

The analytical recoveries of the active ingredient in the freshly prepared solutions ranged between 106.3 % and 108.1 % of the nominal values (mean value: 107.3 %).
After 72 hours of exposure, for all tested concentrations, the analytical recoveries were stable being in the range 105.3 % and 108.3 % (mean value: 106.7 %).

The analytical recoveries were referred to the active ingredient content (declared purity: 9.5 % w/w).

As recommended by the OECD Guidance Document on testing of difficult substances Nr 23 (2000), since the a.i. analytical recoveries during the test period were in the range 80 % – 120 %, the biological results refer to the nominal concentrations of the active ingredient, and then, of the test item,
Algal cell density was measured every 24 hours by fluorescent reading with a spectrofluorophotometer in few millilitre samples taken from each test concentration replicate and from negative controls.

At the beginning of the test, the pH of the test medium was in the range 7.98 – 8.03 for test item solutions and 8.02 for the negative control.

At the end of the test the pH values of negative control ranged between 9.10 – 9.21, with a difference lower than 1.5 units, if compared with the values at the test start, as provided by OECD 201/2006 guideline. After 72 hours the pH mean values of test item solutions were in the range 9.00 – 9.18.

According to the OECD's recommended range (24 ± 2 °C), the room temperature was in the range 24.0 – 25.8 °C during the test period.

Light intensity during the test period was measured once a day and it was in the range 5352 - 5601 Lux (within the OECD required range 4440 - 8880 Lux).

In the negative control the cell density increased on average by a factor of 227. This value complies with the validity criterion of the test, according to the mentioned guideline. The negative control also met the other validity criterion, with a coefficient of variation of daily growth rates at 72 hours of 34.4 % and a coefficient of variation of average growth in replicates negative control cultures during the test period of 2.6 %.

After 72 hours of exposure only the highest test item concentration (100 mg/L) showed an inhibition equal to 1.3% for growth rate (r) and equal to 7.1 for yield(y). The lowest concentrations (range: 6.3 - 50 mg/L) did not show any effect due to test item.

The 72-hour EC_{10}, EC_{20} and EC_{50} with their 95% confidence limits calculated in terms of nominal concentrations of test item, for the two end-points, are reported in the following table:

Endpoint	72 h EC_{10} (mg/L)	72 h EC_{20} (mg/L)	72 h EC_{50} (mg/L)
Growth rate	> 100.0	> 100.0	> 100.0
Yield	81.3 (74.6 – 86.6)	> 100.0	> 100.0

The 72-hour EC_{10}, EC_{20} and EC_{50} with their 95% confidence limits calculated in terms of nominal active ingredient concentrations, for the two end-points, are reported in the following table:

Endpoint	72 h EC_{10} (mg a.i./mL)	72 h EC_{20} (mg a.i./mL)	72 h EC_{50} (mg a.i./mL)
Growth rate	> 9.5	> 9.5	> 9.5
Yield	7.7 (7.0 – 8.3)	> 9.5	> 9.5

Data requirement
zRMS updated dRR B9 in terms of tabular information on the dose response, e.g. a table listing dose and effect data, and recovery to the dRR for the sake of transparency:

Analytical recovery of the measured test concentrations to the nominal ones

Time (hours)	Nominal a.i. Clopyralid concentration [mg a.i./mL]	Actual a.i. Clopyralid concentration [mg a.i./mL]	analytical recovery [%]
0	0.60	0.64	107.0
0	1.19	1.26	106.3
0	2.38	2.56	107.8
0	4.75	5.09	107.1
0	9.50	10.27	108.1
72	0.60	0.63	105.3
72	1.19	1.26	106.0
72	2.38	2.57	108.3
72	4.75	5.10	107.4
72	9.50	10.13	106.6

Effect of PP-113H (Clopyralid 10 % w/v SL) on the growth of *Pseudokirchneriella subcapitata*

Nominal test item concentration [mg/L]	replicate	Cell density (n. cells x 10^4/mL)		
		24 h	48 h	72 h
0.00 (negative control)	A	7.5424	81.0440	229.7490
	B	8.5282	76.6760	225.4490
	C	8.8981	74.4770	229.6490
	D	7.2521	77.6950	225.4490
	E	8.2179	74.3820	235.0490
	F	8.7228	72.8580	218.9490
	Average value	**8.1936**	**76.1887**	**227.3823**
6.3	A	10.3269	89.5940	249.4610
	B	11.4366	79.6850	270.3610
	C	11.2761	94.2160	255.9610
	Average value	**11.0132**	**87.8317**	**258.5943**
12.5	A	9.7100	119.1390	266.9940
	B	7.2453	118.6790	255.2940
	C	8.9834	120.6490	253.6940
	Average value	**8.6462**	**119.4890**	**258.6607**
25.0	A	14.2047	119.4200	260.5790
	B	17.0577	122.1900	251.1790
	C	16.0422	121.9000	256.8790
	Average value	**15.7682**	**121.1700**	**256.2123**
50.0	A	14.7400	113.7210	264.7220
	B	15.6516	116.5210	246.5220
	C	16.6159	117.3610	255.6220
	Average value	**15.6692**	**115.8677**	**255.6220**
100.0	A	15.0429	109.8850	207.3200
	B	16.3115	109.1260	213.7200
	C	14.1313	112.0140	213.0200
	Average value	**15.1619**	**110.3417**	**211.3533**

A 2.2.1.3 Study 3: Toxicity to Lemna

Comments of zRMS:	The study is considered as acceptable . The validation criteria should be met: OECD 221: For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d-1.

All validity criteria were met.

The validity criterion was accomplished as follows:
1. According to the guideline, the doubling time of the frond number in the control must be less than 2.5 d (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 d^{-1}.
2. The measured doubling time of the frond number in the control was in average 2.4 days corresponding to a 15-fold increase in dry weight (1.03 mg to 15.8 mg dry weight in the control vessels).
3. The average specific growth rate in the control was 0.294 d^{-1} for frond number. Therefore, the validity criterion was met in this study.
4. The E_rC_{50} (frond number) value for the reference item (toxic standard) 3,5-dichlorophenol was 2.64 mg /L. This value is included in the range 2.2 - 3.8 mg 3,5-dichlorophenol/L as stated in the Guideline ISO 20079, demonstrating that the test system was sensitive.

The study was performed in compliance with the GLP principles.

Agreed endpoints:

Effect concentration	**Average specific growth rate inhibition**		**Yield inhibition**	
(mg/L)	**Frond number**	**Biomass**	**Frond number**	**Biomass**
EC_{50} 95 % confidence limits (lower – upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
test item, nominal	**1968.6** (1408.0 – 3224.8)	**2518.3** (2265.3 – 2841.1)	**673.1** (448.5 – 1079.2)	**1520.5** (1188.1 – 1925.1)
a.i., nominal	**185.3** (132.6 – 303.6)	**237.1** (213.3 – 267.5)	**63.4** (42.2 – 101.6)	**143.1** (111.9 – 181.2)
NOEC				
test item, nominal	**79.4**	**812.5**	**24.8**	**79.4**
a.i., nominal	**7.5**	**76.5**	**2.3**	**7.5**
LOEC				
test item, nominal	**253.9**	**2600.0**	**79.4**	**253.9**
a.i., nominal	**23.9**	**244.8**	**7.5**	**23.9**

Reference KCP 10.2.1/03

Report: Juckeland D, 2012, Effects of PP-113H (Clopyralid 10% w/v SL) on Lemna minor in a growth inhibition test under static test conditions

Guidelines OECD 221

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) Not applicable

The purpose of this study was to determine the effects of PP-113H (Clopyralid 10% w/v SL) on the freshwater aquatic plants of the genus *Lemna* (duckweed) under static test concentrations.
The EC_{50}, LOEC and NOEC for the endpoints based on the inhibition of *Lemna* growth (total frond number, dry weight, growth rate) over a period of 7 days were determined. The test was performed according to the OECD Guideline 221 (2006)
The test was valid because the doubling time of frond number in the control was less than 2.5 days (actually 2.4 days for untreated control) as requested by OECD 221 Guideline (2006).

The measured concentrations of Clopyralid remained within a range of 93 - 96 % of nominal values at the start of the test (day 0) and within a range of 90 - 96 % of nominal values at the end of the test (day 7).

Therefore, the toxicity results are based on the nominal concentrations.

Table I: EC_{50}, LOEC and NOEC values of PP-113H (Clopyralid 10% w/v SL) regarding growth rate and yield based on frond number and biomass of *Lemna minor*

Effect concentration	**Average specific growth rate inhibition**		**Yield inhibition**	
(mg/L)	**Frond number**	**Biomass**	**Frond number**	**Biomass**
EC_{50} 95 % confidence limits (lower – upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
test item, nominal	**1968.6** (1408.0 – 3224.8)	**2518.3** (2265.3 – 2841.1)	**673.1** (448.5 – 1079.2)	**1520.5** (1188.1 – 1925.1)
a.i., nominal	**185.3** (132.6 – 303.6)	**237.1** (213.3 – 267.5)	**63.4** (42.2 – 101.6)	**143.1** (111.9 – 181.2)
NOEC				
test item, nominal	**79.4**	**812.5**	**24.8**	**79.4**
a.i., nominal	**7.5**	**76.5**	**2.3**	**7.5**
LOEC				
test item, nominal	**253.9**	**2600.0**	**79.4**	**253.9**
a.i., nominal	**23.9**	**244.8**	**7.5**	**23.9**

Table II: Effects of PP-113H (Clopyralid 10% w/v SL) on growth rate and yield of *Lemna minor*

Treatment group mg test item/L, nominal	**Final frond number** replicate mean day 7	**Biomass (dry weight)** replicate mean day 7 (mg)	**% Inhibition**			
			Average specific growth rate (% I_r)		**Yield (% I_y)**	
			frond number	biomass	frond number	biomass
Control	61.7	15.8	-	-	-	-
24.8	63.7	15.4	0.0 (-1.3) [1]	1.0	0.0 (-3.2)[1]	3.0
79.4	46.0	14.6	12.3	3.1	25.4 +	8.7
253.9	40.3	13.8	17.5 +	4.9	34.6 +	13.6 +
812.5	36.7	13.6	21.2 +	5.5	40.5 +	15.2 +
2600.0	11.0	3.9	61.6 +	51.8 +	82.2 +	80.7 +

+ statistically significantly different to the untreated control (Williams t-test; Welch's-t-test, $p \leq 0.05$, one-sided)
[1] negative values mean a higher growth compared to the control
Average specific growth rate (based on frond number) and yield (based on biomass)

No statistically significant effects on the average specific growth rate based on frond number and yield based on biomass were observed at nominal concentrations ≤ 79.4 mg test item/L, whereas statistically significant effects ($p \leq 0.05$) were calculated for nominal concentrations ≥ 253.9 mg test item/L.

As a result, the NOEC regarding the average specific growth rate based on frond number and yield based on biomass was determined to be 79.4 mg test item/L, whereas the LOEC was determined to be 253.9 mg test item/L for nominal concentrations.

Based on nominal concentrations the calculated average specific growth rate value (ErC50, 0-7 d) was 1968.6 mg test item/L for frond number.

Based on nominal concentrations the calculated yield value (EyC50, 0-7 d) was 1520.5 mg test item/L for frond number.

Average specific growth rate (based on biomass)

No statistically significant effects on the average specific growth rate of Lemna based biomass were observed at nominal concentration ≤ 812.5 mg test item/L.

As a result, the NOEC regarding the average specific growth rate and yield for biomass was determined to be 812.5 mg test item/L, whereas the LOEC was determined to be 2600.0 mg test item/L for nominal concentrations.

Based on nominal concentrations the calculated average specific growth rate value (ErC50, 0-7 d) was 2518.3 mg test item/L for biomass.

Yield (based on frond number)

No statistically significant effects on yield based on frond number were observed at nominal concentration of 24.8 mg test item/L.

As a result, the NOEC regarding the average specific growth rate and yield for biomass was determined to be 24.8 mg test item/L, whereas the LOEC was determined to be 79.4 mg test item/L for nominal concentrations.

Based on nominal concentrations the calculated yield value (EyC50, 0-7 d) was 673.1 mg test item/L for frond number.

The validity criterion was accomplished as follows:
According to the guideline, the doubling time of the frond number in the control must be less than 2.5 d (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 d-1.

The measured doubling time of the frond number in the control was in average 2.4 days corresponding to a 15-fold increase in dry weight (1.03 mg to 15.8 mg dry weight in the control vessels). The average specific growth rate in the control was 0.294 d-1 for frond number. Therefore, the validity criterion was met in this study.

The ErC50 (frond number) value for the reference item (toxic standard) 3,5-dichlorophenol was 2.64 mg /L. This value is included in the range 2.2 - 3.8 mg 3,5-dichlorophenol/L as stated in the Guideline ISO 20079, demonstrating that the test system was sensitive.

The study was performed in compliance with the GLP principles.

Data requirement
zRMS updated dRR B9 in terms of tabular information on the dose response, e.g. a table listing dose and effect data, and recovery to the dRR for the sake of transparency:

PP-113H (Clopyralid 10 % w/v SL) time 0 analysis – Clopyralid content in water samples from the static growth inhibition test

Vial Identification	ChemService specimen identification	A_S	C_S (mg/L)	D_S	Clopyralid Found* (µg/mL)	Clopyralid Add** (µg/mL)	Recovery (%)
Spike Low (QC)	-	271857	0.16	1.00	0.16	0.15	105.45
Spike High (QC)	-	2670705	1.54	1.00	1.54	1.52	101.49
Control t0	2907849-001	0	n.d.	1.00	n.d.	0.00	-
24.8 mg/L t0	2907849-002	3934485	2.27	1.00	2.27	2.36	96.23
79.4 mg/L t0	2907849-003	6273261	3.61	2.00	7.23	7.54	95.79
253.9 mg/L t0	2907849-004	3978384	2.29	10.00	22.93	24.12	95.04
812.5 mg/L t0	2907849-005	6255327	3.60	20.00	72.05	77.19	93.34
2600.0 mg/L t0	2907849-006	4072743	2.35	100.00	234.68	247.00	95.01

A_S : Sample peak area
C_S : injected concentration
D_S : Dilution applied to the sample

(*) Spike and samples were quantified with the linear calibration reported in Table 2a.
(**) Nominal add of clopyralid was calculated considering its title in test item PP-113H (Clopyralid 10 % w/v SL) determined in ChemService study CH-397/2011 ; 9.5 ± 0.1 % w/w.
n.d. not detected, lower than L.O.D. (0.05 µg/mL)

PP-113H (Clopyralid 10 % w/v SL) time 7 analysis – Clopyralid content in water samples from the static growth inhibition test

Vial Identification	ChemService specimen identification	A_S	C_S (mg/L)	D_S	Clopyralid Found* (µg/mL)	Clopyralid Add** (µg/mL)	Recovery (%)
Spike Low (QC)	-	247431	0.14	1.00	0.14	0.15	91.51
Spike High (QC)	-	2505372	1.42	1.00	1.42	1.52	93.70
Control t7	2907926-001	0	n.d.	1.00	n.d.	0.00	-
24.8 mg/L t7	2907926-002	3892845	2.21	1.00	2.21	2.36	93.81
79.4 mg/L t7	2907926-003	6000660	3.41	2.00	6.82	7.54	90.36
253.9 mg/L t7	2907926-004	3992445	2.27	10.00	22.67	24.12	93.98
812.5 mg/L t7	2907926-005	6203025	3.52	20.00	70.46	77.19	91.28
2600.0 mg/L t7	2907926-006	4155654	2.36	100.00	235.96	247.00	95.53

A_S : Sample peak area
C_S : injected concentration
D_S : Dilution applied to the sample

(*) Spike and samples were quantified with the linear calibration reported in Table 2b.
(**) Nominal add of clopyralid was calculated considering its title in test item PP-113H (Clopyralid 10 % w/v SL) determined in ChemService study CH-397/2011 ; 9.5 ± 0.1 % w/w.
n.d. not detected, lower than L.O.D. (0.05 µg/mL)

Study 4: Toxicity to *Myriophyllum*

Comments of zRMS:	The study was accepted in dRR B9 for plant product protection Faworyt 300 SL in 07.2022 by PL zRMS. Agreed endpoints:

The results of the definitive test calculated by ToxRat Professional

Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	NOEC [mg/L]	LOEC [mg/L]
Yield for fresh weight	13.385 (7.101 - 25.231)*	15.002 (7.907 - 28.334)*	18.659 (7.592 - 43.742)*	9.530	17.150
Average specific growth rate for fresh weight	16.150 (0.000 - n.d.)*	16.684 (0.000 - n.d.)	17.755 (n.d. - n.d.)*	9.530	17.150
Yield for dry weight	8.094 (3.127 - 20.950)*	9.008 (3.444 - 23.552)*	11.053 (2.774 - 41.008)*	9.530	17.150
Average specific growth rate for dry weight	9.110 (0.000 - n.d.)*	9.419 (n.d. - n.d.)*	10.038 (n.d. - n .d.)*	9.530	17.150
Yield for total shoot length after 14 days	21.953 (10.052 - 47.940)*	26.739 (12.655 - 56.444)*	38.994 (15.677 - 95.802)*	30.860	55.560
Average specific growth rate for total shoot length after 14 days	21.233 (7.770 - 57.966)*	28.707 (10.965 - 75.269)*	51.159 (15.689 - 163.034)*	30.860	55.560

EC_{10} concentration of test item causing symptoms of intoxication in 10% of the population
EC_{20} concentration of test item causing symptoms of intoxication in 20% of the population
EC_{50} concentration of test item causing symptoms of intoxication in 50% of the population
NOEC highest non observe effective concentration cause no statistically significant differences in comparison to the control
LOEC lowest observe effective concentration cause statistically significant differences in comparison to the control
*) the lower and upper 95% confidence limits are given in brackets
n.d. not determined due to mathematical reasons

Reference:	KCP 10.2.1
Report	Faworyt 300 SL Water-sediment *Myriophyllum spicatum* toxicity test according to OECD 239, Aleksandra Kamińska, 2019, STUDY CODE: 0016/0061/E, SORBOLAB Research Laboratory, Poland
Guideline(s):	Yes. According to the OECD Guideline No. 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. MATERIALS

1. Test material

Description	clear liquid, yellow in color with a characteristic weak odor
Lot/Batch #	201805002
Content of active substance	Clopyralid: 302.7 g/L
Density:	1.161 g/mL
Expiry date	15.05.2021

2. Test organism

Species	spiked water-milfoil *Myriophyllum spicatum*
Source	Culture grown in the SORBOLAB Research Laboratory.
Culturing	At 20 ± 2 °C under constant illumination in Smart & Barko medium.
Acclimation period	Culturing was done under test conditions.
Test units	Plastic pots with sediment were placed in bakers in which three shoot apices were planted and the baker was filled with Smart&Barko medium

3. Environmental conditions

Test water Smart & Barko medium containing the following constituents:

Substance	Concentration [mg/L]
$CaCl_2 \cdot 2\ H_2O$	91.7
$MgSO_4 \cdot 7\ H_2O$	69.0
$NaHCO_3$	58.4
$KHCO_3$	15.4
pH	7.9

Water temperature	21.583°C, minimal temperature 19.60°C, maximal temperature 23.30°C
pH	The pH value in the experimental vessels was maintained at 7.81-7.98 at the start, 7.89-8.02 at the end and did not fluctuate by more than 1.5 units during the experiment (OECD 211 requirements: pH 6-9)
Lighting	Photoperiod 16 h day/8 h night with a light intensity 10700-115700 lux

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

Test design B: one pot per one test vessel with tree shoots
All concentrations of the test item were prepared in four replicates and control in six. Plastic pots with sediment were placed in bakers in which three shoot apices were planted and the baker was filled with Smart&Barko medium. Rooting phase lasted 13 days. Exposition phase to tested item lasted 14 days. The test was carried out in 2 L glass bakers filled with 1.7 L of Smart&Barko medium

The aim of the study was to determine the effect of the test item Faworyt 300 SL on growth of spiked water-milfoil Myriophyllum spicatum, expressed as increase in yield (EyCx) and average specific growth rate (ErCx) for fresh and dry weight and total shoot length after 14 days. End points of the study are ECx values for tested parameters. Also statistically were determined NOEC and LOEC values

Concentrations tested

Product was tested at nominal concentrations of 9.53 mg/L; 17.15 mg/L; 30.86 mg/L; 55.56 mg/L; 100 mg/L in four repetitions mg/L.. Test definitive test concentrations were chosen based on range finding test results. A control group with untreated test medium was used. The reference item was tested in a separate study

Treatment/Application

A stock solution was prepared by weighing 2 000 mg of the test item and filling it up to 1000 mL test medium. The test solutions were prepared by dilution of the respective amount of the stock solution with test medium.

Analytics

The actual content of test item measured as clopyralid was determined in one replicate of each test item concentration and the control at test start and test end using HPLC with photodiode array detector (PDA). The method was validated according to SANCO/3029/99 rev.4

2. Sampling and measurements

~~Test was conducted in semi-static system; test item solution were renewal every 72 hours (on the basis of results of stability test). The main objective of the study was to determine the effect of the test item on daphnia reproduction, furthermore, mortality of adults, adult length, age of first offspring production, development rate and intrinsic rate. The end points of the experiment are ECx, NOEC and LOEC values.~~

The aim of the study was to determine the effect of the test item Faworyt 300 SL on growth of spiked water-milfoil Myriophyllum spicatum, expressed as increase in yield (EyCx) and average specific growth rate (ErCx) for fresh and dry weight and total shoot length after 14 days. End points of the study are ECx values for tested parameters. Also statistically were determined NOEC and LOEC values.

3. Calculation of toxicity

The end points of the experiment are ECx, NOEC and LOEC values.

4. Statistics

Based on the obtained data, a statistical analysis was carried out in accordance with the OECD 211 using the ToxRat Professional statistical program.

Results and discussions

A. ANALYTICAL RESULTS

Analysis of the concentrations determination was performed by high-performance liquid chromatography with diode UV detection. The identification of active substances in the tested material was made by comparing the UV spectra and the retention times of the reference substance and the sample of the item being tested . Analytically measured concentrations of Faworyt 300 SL measured as clopyralid were within the range of 80 – 120% of nominal.

Results of analytical determinations

Date	Sample ID in Physicochemistry and Analytics Laboratory	Sample ID in Ecotoxicology Laboratory	Determined concentration of test item [mg/L]	Concentration of test item in solution after correction of dilution [mg/L]	Average concentration of test item in solution [mg/L]
23.04.2019	449_2019 01	control	0.00000	0.00000	0.00
	449_2019 02		0.00000	0.00000	
	450_2019 01	9.53 mg/L	0.88474	8.84740	8.88
	450_2019 02		0.89071	8.90710	
	451_2019 01	17.15 mg/L	0.76734	15.34680	15.57
	451_2019 02		0.78949	15.78980	
	452_2019 01	30.86 mg/L	0.26560	26.56000	26.62
	452_2019 02		0.26680	26.68000	
	453_2019 01	55.56 mg/L	0.50484	50.48400	50.91
	453_2019 02		0.51333	51.33300	
	454_2019 01	100 mg/L	0.93190	93.19000	92.92
	454_2019 02		0.92651	92.65100	
07.05.2019	497_2019 01	control	0.00000	0.00000	0.00
	497_2019 02		0.00000	0.00000	
	498_2019 01	9.53 mg/L	0.87697	8.76970	8.76
	498_2019 02		0.87439	8.74390	
	499_2019 01	17.15 mg/L	0.74912	14.98240	15.33
	499_2019 02		0.78379	15.67580	
	500_2019 01	30.86 mg/L	0.23391	23.39100	23.79
	500_2019 02		0.24196	24.19600	
	501_2019 01	55.56 mg/L	0.50233	50.23300	49.53
	501_2019 02		0.48817	48.81700	
	502_2019 01	100 mg/L	0.89209	89.20900	89.10
	502_2019 02		0.88996	88.99600	

B. RESULTS

The aim of the conducted study was to determine a toxicological impact of the test item on spiked water-milfoil (Myriophyllum spicatum) in yield (EyCx) and average specific growth rate (ErCx) for fresh and dry weight and total shoot length at tested concentrations. Statistically NOEC and LOEC values for all parameters were calculated. The obtained EC10, EC20, EC50 and NOEC and LOEC values after 14 days from the application of the tested item, testify to the ecotoxic effect of the tested material in concentrations of 17.15 mg/L, 30.86 mg/L, 55.56 mg/L, 100 mg/L for yield of fresh and dry weight, average specific growth rate for fresh and dry weight and for 55.56 mg/L, 100 mg/L concentrations for yield of the total shoot length and the average specific growth rate for total shoot length

C. VALIDITY CRITERIA

The validity criteria in accordance with OECD Guideline 239 were met:

– the fresh weight factor for control plants was 2.2 (requirements according to OECD 239: minimum 2)

– the total shoot length factor for control plants was 2.3 (requirements according to OECD 239: minimum 2

– the coefficient of variations for yield based on fresh weight was 31.1% (requirements according to OECD 239: ≤35%)

Conclusion

The results of the definitive test calculated by ToxRat Professional					
Rated value	**EC_{10} [mg/L]**	**EC_{20} [mg/L]**	**EC_{50} [mg/L]**	**NOEC [mg/L]**	**LOEC [mg/L]**
Yield for fresh weight	13.385 (7.101 - 25.231)*	15.002 (7.907 - 28.334)*	18.659 (7.592 - 43.742)*	9.530	17.150
Average specific growth rate for fresh weight	16.150 (0.000 - n.d.)*	16.684 (0.000 - n.d.)	17.755 (n.d. - n.d.)*	9.530	17.150
Yield for dry weight	8.094 (3.127 - 20.950)*	9.008 (3.444 - 23.552)*	11.053 (2.774 - 41.008)*	9.530	17.150
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Yield for total shoot length after 14 days	21.953 (10.052 - 47.940)*	26.739 (12.655 - 56.444)*	38.994 (15.677 - 95.802)*	30.860	55.560
Average specific growth rate for total shoot length after 14 days	21.233 (7.770 - 57.966)*	28.707 (10.965 - 75.269)*	51.159 (15.689 - 163.034)*	30.860	55.560

EC_{10} concentration of test item causing symptoms of intoxication in 10% of the population
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NOEC highest non observe effective concentration cause no statistically significant differences in comparison to the control
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*) the lower and upper 95% confidence limits are given in brackets
n.d. not determined due to mathematical reasons

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 2.3.1.1.1.1 Study 1: Acute oral toxicity to honey bees

Comments of zRMS:	The study is considered as acceptable . All validity criteria were met. **Agreed endpoints** **Acute oral toxicity test** **48-h LD_{50} > 100.00 µg product formulated BARICHLORE/bee** (equivalent to 9.41 µg of clopyralid/bee); no repellent effect of the test item on bees was observed. The statistical analysis showed no statistically significant difference between the control and the test item (p-level = 0.27) **Acute contact toxicity test** 48-h LD_{50} > 100.00 µg product formulated **BARICHLORE**/bee (equivalent to 9.41 µg of clopyralid/bee); no repellent effect of the test item on bees was observed. The statistical analysis showed no statistically significant difference between the control and the test item (p-level = 0.46)

Reference KCP 10.3.1/01

Report: Barcarotti M. , 2011
EFFECTS, ACUTE ORAL AND ACUTE CONTACT TOXICITY, OF PP-113H (Clopyralid 10% w/v SL) ON THE HONEYBEE APIS MELLIFERA L. IN THE LABORATORY (LIMIT TEST)
Report Nº BT102/11

Guidelines: OECD 213 (1998)

Deviation None.

GLP: Yes (Certified laboratory)

Executive Summary

The effects, acute oral and acute contact toxicity, of the test item PP-113H (Clopyralid 10% w/v SL) on the honeybees, were tested in a laboratory study according to GLP regulations

.I. MATERIALS AND METHODS

A. MATERIALS

1. Test item	
Description	Herbicide
Lot/Batch #	20110713
Purity	99 ± 1g/L
Stability of the test item	Stable
2.Test system	
Species	*Apis mellifera*
Strain	Not relevant
Age	Adult worker
Weight at dosing	Not relevant
Source	Healthy colony (derived from swarms purchased by local beekeeper in May

	2010) maintained at Biotecnologie BT S.r.l.
Acclimation	90 min at 25 ± 2°C for the oral test – 30 min at 25 ± 2°C for the contact test
Diet	50% (w/v) aqueous sucrose solution
Water	Not relevant
3. Experimental conditions	
Temperature	23.33 – 25.00 °C
Humidity	60.00 – 69.50%
Photoperiod	24 h darkness (except during observations)
Air changes	Not relevant

B. STUDY DESIGN AND METHODS

1. Experimental period	11st - 13rd October 2011
2a. ORAL TEST: Experimental treatment	Five replicates of 10 bees each were prepared for each experimental group. PP-113H (a.s. 10% w/v SL) was tested at a single concentration (see Table 1). A test with Reference Item (a.s. dimethoate) at a single concentration (0.20 µg a.s/bee) was simultaneously performed. Bees were anaesthetised at 5°C for 30'. Each group of bees was provided for 4 hours with 200 µL of 50% (w/v) sucrose-water solutions containing the test item. Then the test item was replaced with 50% (w/v) aqueous sucrose solution (*ad libitum*).
2b. CONTACT TEST: Experimental treatment	Five replicates of 10 bees each were prepared for each experimental group. PP-113H (a.s. 10% w/v SL) was tested at a single concentration (see Table 2). A test with Reference Item (a.s. dimethoate) at a single concentration (0.20 µg a.s./bee) was simultaneously performed (the substance was dissolved in deionised water). Bees were anaesthetised at 5°C for 30 min and then individually treated by topical application with a micro applicator. 1µL of test item has been applied to the dorsal side of the thorax of each bee. After application the bees were returned to the cages and fed with 50% aqueous sucrose solution (*ad libitum.*).
3. Study design	**Table 1. Oral test, study design** **Treatment** / **µg product/bee** / **µg a.s./bee** control: 0.00 / 0.00 test item: 100.00 / 9.41 reference item: 0.50 / 0.20 **Table 2. Contact test, study design** **Treatment** / **µg product/bee** / **µg a.s./bee** control: 0.00 / 0.00 test item: 100.00 / 9.41 reference item: 0.50 / 0.20
4. Observations	Assessments on mortality and behavioural abnormalities were performed at 4, 24 and 48 h after treatment.
5. Statistics	Significance with T- Student Test

II. RESULTS

A. FINDINGS

Mortality:

Table 3. Oral toxicity test

Product	Concentrations µg product/bee	Concentrations µg a.s./bee	%Corrected Mortality 4h	%Corrected Mortality 24h	%Corrected Mortality 48h
control	0.00	0.00	n.a.	n.a.	n.a.
test item	100.00	9.41	18.37	11.11	11.11
reference item	0.50	0.20	34.69	68.89	84.44

Table 4. Contact toxicity test

Product	Concentrations µg product/bee	Concentrations µg a.s./bee	%Corrected Mortality 4h	%Corrected Mortality 24h	%Corrected Mortality 48h
control	0.00	0.00	n.a.	n.a.	n.a.
test item	100.00	9.41	0.00	0.00	4.26
reference item	0.50	0.20	33.33	82.98	87.23

III. CONCLUSIONS

In the acute oral toxicity test the observed mortality after 48 hours caused by PP-113H (Clopyralid 10% w/v SL) compared to the control group, resulted in LD_{50} > 100.00 µg product formulated/bee (equivalent to 9.41 µg of Clopyralid/bee); no repellent effect of the test item on bees was observed. The statistical analysis showed no statistically significant difference between the control and the test item (p-level = 0.27)

In the acute contact toxicity test the observed mortality after 48 hours caused by PP-113H (Clopyralid 10% w/v SL) compared to the control group resulted in LD_{50} > 100.00 µg product formulated/bee (equivalent to 9.41 µg of Clopyralid/bee); no repellent effect of the test item on bees was observed. The statistical analysis showed no statistically significant difference between the control and the test item (p-level = 0.46)

A 2.3.1.1.1.2 Study 1: Acute contact toxicity to honey bees

Please see above

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1: Chronic toxicity to the honey bee

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. - Bee mortality in control after 10 days was 8% (acceptable ≤15%) - Bee mortality in the reference test after 10 days was 100% **Agreed endpoints:** The LDD_{50}-value (Lethal Dietary Dose that kills 50 % of exposed individuals) for **BARILOCHE** was empirically estimated to be higher than the highest co sumed dose of 54.20 µg clopyralid/bee/day. The LC_{50}-value (Lethal Concentration that kills 50% of exposed individuals) was empirically estimated to be higher than the highest concentration tested of 4000 mg clopyralid/L diet. Since no dose response was obtained, the LDD_{10}/LC_{10} and LDD_{20}/LC_{20} values (Lethal Dietary Doses / Concentrations that kill 10 and 20% of exposed individuals, respectively) for **BARILOCHE** could not be estimated. The NOEDD (No Observed Effect Dietary Dose), based on actual consumption of the test item feeding solutions, was determined to be to the highest consumed dose of 54.20 µg clopyralid/bee/day. After 10 days the NOEC value was determined to be 4000 mg clopyralid/L diet. Symptoms of intoxication were observed sporadically for few bees of the three highest treatments, with no apparent relationship with the exposure to the test item. The results obtained with the toxic reference item, dimethoate, confirmed the sensitivity of the test system.

Reference:	KCP 10.3.1/02
Report	Ansaloni, T. 2020. PP-113H (Clopyralid 100 g/L SL): Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee, Apis mellifera L. under Laboratory Conditions Trialcamp S.L.U., Report Nº S19-03760
Guideline(s):	OECD 245
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No - not appropriate

Executive Summary

The study was conducted to Determine the effects of PP-113H (Clopyralid 100 g/L SL) on the honey bee Apis mellifera L. from chronic oral exposure to estimate the median lethal dietary dose (LDD50), the respective median lethal concentration (LC50), the no observed effect daily dose (NOEDD) and the respective no observed effect concentration (NOEC) values, where possible. In Addition, estimate the daily Lethal Dietary Doses and the Lethal Concentrations that caused 10 and 20% mortality (LDD10/20/LC0/20), where possible

Young adult worker honey bees were orally exposed to 5 different PP-113H (Clopyralid 100 g/L SL) concentrations in 50 % (w/v) aqueous sucrose solution ad libitum for 10 consecutive days. The control

group C was fed with untreated 50 % (w/v) aqueous sucrose solution. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period.

The mortalities for PP-113H (Clopyralid 100 g/L SL) at the dose levels of 1632.6, 3265.2, 65.30.4, 13060.8 and 26121.6 mg item/kg diet were 2.0, 2.0, 2.0, 12.0, 94.0 and 100.00% (corrected mortality 0.0, 0.0, 10.2, 93.88 and 100.0%), respectively. In the control group 2.0% mortality was observed at the final assessment after 10 days. There was 100% mortality observed at day 6 for reference item BAS 152 11 I (dimethoate) at the dose level of 0.107 µg/bee/day.

The overall mean daily consumption (the average consumption/bee over 10 days) of feeding solutions in the test item concentrations of 1632.6, 3265.5, 6530.4, 13060.8 and 26121.6 mg test item/kg feeding solution was 19.47, 21.14, 22.04, 44.88 and 22.23 µL/bee/day, respectively. For the control and reference item groups 21.31 and 24.34 µL/bee/day, respectively. After 10 days continuous exposure the mean accumulated uptake of test item at the concentrations of 1632.6, 3265.5, 6530.4, 13060.8 and 26121.6 mg test item/kg feeding solution was 378.33, 821.4, 1713.15, 6975,74 and 1381.92µg test item/bee, respectively. The corresponding average daily dose was therefore 37.83, 82.14, 171.32, 697.57 and 690.96 µg test item/bee/day.

The LDD50-value (Lethal Dietary Dose that kills 50 % of exposed individuals) for PP-113H (Clopyralid 100 g/L SL) was empirically estimated to be higher than the highest consumed dose of 54.20 µg Clopyralid/bee/day. The LC50-value (Lethal Concentration that kills 50 % of exposed individuals) was empirically estimated to be higher than the highest concentration tested of 4000 mg Clopyralid/L diet.
Since no dose response was obtained, the LDD10 / LC10 and LDD20 / LC20 values (Lethal Dietary Doses / Concentrations that kill 10 and 20 % of exposed individuals, respectively) for PP-113H (Clopyralid 100 g/L SL) could not be estimated.
The NOEDD (No Observed Effect Dietary Dose), based on actual consumption of the test item feeding solutions, was determined to be to the highest consumed dose of 54.20 µg Clopyralid/bee/day. After 10 days the NOEC value was determined to be 4000 mg Clopyralid/L diet.
Symptoms of intoxication were observed sporadically for few bees of the three highest treatments, with no apparent relationship with the exposure to the test item.
The results obtained with the toxic reference item, dimethoate, confirmed the sensitivity of the test system.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Dark yellow liquid
Lot/Batch #:	20190506
Purity:	Clopyralid: 1702-17-6 (analysed)
Stability of test compound:	Stable
Positive control:	Positive control: BAS 152 11 I (400g/L dimethoate)

3. Test animals -

Species:	Honey bee (*Apis mellifera*)
Age:	Bees from a healthy colony were two days old
Source:	Commercial bee hives maintained by Trialcamp S.L.U.
Acclimation period:	20 hours
Feeding:	50% (w/v) aqueous sucrose solution [each cage was provided daily with 1 mL of the corresponding feeding solution (0.10 mL/bee) for

	10 days]
Water	N/A.
Housing:	Bees were transferred into stainless steel cages (approximate measures base: 8.5 cm x 4.5 cm; height: 6.5 cm). The front side of the cage was equipped with a transparent pane to enable observation. The bottom of the cage consists of perforated steel to ensure sufficient air supply. Two holes at the top of the cage allow for the use of feeders. The cages were lined with filter paper
Test duration:	10 days
4. Environmental conditions -	
Temperature:	32.4 – 34.0 °C
Humidity:	57.2 – 69.0%
Photoperiod:	Dark except during feeding and assessments

B. STUDY DESIGN AND METHODS:

1. In life dates: 14 Jun 2019 – 29 Jun 2019

2.Animal assignment and treatment:

The colonies are examined for reportable bee epidemics by an authorised bee specialist and are inspected periodically, according to the standard bee- keeping practices, by an experienced apiarist.
The hives used for honey bees' collection for this test will be queen-right, adequately fed, healthy and as far as possible disease-free.
No chemical substances (such as antibiotics, anti-Varroa treatments, pesticides, etc.) have been used in the hive for at least one month prior to this test.

Two days before the beginning of the test, frames with capped cells are transferred from the hive to an incubator, transported to Trialcamp facilities and located in a bioclimatic chamber. One day prior to test start, the bees will be randomly collected directly from the frames, introduced into the test units and kept under test conditions until start of the test.
Acclimatisation period lasts from honey bee collection to the start of the test. During this period the bees will be fed ad libitum with 50 % w/v sucrose solution.
Moribund bees will be rejected and replaced by healthy bees before starting the test, if applicable

The chronic feeding test was carried out as a dose-response test with duration of 10 days. The test comprised one control treatment group, five test item treatment groups and one reference item treatment group. Each treatment group consisted of 50 test organisms (divided into 5 replicates, containing 10 test organisms each).
Additionally, 5 test units without bees but with food syringes containing pure 50 % (w/v) aqueous sucrose solution were placed in the climatic chamber for the evaluation of the evaporation.
Two sets of samples of the control group (C), the stock solution (St) and the highest (T5) and lowest (T1) concentration of the test item feeding solutions were taken on day 0, directly after preparation. Samples were stored in a freezer at the target temperature of ≤ - 18 ºC until shipment and delivery to the analytical laboratory for analytical determination of the actual concentration of the test chemical.

The stock solution (St) was prepared daily by mixing a defined amount of the test item with a defined amount of 50 % (w/v) sucrose solution, and this solution was used for the preparation of the test item treatment solutions (T1 – T5). The amount of test item needed for the daily preparation of the stock solution was measured using a calibrated balance. Aliquots of the stock solution St were mixed daily with 50 % (w/v) sucrose solution up to a defined volume to obtain the treatment solutions.
For the reference item treatment a stock solution was prepared daily using 50 % (w/v) aqueous su-

crose solution as solvent. The definitive feeding solution was prepared daily by mixing an aliquot of the stock solution with 50 % (w/v) aqueous sucrose solution.
Details on the preparation of the feeding solutions are described in Table 1 to Table 2 (Appendix A 1). The test item concentrations in the feeding solution that have been used in the study are presented in the application schedule.
The feeding solutions were offered to the test organisms of each test unit with feeders (plastic syringes, approx. 5 mL). The tip of each feeder was removed in order to give the bees access to the feeding solution.
The feeding volume of 1 mL per replicate was offered daily to the bees ad libitum.
The bees in one cage shared the feeding solution and thus received similar doses (trophallaxis).
Freshly prepared feeding solution replaced the feeding solution of the previous day by changing the feeders.
The application order was control (C), test item at increasing concentrations (T1 to T5) and reference item (R).
The amount of feeding solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.
Syringes of 5 additional cages were filled with 1 mL of pure 50 % (w/v) aqueous sucrose solution and weighed daily for the determination of the evaporation.

3. Observations:

Mortality and behavioural abnormalities were recorded every 24 hours (± 2 hours) before each application (start of feeding). At each assessment time dead bees were removed for sanitary reasons.

4. Statistics:

Statistical calculations were made with the statistical program ToxRatPro Version 3.2.1. For the estimation of the LDx/LCx values (i.e., LD10/LC10,LD20/LC20 and LD50/LC50), where possible, a Probit regression analysis will be performed, and the robustness of the result obtained should be documented by providing the 95 % confidence limits and the statistical significance of the data to the regression model used. A heterogeneity factor could be used if goodness-of-fit test (Pearson $\chi 2$-test) is significant at the 10 % level. A determination of the LDx/LCx values by interpolation or other appropriate statistical method may be performed in case that no Probit regression analyisis with 95 % confidence limits could be calculated.
The determination of the NOEDD/NOEC values will be performed by using a suitable statistical method

II. RESULTS AND DISCUSSION

A ANALYTICAL DATA

The measured concentrations for the active ingredient Clopyralid in the treated sucrose solution were within 80-120 % of nominal for the treatment groups that were analysed (between 99 and 102 %).

B. MORTALITY

Validity criterion for the control C (untreated 50 % (w/v) aqueous sucrose solution) was fulfilled (mortality < 15% after 10 days of exposure), with 8.0 % mean mortality after 10 days of continuous exposure.
In the reference item group, the validity criterion was fulfilled with 100.00 % mortality after 10 days of continuous exposure. Since validity criteria were fulfilled, the test was considered valid.
In the test item groups of 4.12, 7.68, 15.49, 30.84 and 54.20 consumed µg Clopyralid/bee/day cumulative mortalities of 8.0, 8.0, 6.0, 10.0, and 4.0 % were observed, respectively, at the final assessment after 10 days of exposure.
Symptoms of intoxication were observed sporadically for few bees of the three highest treatments, with no apparent relationship with the exposure to the test item. By the end of the test (D10), one affected bee was observed in treatment T3, and three affected bees and one moribund bee were observed in treatment T4. No affected bees were observed in the control group throughout the test

C. OTHER EFFECTS (BEHAVIOURAL OBSERVATION)

The behavioural abnormalities observed were apathetic and moribund in all test item treatment groups on different assessment days from day 1 until the end of the 10 day test period.

Behavioural abnormalities in the reference item treatment group were not recorded since the reference item is known to be toxic to honeybees and therefore effects are expected. Moreover, validity criteria for reference item group were met.

D. FOOD CONSUPTION

The overall mean daily consumption of feeding solution was 19.00 mg/bee/day in the control group C (untreated 50 % (w/v) aqueous sucrose solution). The overall mean daily consumption of feeding solution for the test item doses of 25, 50, 100, 200 and 400 µg Clopyralid/bee/day was 19.62, 18.28, 18.43, 18.35 and 16.12 mg/bee/day, respectively.
The overall mean daily consumption of feeding solution in the reference item treatment group was 14.16 mg/bee/day.
The mean daily uptake for the test item doses of 25, 50, 100, 200 and 400 µg Clopyralid/bee/day (nominal) was 4.12, 7.68, 15.49, 30.84 and 54.20 µg Clopyralid/bee/day, respectively, and cumulative uptake was of 41.21, 76.82, 154.85, 308.44 and 541.97 µg Clopyralid/bee, respectively.

E. DEFFICIENCIES

None

F. VALIDITY CRITERIA

Control mortality <15% actual 8%.
Reference standard - 100% mortality after 10 days

III. CONCLUSIONS

All validity criteria for OECD 245 were met.

Cumulative and corrected cumulative mortality in the control, the test item and reference item treatment groups

Treatment	Nominal dose (µg a.i./bee/day)	Total number of bees dosed	Final Mortality (cumulative %)	SE	Abbotts' transformed mortality (%)
C	--	50	8.00	3.74	--
T1	25	50	8.00	3.74	0.00
T2	50	50	8.00	5.83	0.00
T3	100	50	6.00	2.45	-2.17
T4	200	50	10.00	7.75	2.17
T5	400	50	4.00	2.45	-4.35
R	0.170	50	100.00	0.00	100.00

Symptoms of intoxication were observed sporadically for few bees of the three highest treatments, with no apparent relationship with the exposure to the test item. By the end of the test (D10), one affected bee was observed in treatment T3, and three affected bees and one moribund bee were observed in treatment T4. No affected bees were observed in the control group throughout the test.

Behavioural Abnormalities

Treatment	Nominal dose (µg a.i./bee/day)	% of affected bees									
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
C	--	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T2	50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.13
T4	200	0.00	0.00	0.00	0.00	4.17	0.00	0.00	0.00	0.00	8.89
T5	400	0.00	0.00	2.04	0.00	4.08	0.00	0.00	0.00	0.00	0.00

The LDD50-value (Lethal Dietary Dose that kills 50 % of exposed individuals) for PP-113H (Clopyralid 100 g/L SL) was empirically estimated to be higher than the highest consumed dose of 54.20 µg Clopyralid/bee/day. The LC50-value (Lethal Concentration that kills 50 % of exposed individuals) was empirically estimated to be higher than the highest concentration tested of 4000 mg Clopyralid/L diet.
Since no dose response was obtained, the LDD10 / LC10 and LDD20 / LC20 values (Lethal Dietary Doses / Concentrations that kill 10 and 20 % of exposed individuals, respectively) for PP-113H (Clopyralid 100 g/L SL) could not be estimated.

The NOEDD (No Observed Effect Dietary Dose), based on actual consumption of the test item feeding solutions, was determined to be to the highest consumed dose of 54.20 µg Clopyralid/bee/day. After 10 days the NOEC value was determined to be 4000 mg Clopyralid/L diet.
Symptoms of intoxication were observed sporadically for few bees of the three highest treatments, with no apparent relationship with the exposure to the test item.

The results obtained with the toxic reference item, dimethoate, confirmed the sensitivity of the test system.

Data requirement
zRMS updated dRR B9 in terms of tabular information on the dose response, e.g. a table listing dose and effect data, and recovery to the dRR for the sake of transparency:

Test Conditions during Acclimatisation and Exposure

Timing	Incubation* (actual min. - max.)	Acclimatisation (actual min. - max.)	Exposure (actual min. - max.)
Temperature (target: 33 ± 2 °C)	33.0 – 34.1 °C	33.0 – 34.0 °C	32.4 – 34.0 °C
Relative humidity (target: 50 – 70 %)	56.5 – 68.2 %	56.2 – 64.4 %	57.2 – 69.0%

*Incubation period lasted from collection of the frames to the introduction of bees into test units.

Analytical results:

The measured concentration of clopyralid in the treated sucrose solution were within 80-120% of nominal for both treatment groups. Results of the analytical verification are given in the following table:

Sampling Code	Treatment No	Analysed concentration of Clopyralid (mg/kg diet)*	Clopyralid nominal concentrations (mg/kg diet)*	Recovery (%)
S19-3760-D0-C-AS	C	Not detected	--	--
S19-3760-D0-St-AS	St	6865.44	6722.88	102
S19-3760-D0-T1-AS	T1	208.30	210.08	99
S19-3760-D0-T5-AS	T5	3364.78	3361.34	100

* Density of the sucrose solution 1.19 g/mL

Cumulative mortal dietary dose (DD), and LDD_x/LC_x.

Treatment	1 cur mo
Control(s):	
C (0)	
Reference item: Dimeth	
R (0.170)	
Test item: PP-113H (Clo	
T1 (25)	
T2 (50)	
T3 (100)	
T4 (200)	
T5 (400)	
NOEDD	
LDD_{50} (95% CI)	
NOEC	
LC_{50} (95% CI)	

[a] Based on actual measu
[b] Over 7 days of exposu

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: Toxicity to honey bee larvae

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. **Validity criteria of the study:** ☑ The study was considered valid since validity criteria for the control group and reference item group were met. ☑ Control Mortality: The cumulative larval mortality from day 4 (D4) to the day 8 (D8) was ≤ 15% across all replicates in control group (actual value 2.08). On day 22 (D22) the adult emergence rate was ≥ 70% across all replicates of the control group (actual 85.42 %). ☑ Reference Item Mortality: The cumulative larval mortality was ≥ 50 % across all replicates on day 8 (D8) (actual 91.67 %). With the exception of sample for treatment T1 taken on D3, which gave recoveries of 133.9 % (A sample) and 130.8 % (R sample), recovery of the analysed samples resulted within ± 20 % of nominal. Since the endpoints corresponded to higher doses than T1, the applied test doses/concentrations were verified and no correction for the measured of treatment T1 concentration was needed. **Agreed endpoints:** The 22-Day NOED was determined to be 95.83 µg a.i./larva/developmental period. The 22-Day NOEC was determined to be 622.28 mg a.i./kg diet. The 22-Day LOED was determined to be 210.83 µg a.i./larva/developmental period. The 22-Day LOEC was determined to be 1369.03 mg a.i./kg diet. The 22-Day ED_{10}/EC_{10} and ED_{20}/EC_{20} could not be estimated due to the lack of a clear dose/response. The 22-Day ED_{50} was empirically estimated to be > 95.83 µg a.i./larva/developmental period. The 22-Day EC_{50} was empirically estimated to be > 622.28 mg a.i./kg diet.

Reference KCP 10.3.1/02

Report	Ansaloni, T. 2020, PP-113H (Clopyralid 100 g/L SL): Honey Bee (Apis mellifera L.) Larval Toxicity Test Following Repeated Exposure Under Laboratory Conditions Trialcamp S.L.U., Report Nº S19-03761,
Guideline(s):	ENV/JM/MONO (2016) 34: Guidance Document on Honey bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (OECD 239).
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No - not appropriate

Executive Summary

The The objective of this study was to determine the effects of PP-113H (Clopyralid 100 g/L SL) on the honey bee larvae, Apis mellifera L., from repeated feeding exposure in an 22 day in vitro test
he study was conducted with test item doses of 58.44, 128.57, 282.86, 622.28 and 1369.03 mg a.i./kg diet, equivalent to cumulative doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva; 1 reference item group with 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva.
The validity of the test system was checked with a reference item dimethoate at the dose levels of 48.00 mg/kg diet (Equivalent to 7.39 µg dimethoate/larva) and found to be valid. The untreated control group was treated with deionised water only. There were three replicates for each treatment group. Each replicate consisted of 16 larvae. The test item was provided with diet.

At day 8 (D8) of the test in the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortality were; 6.25, 8.33, 4.17, 2.08 and 12.50 %, respectively. Two larvae in the control group and one larva in treatment T4 with presence of uneaten food were observed at day 8 (D8).

At day 15 (D15) of the test in the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortality were 8.33, 14.58, 18.75, 10.42 and 35.42 %, respectively.

In the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortalities at 22 days (D22) after grafting were 10.42, 16.67, 20.83, 12.50 and 47.92 %, respectively. Consequently, the
mean emergence rates were 89.58, 83.33, 79.17, 87.50 and 52.08 %, respectively. No affected emerged bees were recorded on day 22 (D22).
With the exception of sample for treatment T1 taken on D3, which gave recoveries of 133.9 % (A sample) and 130.8 % (R sample), recovery of the analysed samples resulted within ± 20 % of nominal. Since the endpoints corresponded to higher doses than T1, the applied test doses / concentrations were verified and no correction for the measured of treatment T1 concentration was needed.

The 22-Day NOED was determined to be 95.83 µg a.i./larva/developmental period. The 22-Day NOEC was determined to be 622.28 mg a.i./kg diet.

The 22-Day LOED was determined to be 210.83 µg a.i./larva/developmental period. The 22-Day LOEC was determined to be 1369.03 mg a.i./kg diet.

The 22-Day ED10 / EC10 and ED20 / EC20 could not be estimated due to the lack of a clear dose / response. The 22-Day ED50 was empirically estimated to be > 95.83 µg a.i./larva/developmental period.

The 22-Day EC50 was empirically estimated to be > 622.28 mg a.i./kg diet The study was deemed valid since all validity criteria were met.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Dark yellow liquid
Lot/Batch #:	20190506
Purity:	Clopyralid: 10.2 ± 0.1 (as clopyralid)
Stability of test compound:	Stable
Positive control:	Positive control: BAS 152 11 I (400g/L dimethoate)

3. Test animals -

Species:	Honey bee (*Apis mellifera*)
Age:	First instar larvae, L1 (not older than 30 hours at grafting time).
Source:	Commercial bee hives maintained by Trialcamp S.L.U
Feeding:	The larval diet was prepared prior to the test and stored in a fridge (≤ 5 ºC but not frozen). Each larva was fed once a day (except on day 2 (D2)) with a standardized amount of artificial diet until day 6. For feeding, a multi stepper pipette was used. For feeding of treated diet, a new pipette tip was used for each treatment group. Care was taken to avoid touching and drowning the larvae when feeding them. Food was dropped next to the larva, along the wall of the grafting cell. The diet was prepared with deionised water using the following ingredients: • Diet A (D1, volume administered: 20 µL/larva): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight of fructose • Diet B (D3, volume administered: 20 µL/larva): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight of glucose and 15 % weight of fructose. • Diet C (from D4 to D6, volume administered: 30 µL/larva, 40 µL/larva and 50 µL/larva, respectively): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of glucose and 18 % weight of fructose.
Water	See above.
Housing:	

Larvae were transferred into crystal polystyrene grafting cells (NICOTPLAST) with a diameter of 9 mm. Cells were initially sterilised by emerging for 30 min in ethanol 70 % (v/v), and then dried. Each cell was placed into a well of a sterile 48-well cellular culture plate (Greiner Bio One), and the so prepared experimental units were placed under UV light for 15 minutes. The open plates of the control groups, of all test item groups and the reference item group were individually placed into hermetically sealed Plexiglas desiccators, containing dishes filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from day 1 (D1) to day 7 (D7), when the well plates were transferred to another Plexiglas desiccator, containing a saturated sodium chloride (NaCl) solution in order to keep the established relative humidity until day 15 (D15). All desiccators were placed into the same incubator with forced air circulation. After the assessment on day 15 (D15), the test units were allocated into to an emergence box (plastic polypropylene 18 x 13 x 7 cm, all approximate) and placed inside a climatic chamber. Each emergence box was supplied with 50 % (w/v) aqueous sucrose *ad libitum*.

Loading:

The study was conducted as a dose response test with a duration of 22 days from grafting on day 1 (D1) to the final assessment on day 22 (D22). It comprised 1 control group; 5 test item groups with the cumulative doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva and 1 reference item group with a cumulative dose of 7.39 µg dimethoate/larva.

For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate, 16 larvae from each replicate were used.

Test duration: 22 days

4. Environmental conditions -

Temperature: 29.7- 35.1°C

Humidity: 44.6 to 97.8%

Photoperiod: Dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 11/12/19 to 24/12/19

2.Animal assignment and treatment:

The colonies were examined for reportable bee epidemics by an authorized bee specialist and are inspected periodically according to the standard bee-keeping practices by an experienced apiarist. The hives used for honey bee larvae collection were adequately fed, healthy, as far as possible parasite-free and queen-right. No chemical substances (such as antibiotics, anti-Varroa treatments, pesticides, etc.) have been used in the hive within 4 weeks preceding the start of the test.

Four days prior to grafting of larvae (D-3), in order to synchronize the age of larvae used for the test, the queens of at least three colonies were confined in their own colony in an excluder cage containing a comb with empty cells. Three days prior to the grafting (D-2), maximum 30 hours after encaging, the queens were released from the cages. The combs containing eggs were left in the excluder cages during the incubation stage until hatching on day 1 (D1). At D1, three combs were transferred to the laboratory using an insulated container in order to avoid temperature variation. Once in the laboratory, first instar larvae were selected for grafting. On D1 the test was initiated with excess larvae with 3 reserve plates. Before the first application of the test item on day 3 (D3), it was assured that all larvae used for the test were of similar size and alive

The study was conducted as a dose response test with a duration of 22 days from grafting on day 1 (D1) to the final assessment on day 22 (D22). It comprised 1 control group; 5 test item groups with the cumulative doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva and 1 reference item group with a cumulative dose of 7.39 µg dimethoate/larva.
For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate, 16 larvae from each replicate were used.

3. Observations:

Assessment of larval mortality was conducted before feeding on day 4 (D4), day 5 (D5), day 6 (D6), day 7 (D7), day 8 (D8). On day 15 (D15) larvae that had not transformed into pupae were re corded as dead. Assessment of adult emergence was carried out on day 22 (D22). With assistance of a stereo microscope, when necessary, larvae were recorded as dead if no respiration (movement of spiracles) was observed. At each assessment time, dead larvae were removed for sanitary reasons. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group. On day 8 (D8) during the assessment of mortality, the presence of uneaten food was qualitatively recorded.

4. Statistics:

Statistical calculations were made with the statistical program ToxRatPro Version 3.2.1.
Step-down Cochran-Armitage Test Procedure was used to calculate the 22-Day, NOED / NOEC and LOEC / LOED values.
Due to the lacking of a clear dose/response, valid EDx/ECx values could not be calculated.

II. RESULTS AND DISCUSSION

A. MORTALITY

In the control group, cumulative larval mortality from day 4 (D4) until day 8 (D8) was 2.08. On day 22 (D22), the adult emergence rate in the control group was 85.42 % of the initial grafted larvae. Cumulative mortality in the Reference Item treatment group was 91.67 % by D8. Therefore, the validity criteria were met.

At day 8 (D8) of the test in the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortality were; 6.25, 8.33, 4.17, 2.08 and 12.50 %, respectively. Two larvae in the control group and one larva in treatment T4 with presence of uneaten food were observed at day 8 (D8).

At day 15 (D15) of the test in the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortality were 8.33, 14.58, 18.75, 10.42 and 35.42 %, respectively.

In the test item doses of 9.00, 19.80, 43.56, 95.83 and 210.83 µg a.i./larva, the cumulative mean mortalities at 22 days (D22) after grafting were 10.42, 16.67, 20.83, 12.50 and 47.92 %, respectively. Consequently, the

mean emergence rates were 89.58, 83.33, 79.17, 87.50 and 52.08 %, respectively. No affected emerged bees were recorded on day 22 (D22).

B. OTHER EFFECTS (BEHAVIOURAL OBSERVATION)

Two larvae in the control group and one larva in treatment T4 with presence of uneaten food were observed at day 8 (D8).

C. VALIDITY CRITERIA

The study was considered valid since validity criteria for the control group and reference item group were met.

Control Mortality: The cumulative larval mortality from day 4 (D4) to the day 8 (D8) was ≤ 15% across all replicates in control group (actual value 2.08). On day 22 (D22) the adult emergence rate was ≥ 70% across all replicates of the control group (actual 85.42 %).

Reference Item Mortality: The cumulative larval mortality was ≥ 50 % across all replicates on day 8 (D8) (actual 91.67 %).

D DEFICIENCIES

None

III. CONCLUSIONS

The repeated exposure of PP-113H (Clopyralid 100 g/L SL) to honey bee larval was tested under laboratory conditions over a period of 22 days.

With the exception of sample for treatment T1 taken on D3, which gave recoveries of 133.9 % (A sample) and 130.8 % (R sample), recovery of the analysed samples resulted within ± 20 % of nominal. Since the endpoints corresponded to higher doses than T1, the applied test doses / concentrations were verified and no correction for the measured of treatment T1 concentration was needed.

The 22-Day NOED was determined to be 95.83 µg a.i./larva/developmental period. The 22-Day NOEC was determined to be 622.28 mg a.i./kg diet.

The 22-Day LOED was determined to be 210.83 µg a.i./larva/developmental period. The 22-Day LOEC was determined to be 1369.03 mg a.i./kg diet.

The 22-Day ED10 / EC10 and ED20 / EC20 could not be estimated due to the lack of a clear dose / response. The 22-Day ED50 was empirically estimated to be > 95.83 µg a.i./larva/developmental period.

The 22-Day EC50 was empirically estimated to be > 622.28 mg a.i./kg diet The study was deemed valid since all validity criteria were met.

Table A 2.3.1.2-1: Corrected cumulative mortality by the control group in the test item and reference item treatment groups

Treatment Group	Dose		Corrected Mortality [%]						
			D4	D5	D6	D7	D8	D15	D22
Control	---	---	---	---	---	---	---	---	---
Test item PP-113H (Clopyralid 100 g/L SL)	9.00	[µg a.i./larva]	-2.13	2.13	2.13	2.13	4.26	-7.32	-4.88
	19.80		2.13	4.26	4.26	4.26	6.38	0.00	2.44
	43.56		-2.13	-2.13	0.00	0.00	2.13	4.88	7.32
	95.83		-2.13	-2.13	0.00	0.00	0.00	-4.88	-2.44
	210.83		-2.13	-2.13	6.38	8.51	10.64	24.39	39.02
Reference Item (dimethoate)	7.39	[µg dimethoate/larva]	31.91	61.70	87.23	87.23	91.49	97.56	97.56

Table A 2.3.1.2-2: Emergence rate in the control, the test item and reference item treatment groups

Treatment Group	Dose		Emergence % D22
Control	---	---	85.42
Test item PP-113H (Clopyralid 100 g/L SL)	9.00	[µg a.i./larva]	89.58
	19.80		83.33
	43.56		79.17
	95.83		87.50
	210.83		52.08
Reference Item (dimethoate)	7.39	[µg dimethoate/larva]	2.08

Table A 2.3.1.2-3: Study endpoint summary

Endpoint	µg a.i./larva/developmental period
22-Day NOED[1]	95.83
22-Day LOED[1]	210.83
22-Day ED10 [95 % I.C.]	Not estimated [2]
22-Day ED20 [95 % I.C.]	Not estimated [2]
22-Day ED50 [95 % I.C.]	> 95.83
Endpoint	**mg a.i./kg diet**

22-Day NOEC[1]	622.28
22-Day LOEC[1]	1369..03
22-Day EC10 [95 % I.C.]	Not estimated [2]
22-Day EC20 [95 % I.C.]	Not estimated [2]
22-Day EC50 [95 % I.C.]	> 622.28

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing and aged residue studies

A 2.3.2.2.1 Study 1: Extended laboratory - *Aphidius rhopalosiphi*

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. **Agreed endpoints:** The results obtained in the trial showed that the test item **BARILOCHE** caused a light mortality increase of the test system *Aphidius rhopalosiphi* when applied to wheat plants at increasing rates. The wasp's mortality at the maximum rate tested equivalent to 3154.80 g of formulated product/ha was 28.57%. Then the LR_{50} of the test item evaluated after 48 hours of exposure was higher than 3154.80 g of formulated product/ha equivalent to 299.71 g of Clopyralid/ha. During the reproduction performance, the surviving females from the treatments had a reduced reproductive capacity respect to the surviving females of the control group. At the maximum rate tested the reduction in reproduction of the treated wasp's respect the wasps of the control group was 67.87%. The calculated ER_{50} value of the test item was 993.22 g of formulated product/ha (confidential limits: 794.43 – 1267.95) equivalent to 94.36 g of clopyralid/ha.

The test item **BARILOCHE** had no effect on the behaviour of the treated parasitoids and had no repellent effects to the wasps at the maximum rate tested.

FINDINGS

Mortality:

Table 2. Mean mortality of the wasps after 48 hours of exposure

Product	g product/ha	g a.i./ha	% Mean Mortality	% Mean Corrected Mortality
Control	0.00	0.00	6.67	n.a.
Test item - T1	197.17	18.73	3.33	-3.58
Test item - T2	394.35	37.46	6.67	0.00
Test item - T3	788.70	74.93	6.67	0.00
Test item - T4	1577.40	149.85	13.33	7.14
Test item - T5	3154.80	299.71	33.33	28.57
Reference item	10.00	3.65	90.00	89.29

Reproduction:

Table 3. Reproduction performance of the treated wasps

Product	g product/ha	g a.i./ha	Mean N° of mummies/♀	±SD	% R
Control	0.00	0.00	10.80	3.10	n.a.
Test item - T1	197.17	18.73	9.13	2.72	15.46
Test item - T2	394.35	37.46	7.20	2.57	33.33
Test item - T3	788.70	74.93	5.13	2.50	52.50
Test item - T4	1577.40	149.85	4.27	2.60	60.46
Test item - T5	3154.80	299.71	3.47	2.47	67.87

%R = % reduction in reproduction

Reference	KCP 10.3.2/01
Report:	Colli, M.,2011 Effects of the product PP-113H (CLOPYRALID 10% w/v SL) on the aphid parasitoid Aphidius rhopalosiphi De Stefani-Perez (Hymenoptera: Braconidae) under Extended Laboratory Conditions (Rate Response Test). Report Nº BT098/11
Guidelines	IOBC (Mead-Briggs et al., 2000).
Deviation	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of residues of the *product PP-113H (CLOPYRALID 10% w/v SL)* on the mortality and reproduction of the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae) were tested with an extended laboratory study, according to GLP regulations.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Item	
Name	**PP-113H (CLOPYRALID 10% w/v SL)**

Description	Herbicide
Lot/Batch #	20110713
Purity	CLOPYRALID: 9.50% w/w – 99.00 g/L
Stability of the test compound	Stable
2.Test system	
Species	*Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae)
Strain	Not relevant
Age	Adults (24 hours old)
Weight at dosing	Not relevant
Source	Biotecnologie BT S.r.l.
Acclimation	Not relevant
Diet	Water/honey solution
Water	Not relevant
3. Experimental conditions	
Temperature	exposure phase: 21.00 – 21.67°C / fecundity test: 18.00 – 21.33°C / mummies maturation period: 19.67 – 21.33°C
Humidity	exposure phase: 73.00 – 81.50%
Light Intensity	16 h light and 8 h darkness
Photoperiod	phase: 675 - 1125 lux / fecundity test 2500 - 3700 lux / mummies maturation period: 20000 lux

B. STUDY DESIGN AND METHODS:

1. Experimental period

31st October – 15th November 2011

2. Experimental treatment

The test item was sprayed on wheat plants with calibrated spray equipment at five rates. The formulation Perfekthion was used as reference item, whereas deionised water was used as control. Six replicates for each treatment were performed. The sprayed test items were allowed to dry for about 1 hour, and then in each cage five adults were introduced. After 48 hours of exposure to the treated plants, the surviving parasitoids were removed from the exposure cages and the parasitic capacity per female was evaluated in the fecundity test.

3. Application rates

The test was carried out on 7 treatment groups.

Table 1. Trial layout

Treatment	Application Rates		N° adults/ cage	N° replicates
	Product g/ha	a.s. g/ha		
Control	0.00	0.00	5	6
Test item - T1	197.17	18.73	5	6
Test item - T2	394.35	37.46	5	6
Test item - T3	788.70	74.93	5	6
Test item - T4	1577.40	149.85	5	6
Test item - T5	*3154.80	299.71	5	6
Reference item	10.00	3.65	5	6

*Worst case application rate 2L/ha + 1L/ha
Density: 1.0516 g/mL

4. Observations	The conditions of the exposed parasitoids were recorded at approximately 2 and 48 hours after their introduction (due to technical reason the assessment at 2 hours after wasps introduction was not carried out; see Deviation N° 1). Counting of parasitized aphids was carried out 13 days after the start of the fecundity test.
5. Statistics	The software SPSS Statistics 17.0 performed the statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality of the wasps after 48 hours of exposure

Product	g product/ha	g a.s./ha	% Mean Mortality	% Mean Corrected Mortality
Control	0.00	0.00	6.67	n.a.
Test item - T1	197.17	18.73	3.33	-3.58
Test item - T2	394.35	37.46	6.67	0.00
Test item - T3	788.70	74.93	6.67	0.00
Test item - T4	1577.40	149.85	13.33	7.14
Test item - T5	3154.80	299.71	33.33	28.57
Reference item	10.00	3.65	90.00	89.29

B. REPRODUCTION PERFORMANCE

Reproduction performance of the treated wasps

Product	g product/ha	g a.s./ha	Mean N° of mummies/♀	±SD	% R
Control	0.00	0.00	10.80	3.10	n.a.
Test item - T1	197.17	18.73	9.13	2.72	15.46
Test item - T2	394.35	37.46	7.20	2.57	33.33
Test item - T3	788.70	74.93	5.13	.50	52.50
Test item - T4	1577.40	149.85	.27	2.60	60.46
Test item - T5	3154.80	299.71	3.47	2.47	67.87

%R = % reduction in reproduction

C. DEFICIENCIES

None

III. CONCLUSIONS

The results obtained in the trial showed that the test item **PP-113H (CLOPYRALID 10% w/v SL)** caused a light mortality increase of the test system *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae) when applied to wheat plants at increasing rates. The wasp's mortality at the maximum rate tested equivalent to 3154.80 g of formulated product/ha was 28.57%. Then the LR_{50} of the test item evaluated after 48 hours of exposure was higher than 3154.80 g of formulated product/ha equivalent to 299.71 g of Clopyralid/ha.

During the reproduction performance, the surviving females from the treatments had a reduced reproductive capacity respect to the surviving females of the control group. At the maximum rate tested the reduction in reproduction of the treated wasp's respect the wasps of the control group was 67.87%. The calcu-

lated ER_{50} value of the test item was 993.22 g of formulated product/ha (confidential limits: 794.43 – 1267.95) equivalent to 94.36 g of Clopyralid/ha.
The test item had no effect on the behaviour of the treated parasitoids and had no repellent effects to the wasps at the maximum rate tested.

A 2.3.2.2.2 Study 1a: Extended laboratory - *Aphidius rhopalosiphi*

Comments of zRMS:

The study was accepted in dRR B9 for plant product protection Faworyt 300 SL in 07.2022 by PL zRMS.

All validity criteria were met.

- Control Mortality: 0.0 % (should not exceed 13%)
- Reference Item Mortality: 100.0 % corrected mortality (should result in at least 50% corrected mortality)
- Control Reproduction Rate: 41.0 mummies per female (mean value) (should be ≥ 5 female)
- There was no parasitoid producing zero values (there should be no more than 2 parasittoids producing zero values)

Agreed endpoints:

	Rate [1] [mL/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [mummies/female]	Effect on reproduction [5] [%]
Control	0	0.0	--	41.0	--
Faworyt 300 SL	4.94	2.5 n.s.	2.5	45.6 n.s.	-11.3
Faworyt 300 SL	14.8	5.0 n.s.	5.0	30.8 *	24.8
Faworyt 300 SL	44.4	0.0 n.s.	0.0	32.6 *	20.5
Faworyt 300 SL	133	0.0 n.s.	0.0	29.4 *	28.3
Faworyt 300 SL	400	10.0 n.s.	10.0	22.0 *	46.3
Endpoints					
LR_{50}: > 400 mL product/ha (equivalent to 121 g a.s./ha) ER_{50}: > 400 mL product/ha (equivalent to 121 g a.s./ha)					

1) Application rate in 200 L water/ha
2) Mortality: after 48 hours of exposure to spray residues on glass plates, (Bonferroni-Holm Fisher's Exact Test, α = 0.05; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of parasitised aphids/female, (Williams t-test, α = 0.05; n.s. = not significant, * = significant)
5) Calculated on the exact raw data; negative values indicate better performance compared to the control

Reference:	KCP 10.3.2.1/01
Report:	Faworyt 300 SL: Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Dose Response Test, Moll M., 2019, 140601001
Guideline(s):	IOBC (Mead-Briggs et al., 2000 and 2010)
Deviations:	None

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Material and methods:

Test Item:	Faworyt 300 SL; batch no.: 201805002; content of a.s.: 302.7 g/L Chlopyralid.
Test Species:	Parasitoid (Aphidius rhopalosiphi), adults not older than 48 hours; source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 4 replicates each containing 10 adult parasitoids. The parasitoids were exposed to dried residues on treated glass plates. Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups where the corrected mortality was < 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 - 12 days before the numbers of aphid mummies that had developed were assessed.
Endpoints:	Mortality of exposed parasitoids; LR50: lethal rate producing 50 % mortality after 48 h of exposure. Additionally reproductive capacity for female survivors.
Reference Item:	Perfekthion (nominal: 400 g dimethoate/L).
Test Rates:	Control, 4.94, 14.8, 44.4, 133 and 400 mL product/ha (equivalent to 1.50, 4.48, 13.4, 40.3 and 121 g a.s./ha) and reference item. The reference item was applied at an application rate of 0.3 mL Perfekthion/ha. All treatments were applied in 200 L water/ha. The spraying dilutions were sprayed onto glass plates via laboratory spraying equipment, which were then air dried.
Test Conditions:	Temperature: 19 - 21 °C; relative humidity: 69 - 72 % (acclimatisation and exposure period), 77 - 84 % (post-exposure period, within the test units); photoperiod: 16 h light : 8 h dark; light intensity: 1140 - 2060 lux (acclimatisation, exposure and parasitisation period), 8560 - 15110 lux (post-parasitisation period

Findings:

	Rate [1] [mL/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [mummies/female]	Effect on reproduction [5] [%]
Control	0	0.0	--	41.0	--
Faworyt 300 SL	4.94	2.5 n.s.	2.5	45.6 n.s.	-11.3
Faworyt 300 SL	14.8	5.0 n.s.	5.0	30.8 *	24.8
Faworyt 300 SL	44.4	0.0 n.s.	0.0	32.6 *	20.5
Faworyt 300 SL	133	0.0 n.s.	0.0	29.4 *	28.3
Faworyt 300 SL	400	10.0 n.s.	10.0	22.0 *	46.3
Endpoints					
LR_{50}: > 400 mL product/ha (equivalent to 121 g a.s./ha) ER_{50}: > 400 mL product/ha (equivalent to 121 g a.s./ha)					

1) Application rate in 200 L water/ha

2) Mortality: after 48 hours of exposure to spray residues on glass plates,
(Bonferroni-Holm Fisher's Exact Test, α = 0.05; n.s. = not significant)

3) Corrected mortality according to Abbott and improvements by Schneider-Orelli

4) Reproduction: mean number of parasitised aphids/female,
(Williams t-test, α = 0.05; n.s. = not significant, * = significant)

5) Calculated on the exact raw data; negative values indicate better performance compared to the control

Conclusion:
Under worst case laboratory conditions the LR50 of Faworyt 300 SL is estimated to be greater than 400mL product/ha (equivalent to 121 g a.s./ha) in 200 L water/ha.
Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item dose rates. There was no statistically significant effect on reproduction at 4.94 mL product/ha (equivalent to 1.50 g a.s./ha). At 14.8, 44.4, 133 and 400 mL product/ha (equivalent to 4.48, 13.4, 40.3 and 121 g a.s./ha) reproduction was statistically significantly affected, but the effect on reproduction was below the trigger value of 50 % (20.5 - 46.3 %). Therefore it can be summarised that there was no effect on reproduction up to and including 400 mL product/ha (equivalent to 121 g a.s./ha). The ER_{50} is estimated to be greater than 400 mL product/ha (equivalent to 121 g a.s./ha) in 200 L water/ha.

A 2.3.2.2.3 Study 2: Extended laboratory -*Typhlodromus pyri*

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. **Agreed endpoints:** The results obtained in the trial demonstrate that the test item **BARILOCHE** caused a no statistically significant mortality of the test system *Typhlodromus pyri* when applied to bean leaves. The exact value of LR_{50} of **BARILOCHE** could not be calculated, because none of the rates tested caused mortalities above 18.97%. The LR_{50} evaluated after 7 days of exposure, can be assumed to be above the highest rate tested of 3154.80 g of formulated product/ha equivalent to 299.70 g of clopyralid/ha. During the reproductive test, the test item caused a light reduction in reproductive capacity of surviving mites. but this reduction was <50%. Based on these results the ER_{50} of **BARILOCHE** evaluated at 14 days after treatment, was >3154.80 g of formulated product/ha equivalent to 299.70 g of Clopyralid/ha.

Reference	KCP 10.3.2/02
Report:	Colli, M.,2011 Effects of the product PP-113H (CLOPYRALID 10% w/v SL)on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) under Extended Laboratory Conditions (Rate Response Test) Report Nº BT099/11
Guidelines	IOBC/WPRS Guidelines to evaluate side-effects of plant protection products to non-target arthropods (Blümel *et al.*, 2000).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of dry spray deposits of the product **PP-113H (CLOPYRALID 10% w/v SL)** on the mortality and reproduction of the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) were tested with an extended laboratory study, according to GLP regulations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item	
Name	PP-113H (CLOPYRALID 10% w/v SL)
Description	Herbicide
Lot/Batch #	20110713
Purity	Clopyralid: 9.50% w/w – 99.00 g/L
Stability of the test compound	Stable
2.Test system	
Species	*Typhlodromus pyri*
Strain	Not relevant
Age	Protonymphs (24 hours old)
Weight at dosing	Not relevant
Source	Biotecnologie BT S.r.l.
Acclimation	Not relevant
Diet	*Tethranichus urticae*
Water	50% deionised water + 50% tap water
3. Testing conditions	
Temperature	23.00 – 26.00 °C
Humidity	69.00 – 77.00%
Photoperiod	16 h light and 8 h darkness
Air changes	n.a.

B. STUDY DESIGN AND METHODS

1. Experimental period	03rd November – 17th November 2011
2. Experimental treatment	The test item was sprayed on bean plants at five different rates. The formulation Perfekthion was used as reference item, whereas deionised water was used as control. Three replicates for each treatment were performed. The treated plants were allowed to dry for about 1 hour, and then in each cage 20 protonymphs were introduced. Mites were exposed to treated leaves up to 14 days. At 7 days, the reproductive test was constituted.
3. Application rates	The test item and the Reference item were tested at the following rates:

Table 1. Trial layout

Products	Rates		N° mites/ cage	N° replicates	Identification Code
	g product/ha	g a.i./ha			
control	0.00	0.00	20	3	Ca ÷ Cc
Test item (T1)	197.17	18.73	20	3	T1a ÷ T1c
Test item (T2)	394.35	37.46	20	3	T2a ÷ T2c
Test item (T3)	788.70	74.92	20	3	T3a ÷ T3c
Test item (T4)	1577.40	149.85	20	3	T4a ÷ T4c

	Test item (T5)	*3154.80	299.70	20	3	T5a ÷ T5c
	Reference item	10.00	3.65	20	3	Ra ÷ Rc
	*Worst case application rate 2L/ha + 1L/ha Density: 1.0516 g/mL					
4. Observations	The survival of mites was assessed at day 4 and day 7. During reproduction phase the number of survived females, laid and hatched eggs were recorded at day 7, 11, 13 and 14.					
5. Statistics	Statistical analysis by "SPSS – Statistics 17.0".					

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality:

Table 2. Mean mortality after 7 days of exposure

Product	g product/ha	g a.i./ha	% Mean Mortality	% Mean Corrected Mortality
control	0.00	0.00	3.33	n.a.
Test item (T1)	197.17	18.73	6.67	3.46
Test item (T2)	394.35	37.46	15.00	12.07
Test item (T3)	788.70	74.92	20.00	17.24
Test item (T4)	1577.40	149.85	21.67	18.97
Test item (T5)	3154.80	299.70	20.00	17.24
Reference item	10.00	3.65	98.33	98.27

Reproduction:

Table 3. Reproduction performance after 14 days

Product	g product/ha	g a.i./ha	Mean N° of eggs/♀	±SD	% R
control	0.00	0.00	9.78	1.14	n.a.
Test item (T1)	197.17	18.73	7.30	1.54	25.36
Test item (T2)	394.35	37.46	7.63	1.35	21.98
Test item (T3)	788.70	74.92	7.28	0.81	25.56
Test item (T4)	1577.40	149.85	7.09	1.02	27.51
Test item (T5)	3154.80	299.70	6.89	0.51	29.55

%R = % reduction in reproduction

III. CONCLUSIONS

The results obtained in the trial demonstrate that the test item **PP-113H (CLOPYRALID 10% w/v SL)** caused a no statistically significant mortality of the test system *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) when applied to bean leaves. The exact value of LR_{50} of **PP-113H (CLOPYRALID 10% w/v SL)** could not be calculated, because none of the rates tested caused mortalities above 18.97%. The LR_{50} evaluated after 7 days of exposure, can be assumed to be above the highest rate tested of 3154.80 g of formulated product/ha equivalent to 299.70 g of Clopyralid/ha.

During the reproductive test, the test item caused a light reduction in reproductive capacity of surviving mites. but this reduction was <50%. Based on these results the ER_{50} of **PP-113H (CLOPYRALID 10% w/v SL)** evaluated at 14 days after treatment, was >3154.80 g of formulated product/ha equivalent to 299.70 g of Clopyralid/ha.

A 2.3.2.2.4 Study 3: extended laboratory - *Orius laevigatus*

Comments of zRMS:

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

Control mortality:

- ☑ The mean juvenile mortality in the control group was ≤ 25 % (actual 16.7 %)
- ☑ Toxic reference mortality: The juvenile mortality in the toxic reference group was ≥ 40 % (actual: 92.5 %, 91.0 % corrected for control).
- ☑ Control reproduction: Bugs in the control group produced ≥ 2 eggs per female per day (actual 6.6); no more than 5 bugs failed to produce eggs (1 female not producing eggs) and 70 % of the eggs hatched successfully (actual 97.1 %).

All required validity criteria were met and the sensitivity of the test organisms was conformed. Accordingly, the study was deemed valid.

Agreed endpoints:

Endpoint	Rate	
	[L test item/ha] [a]	[g clopyralid/ha] [b]
$NOER_{mortality}$	≥ 2.50	≥ 255.00
LR_{50}	> 2.50	> 255.00
$NOER_{fecundity}$	≥ 2.50	≥ 255.00
$ER_{50\ fecundity}$	> 2.50	> 255.00
$NOER_{fertility}$	≥ 2.50	≥ 255.00
$ER_{50\ fertility}$	> 2.50	> 255.00

[a] rate in L of formulated product /ha
[b] active ingredient content according to the certificate of analysis (102 g/L)

Reference	KCP 10.3.2/03
Report:	Luna, F., 2020 PP-113H (Clopyralid 100 g/L SL): Toxicity to the Predatory Bug, Orius laevigatus Fieber (Heteroptera, Anthocoridae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits Report Nº S19-03762 May 2020
Guidelines	IOBC (Bakker et al., 2000, modified).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of freshly applied spray deposits of PP-113H (Clopyralid 100 g/L SL) on juvenile mortality and reproduction of Orius laevigatus Fieber under extended laboratory conditions, and to determine the rate producing 50 % mortality (LR50), the No Observed Effect Rate (NOER) and 50 % effect (ER50), where possible. The test item, herbicide, was tested on fragments of leaf of cast iron plant, Aspidistra elatior.

The tested concentrations were 0.15625, 0.3125, 0.625, 1.25 and 2.50 L of test item/ha. [Equivalent to 15.94, 31.88, 63.75, 127.50 and 255.00 g of active ingredient (a.i.)/ha, according to the analysed content] and a reference standard (BAS 152 11 I) - dimethoate 40% w/v EC at a concentration of 2.0 g/ha). Each treatment group included forty replicates, containing two nymphs in each.. The species used for the test was the predatory bug Orius laevigatus Fieber (Heteroptera: Anthocoridae). Second instar nymphs, 4 to 5 days old, from the same cohort were selected for the exposure. Eighty nymphs per treatment were confined within test units; two individuals per unit.

Under the conditions of this study, PP-113H (Clopyralid 100 g/L SL) applied to fragments of leaf of Aspidistra elatior, the No Observed Effect Rate (NOER) for mortality was determined to be equal to or greater than 2.50 L test item/ha, the highest tested rate, equivalent to

255.00 g active ingredient (a.i.)/ha according to the certificate of analysis. The rate producing 50 % mortality (LR50) was assumed as greater than 2.50 L test item/ha (255.00 g a.i./ha).

No substantial delay in development was observed at any of the test item rates as compared to the control.

The NOER for fecundity was determined to be equal to or greater than 2.50 L test item/ha, equivalent to 255.00 g a.i./ha according to the certificate of analysis; the highest tested rate. The Median Effect Rate (ER50) for fecundity could not be calculated since no reduction in fecundity in the studied test item groups compared to control was observed; therefore, it was assumed greater than 2.50 L test item/ha (255.00 g a.i./ha).

The NOER for fertility was determined to be equal to or greater than 2.50 L test item/ha, equivalent to 255.00 g a.i./ha according to the certificate of analysis; the highest tested rate. The ER50 for fertility could not be calculated as reduction in fertility in the studied test item groups compared to control was always below the trigger value of 50 %; therefore, it was assumed greater than 2.50 L test item/ha (255.00 g a.i./ha).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Dark yellow liquid
Lot/Batch :	20190506
Purity:	Clopyralid: 10.2 ± 0.1
CAS #:	Clopyralid: 1702-17-6
Stability of test compound:	Chemically stable under the conditions of storage, handling and use.
	Control: Deionised water
2. Control/Positive Control	Positive control: BAS 152 11 I (dimethoate 40% w/v EC)
3. Test animals:	
Species:	*Orius laevigatus Fieber (Heteroptera, Anthocoridae)*
Age:	4 – 5 days old nymphs (2nd nymphal stage)
Source:	AGROBIO

Feeding:	Untreated Ephestia kuehniella eggs.
Water	Deionised water

Housing:

Exposure unit:

A treated fragment of leaf placed on top of a cotton pad (adaxial surface facing upwards) in a transparent plastic container without lid of approximately 0.5 L (12 × 10 × 5 cm, long × wide × high). The edges of the leaf could be clipped on the cotton pads with a stapler if it is necessary. The cotton pad under the leaf will be wetted during the whole exposure period. To prevent the bugs from escaping, a tangle-foot ® glue barrier will be set up on the centre of the leaf, so that an exposure area of approximate 4 to 8 cm in diameter will be delimited. Before the glue, on one or two sides of the leaf, wet cotton or paper strips will be extended to the water reservoir and therefore the predators will be constantly provided with water.

Mating Box:

One ventilated plastic box of approximately 12.5 L (32.5 × 26.5 × 20 cm, long × wide × high) per treatment group, filled with folded paper to reduce cannibalism. Untreated bean pods will be provided as a water source.

Reproduction unit:

An untreated bean leaflet placed on top of a cotton pad (abaxial surface facing upwards in a Petri dish (diameter 9 cm) will be used as oviposition substrate. To prevent bugs from escaping, a glass ring (approximately 4 × 4 cm, diameter × height) covered with a piece of gauze will be placed on top of the leaflet. The cotton pad under the leaf will be wetted during the whole reproduction period.

Loading:

4. Environmental conditions

Temperature:	24.5 – 25.1 °C
Humidity:	74.9 – 83.1 %
Light intensity	973.3 – 1522.4 lux
Photoperiod:	16 h light / 8 h darkness

B. STUDY DESIGN AND METHODS:

In life dates:	07 Oct 2019 –28 Oct 2019

Animal assignment and treatment:

The species used for the test was the predatory bug Orius laevigatus Fieber (Heteroptera: Anthocoridae). Second instar nymphs, 4 to 5 days old, from the same cohort were selected for the exposure. Eighty nymphs per treatment were confined within test units; two individuals per unit.

The exposure was conducted on fragments of leaf of cast iron plant, Aspidistra elatior Blume, Asparagaceae, collected from untreated plants grown at the testing facility. One fragment of leaf (size ≥ 4 cm diameter) was used per replicate unit. Immediately before application of the test solutions, the fragments of leaf were cut off and placed on top of the application surface with the adaxial surface facing upwards.

Cast iron plants (Aspidistra elatior Blume) were considered as a suitable crop variety for use in this study since the test item is an herbicide with effect on broadleaf plants and the size (width) of its leaves allows a correct preparation of the experimental units and exposure of the target species, Orius laeviagatus

The tested concentrations were 0.15625, 0.3125, 0.625, 1.25 and 2.50 L of test item/ha. [Equivalent to 15.94, 31.88, 63.75, 127.50 and 255.00 g of active ingredient (a.i.)/ha, according to the analysed content] and a reference standard (BAS 152 11 I) - dimethoate 40% w/v EC at a concentration of 2.0 g/ha). Each treatment group included forty replicates, containing two nymphs in each.. The species used for the test was the predatory bug Orius laevigatus Fieber (Heteroptera: Anthocoridae). Second instar nymphs, 4 to 5 days old, from the same cohort were selected for the exposure. Eighty nymphs per treatment were confined within test units; two individuals per unit

The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany). The track-sprayer was calibrated with deionised water by adjusting the spraying pressure, application speed and distance to the target to provide the required output of 200 L ± 10 % per ha (2 mg/cm2 ± 10 %) . Six metal plates were weighed as a reference. The plates, previously tared, were sprayed with deionised water and weighed immediately after. Three consecutive calibration runs within the range of spray tolerance were achieved and the calibration was hence verified.

Following the calibration, the cut fragments of leaf were placed on top of a plain surface and they were sprayed in the order of deionised water (for the control), the test item solutions (from lowest to highest application rate) and the reference item. Between the highest test item rate and the reference item treatment, the sprayer was rinsed several times with deionised water, followed by a detergent solution and finally again with deionised water. Before the application of the fragments of leaf with each particular treatment, the sprayer was thoroughly flushed through with the corresponding solution.

Just before the application of each treatment, a verification run with the corresponding solution was performed to determine that the spray volume was within the required range; with a maximum accepted deviation of 10 % (≤ 10 % of the required volume; 2 ± 0.2 mg/cm2). For that purpose, six metal plates were used as a reference. The plates, previously tared, were sprayed and weighed immediately after to verify the applied volume. Actual deviations on volume and test item applied were less than 10 %. The maximum deviations were observed in the treatment group T3 at the rate of 0.625 L test item/ha, with - 4.17 % relative to the required volume and - 4.15 relative to the required rate of test item.

No problems were observed during product handling and application. The test item solutions didn’t cause visible spray residues (oily residues) on the treated leaves. Phytotoxic effects after application on the treated leaves were not observed

3. Observations:

Mortality: The condition of the test organisms was recorded starting just after the exposure (between 1 and 2 hours after); on days 1 and 4 after the exposure and on a daily basis thereafter until day 8 when all the individuals in the treatments were adult, dead or glued; there were no living or moribund nymphs.

Reproductive: The sub-lethal effects on the reproductive performance, as fecundity and fertility, were evaluated with the control group and all the test item groups since corrected juvenile mortality was less than 50 %.

Prior to the assessment of the reproductive performance, the sex of the surviving adult predatory bugs of each treatment was determined under a stereomicroscope. Twenty females per treatment group were impartially selected from the mating box and transferred individually to the reproduction units. See Table 18 in Appendix E for more details.

The fecundity phase started on day 14 after the exposure. The number of eggs laid per female (fecundity) was determined in two assessments on days 16 and 18 after the start of exposure (two consecutive egg-laying periods of, approximately, 48 hours each). After the first assessment, the oviposition substrate was replaced with a new one.

4. Statistics:

For data evaluation the statistical programme ToxRat Professional 3.2.1, 2001-2015 and Microsoft Office Excel-2010 ® v. 14 software was used. No statistical analysis was performed for the reference item data. All statistical final comparison tests were conducted with a significance level of $\alpha = 0.05$

A qualitative trend analysis by contrasts using proportions ($\alpha = 0.05$) was performed with mortality data not revealing a linear trend. Accordingly, the Chi2 2×2 table test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used to determine a significant increase in the mortality of the test item groups compared to the control group.

The LR50 could not be calculated and it was estimated empirically from the results since corrected mortality in the test item groups was always below 50 % compared to control.

No statistical procedures were used to study a significant decrease in the fecundity since the number of eggs per female per day obtained in all the test item treatments was even higher than in the control group.

A qualitative trend analysis by contrasts using proportions ($\alpha = 0.05$) was performed with fertility data not revealing a linear trend. Accordingly, the Chi2 2×2 table test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used to determine a significant decrease in the fertility of the test item groups compared to the control group. Difference between total eggs and viable eggs as dead was analysed and then, the statistical test searched differences against the control group at one-sided greater; mortality or non viability of eggs.

The ER50 for fecundity and fertility could not be calculated and were estimated empirically from the results since reduction in fecundity and fertility in the test item groups was always below 50 % compared to control group.

II. RESULTS AND DISCUSSION

A MORTALITY

Results from the mortality assessments of the assayed treatments are detailed below

Juvenile mortality of *Orius laevigatus* exposed to fresh and dry residues on leaves

Treatment Code	Product	Rate [L product/ha]	Mortality [a] [%]	Mcorr [b] [%]
C	Control: deionised water	–	16.7	–
T1	Test item: PP-113H (Clopyralid 100 g/L SL)	0.15625	18.8	2.5
T2		0.3125	22.5	7.0
T3		0.625	21.5	5.8
T4		1.25	22.8	7.3
T5		2.50	16.5	-0.3
R	Reference item: Dimethoate 40% w/v EC	0.0047	92.5	91.0

M_{corr}: mortality corrected for control according to Abbott's formula (1925), modified by Schneider-Orelli (1947).

[a] pre-imaginal mortality: stuck + dead nymphs [%].

[b] negative values indicate a decrease in mortality compared to the control group

Endpoint	Rate

	[L test item/ha]	[g clopyralid/ha] [a]
NOER $_{\text{mortality}}$	≥ 2.50	≥ 255.00
LR_{50}	> 2.50	> 255.00

[a] active ingredient content according to the certificate of analysis (102 g/L)

Since the corrected mortality in the test item rates studied was always below 50 % compared to control, the LR50 was assumed as higher than the maximum tested rate; 2.50 L test item/ha.

No behavioural abnormalities, malformations or any pathological symptoms of the test organisms were observed in the control group and in any of the test item groups.

More than 80 % of the survivors were adult in the control and the test item treatments 6 days after the exposure and there were no living or moribund nymphs on day 8 after the exposure.

No substantial delay in development was observed in any of the test item treatments as compared to the control

The test item (PP-113H (Clopyralid 100 g/L SL) did not cause statistically significant effect in mortality at any of the studied test item rates compared to the control group (Chi^2 2×2 table test with Bonferroni correction, one-sided, $\alpha = 0.05$). Accordingly, the NOER (No Observed Effect Rate) for mortality was determined to be equal to or greater than 2.50 L test item/ha.

A REPRODUCTION

Fecundity, as the mean number of eggs produced per female per day, was always above 2 with all the studied test item rates and the control group. The actual minim value, 7.4 eggs per female per day, was obtained with the tested maximum tested rate of 2.50 L test item/ha, although it was even higher than in the control group with 6.6 eggs/female/day.

Therefore, the test item did not cause any reduction in fecundity of *Orius laevigatus* at any of the studied test item rates up to and including 2.50 L test item/ha, the highest test item rate tested, and the NOER for fecundity was determined to be equal to or greater than 2.50 L test item/ha.

The ER_{50} for fecundity could not be calculated as no reduction in fecundity in the studied test item groups compared to control was observed and then, always below the trigger value of 50 %. Therefore, the ER_{50} for fecundity was assumed higher than 2.50 L test item/ha.

Fecundity of *Orius laevigatus* exposed to fresh and dry residues.

Treatment Code	**Product**	**Rate** [L test item/ha]	**Number of replicates**	**Fecundity**		
				eggs/female/day [a]	**SD**	**Reduction** [%] [b]
C	Control: deionised water	–	20	6.6	3.1	–
T1	Test item: PP-113H	0.15625	20	8.1	2.1	-23.2
T2		0.3125	20	8.3	2.5	-25.9
T3		0.625	20	7.8	3.3	-19.2

T4	(Clopyralid 100 g/L SL)	1.25	20	8.1	2.4	-22.7
T5		2.50	20	7.4	3.5	-13.5

SD: Standard deviation.
[a] Mean number of eggs produced per female per day
[b] negative values indicate an increase in fecundity compared to the control group.

Fertility, as the mean percentage of hatched eggs, was always above 70 % with all the tested item rates and the control group. The actual minimum value, 95.2 % hatched eggs, was obtained with the maximum tested rate of 2.50 L test item/ha.

The test item did not cause a statistically significant reduction in fertility of *Orius laevigatus* at any of the studied test item rates up to and including 2.50 L test item/ha, the highest test item rate tested (Chi^2 2×2 table test with Bonferroni correction with non-viable eggs, one-sided greater, $\alpha = 0.05$). Therefore, the NOER for fertility was determined to be equal to or greater than 2.50 L test item/ha.

The ER_{50} for fertility could not be calculated as reduction in fertility in the studied test item groups compared to control was always below the trigger value of 50 %. Therefore, the ER_{50} for fertility was assumed higher than 2.50 L test item/ha.

Fertility of *Orius laevigatus* exposed to fresh and dry residues

Treatment Code	Product	Rate [L test item/ha]	Number of replicates	Fertility	
				Egg viability [%] [a]	Reduction [%] [b]
C	Control: deionised water	–	19	97.1	–
T1	Test item: PP-113H (Clopyralid 100 g/L SL)	0.15625	20	98.4	-1.3
T2		0.3125	19	94.3	2.9
T3		0.625	20	99.1	-2.1
T4		1.25	20	98.9	-1.9
T5		2.50	20	95.2	2.0

[a] Mean % of hatched eggs.
[b] negative value indicates an increase in fertility compared to the control group.

Endpoint	Rate	
	[L test item/ha]	[g Clopyralid/ha] [a]
$NOER_{fecundity}$	≥ 2.50	≥ 255.00
$ER_{50\ fecundity}$	> 2.50	> 255.00
$NOER_{fertility}$	≥ 2.50	≥ 255.00
$ER_{50\ fertility}$	> 2.50	> 255.00

[a] active ingredient content according to the certificate of analysis (102 g/L)

III. CONCLUSIONS

All validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

Under the conditions of this study, PP-113H (Clopyralid 100 g/L SL) applied to fragments of leaf of *Aspidistra elatior*, the No Observed Effect Rate (NOER) for mortality was determined to be equal to or greater than 2.50 L test item/ha, the highest tested rate, equivalent to 255.00 g active ingredient (a.i.)/ha according to the certificate of analysis. The rate producing 50 % mortality (LR_{50}) was assumed as greater than 2.50 L test item/ha (255.00 g a.i./ha).

No substantial delay in development was observed at any of the test item rates as compared to the control.

The NOER for fecundity was determined to be equal to or greater than 2.50 L test item/ha, equivalent to 255.00 g a.i./ha according to the certificate of analysis; the highest tested rate. The median effect rate (ER_{50}) for fecundity could not be calculated since no reduction in fecundity in the studied test item groups compared to control was observed; therefore, it was assumed greater than 2.50 L test item/ha (255.00 g a.i./ha).

The NOER for fertility was determined to be equal to or greater than 2.50 L test item/ha, equivalent to 255.00 g a.i./ha according to the certificate of analysis; the highest tested rate. The ER_{50} for fertility could not be calculated as reduction in fertility in the studied test item groups compared to control was always below the trigger value of 50 %; therefore, it was assumed greater than 2.50 L test item/ha (255.00 g a.i./ha).

Endpoint	Rate	
	[L test item/ha] [a]	[g clopyralid/ha] [b]
$NOER_{mortality}$	≥ 2.50	≥ 255.00
LR_{50}	> 2.50	> 255.00
$NOER_{fecundity}$	≥ 2.50	≥ 255.00
$ER_{50\ fecundity}$	> 2.50	> 255.00
$NOER_{fertility}$	≥ 2.50	≥ 255.00
$ER_{50\ fertility}$	> 2.50	> 255.00

[a] rate in L of formulated product /ha
[b] active ingredient content according to the certificate of analysis (102 g/L)

Data requirement
zRMS updated dRR B9 in terms of tabular information on the dose response, e.g. a table listing dose and effect data, and recovery to the dRR for the sake of transparency:

Environmental chamber conditions summary

Phase	Minimum temperature [°C]	Maximum temperature [°C]	Minimum humidity [%RH]	Maximum humidity [%RH]
Mortality (Exposure)	24.5	25.1	74.9	83.1
Maturation and Mating	24.6	25.0	75.5	80.9
Reproduction (Fecundity and Fertility)	24.5	25.0	75.5	81.4

Calculation for the application

Treatment group		Rate [L product/ha]	Rate [g a.i./ha]	Application volume [L/ha]	Solution volume to prepare [mL]	Required Product [a] [g]
C	Deionised Water (Control)	–	–	200	approx. 250.00	–
T1	PP-113H (Clopyralid 100 g/L SL)	0.15625	15.94	200	250	0.2045
T2		0.3125	31.88	200	250	0.4090
T3		0.625	63.75	200	250	0.8180
T4		1.25	127.50	200	250	1.6359
T5		2.50	255.00	200	250	3.2719
R	Dimethoate 40% w/v EC	0.0047	2.00	200	429	10.00 µL

a.i.: active ingredient
[a] Calculated according the information of the GLP Certificates of Analysis; test item analytical content 102 g/L, density: 1.0470 g/mL; reference item analytical content 429.0 g/L, density: 1.076 g/mL.

Application data

Treatment group		Rate [L product/ha]	Product used [g]	Deionised water weighed [g]	Volume solution prepared [L]	Target fluid on metal plates [g]	Applied fluid on metal plates [a] [g]	Applied volume [L/ha]
C	Deionised Water (Control)	–	–	approx. 250.00 mL	approx. 250.00	1.20	1.21	201.67
T1	PP-113H (Clopyralid 100 g/L SL)	0.15625	0.2044	249.80	0.250	1.20	1.23	205.00
T2		0.3125	0.4089	249.61	0.250	1.20	1.17	195.00
T3		0.625	0.8181	249.22	0.250	1.20	1.15	191.67
T4		1.25	1.6360	248.44	0.250	1.20	1.20	200.00
T5		2.50	3.2719	246.88	0.250	1.20	1.24	206.67
R	Dimethoate 40% w/v EC	0.0047	10.00 µL	428.99	0.429	1.20	1.23	205.00

[a] Verification of the application: The fluid deposited on metal plates (6 plates of 10x10 cm) was weighed in each treatment 600 cm², at volume 200 L/ha (2 mg/cm²) = 1.2 g fluid ± 10 % (1.32 - 1.08 g)

A 2.3.2.2.5 Study 4: Toxicity to *Coccinella septempunctata*

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met and the sensitivity of the test organisms was confirmed: 5.00 % mortality in the control group and 25.80 fertile eggs per female per day. Accordingly, the study was deemed valid. **Agreed endpoints:** *Coccinella septempunctata*

Endpoint	Rate [L test item/ha] [a]	Rate [g clopyralid/ha] [b]
NOER mortality	≥ 2.50	≥ 255.00
LR_{50}	> 2.50	> 255.00

[a] Rate in L of formulated product /ha
[b] Active ingredient content according to the certificate of analysis (102 g/L)

Reference	KCP 10.3.2/04
Report:	Luna, E., 2020 PP-113H (Clopyralid 100 g/L SL): Toxicity to the Ladybird, Coccinella septempunctata L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits Report Nº S19-03763
Guidelines	IOBC (Schmuck R., et al., 2000).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of Clopyralid 100 g/L SL (PP-113H) on the mortality and reproduction *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) were tested under extended laboratory test. The tested concentrations were 15625, 0.3125, 0.625, 1.25 and 2.50 L of test item/ha [Equivalent to 15.94, 31.88, 63.75, 127.50 and 255.00 g of active ingredient (a.i.)/ha. A control group (treated with deionised water) and a reference standard (BAS 152 11 I - dimethoate 40% w/v EC at a concentration of 30 mL/ha). Each treatment group included 40 replicates containing one larva each. Study endpoints were percentage mortality, the mean number of eggs/female/day, the percentage of fertile eggs (hatching rate) and the mean number of fertile eggs/female/day.

The test item, PP-113H (Clopyralid 100 g/L SL), was diluted in deionised water and applied with a laboratory track sprayer to fragments of leaf of cast iron plant at five different rates. One control group treated with deionised water and one reference item group treated with dimethoate were included in the study. All applications were performed with a spray volume of 200 L/ha. After drying of the treated leaves the test units were assembled. Each treatment group included 40 replicates containing one larva each. The larvae were exposed to the dried residues on the fragments of leaf of cast iron plant. The larvae were fed with aphids of the species Acyrthosiphon pisum ad libitum. During the reproduction phase adults were provided with aphids (same species as used for the larvae), honey-water solution (1:1 w/w) and a mixture of unspecified pollen types. The mortality was determined from the larval stage until pupation and emergence of the adults at least every working day.

Under the conditions of this study, PP-113H (Clopyralid 100 g/L SL) applied to fragments of leaf of Aspidistra elatior, the No Observed Effect Rate (NOER) for mortality was determined to be equal to or greater than 2.50 L test item/ha, the highest tested rate, equivalent to 255.00 g active ingredient (a.i.)/ha according to the certificate of analysis. The rate producing 50 % mortality (LR50) was assumed as greater than 2.50 L test item/ha (255.00 g a.i./ha).

It can be assumed that there are no adverse effects on the reproductive performance of the test organism at the tested rates of the test item up to and including 2.50 L test item/ha, since the mean fertility was above the control validity criterion of 2 fertile eggs per female per day.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Dark yellow liquid
Lot/Batch :	20190506
Purity:	Clopyralid: 10.2 ± 0.1 [=102 ± 1 g/L]
CAS #:	Clopyralid: 1702-17-6
Stability of test compound:	Stable
	Control: Deionised water
2. Control/Positive Control	Positive control: BAS 152 11 I (dimethoate 40% w/v EC)
3. Test animals:	
Species:	*Coccinella septempunctata* L. (Coleoptera, Coccinellidae)
Age:	3 to 5 days old
Source:	Katz Biotech AG'
Acclimation period:	Yes
Feeding:	fresh aphids (*Acyrthosiphon pisum*)
Water	Deionised water

Housing:

Plastic vessels (size: 32.5 x 26.5 x 20 cm) closed with gauze covered lids were used as maintenance and breeding units. As soon as the adults are observed, they were transferred, separated by treatment, to the breeding containers. Only those adults emerged when at least 90 % of the viable pupae had hatched, were maintained in the containers; latecomers (emerged adults after 90% had emerged) were discarded for the reproduction phase. The beetles were sexed when the reproduction phase started

On the bottom of the container, a piece of paper or a tissue (to absorb the excreta and to facilitate the cleaning process) was placed. On the top of the tissue, black plastic sheets rolled up to form a cylinder were provided for egg laying.

The adults of C. septempunctata were fed with freshly caught aphids (Acyrthosiphon pisum) on sprouts of broad bean (Vicia fava) and a supplement of pollen and water with honey (50% w/w) was provided. Aphids were supply at least, every working day, and pollen and water with honey were replaced when necessary, at least every two or three days. Pollen was supply as loose, and water with honey on a cotton pad

Loading:

4. Environmental conditions

Temperature:	24.7 – 25.3 °C
Humidity:	75.2 – 81.4 %
Light intensity	1439 - 2223 lux
Photoperiod:	16 hours light and 8 hours darkness

B. STUDY DESIGN AND METHODS:

In life dates: 08 Jul 2019 – 19 Aug 2019

Animal assignment and treatment:

The test unit to assess toxicity of the product consisted of a fragment of leaf of cast iron plant (Aspidistra elatior) placed with the treated surface (adaxial face) upwards on the top of a wet cotton pad in a Petri dish which was moistened regularly with deionised water throughout the test. Leaves used for the exposure units were without any damages and of good turgor conditions.

Plastic vessels (size: 32.5 x 26.5 x 20 cm) closed with gauze covered lids were used as maintenance and breeding units. As soon as the adults are observed, they were transferred, separated by treatment, to the breeding containers. Only those adults emerged when at least 90 % of the viable pupae had hatched, were maintained in the containers; latecomers (emerged adults after 90% had emerged) were discarded for the reproduction phase. The beetles were sexed when the reproduction phase started.

On the bottom of the container, a piece of paper or a tissue (to absorb the excreta and to facilitate the cleaning process) was placed. On the top of the tissue, black plastic sheets rolled up to form a cylinder were provided for egg laying.

The adults of C. septempunctata were fed with freshly caught aphids (Acyrthosiphon pisum) on sprouts of broad bean (Vicia fava) and a supplement of pollen and water with honey (50% w/w) was provided. Aphids were supply at least, every working day, and pollen and water with honey were replaced when necessary, at least every two or three days. Pollen was supply as loose, and water with honey on a cotton pad.

The tested concentrations were 15625, 0.3125, 0.625, 1.25 and 2.50 L of test item/ha [Equivalent to

15.94, 31.88, 63.75, 127.50 and 255.00 g of active ingredient (a.i.)/ha. A control group (treated with de-ionised water) and a reference standard (BAS 152 11 I - dimethoate 40% w/v EC at a concentration of 30 mL/ha). Each treatment group included 40 replicates containing one larva each. Study endpoints were percentage mortality, the mean number of eggs/female/day, the percentage of fertile eggs (hatching rate) and the mean number of fertile eggs/female/day.

The mortality was determined from the larval stage until pupation and emergence of the adults at least every working day.Reproduction was evaluated with 8 synchronisations of egg laying (24-h periods) in two weeks, to calculate the eggs per female and day (fecundity rate) and the larvae emerging from eggs to calculate the percentage of viable eggs (fertility rate). In this way, the mean of fertile eggs per female per day was obtained per treatment.

The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany). The track-sprayer was calibrated with water before application by adjusting the spraying pressure and distance to target to provide an output of 200 L ± 10 % per ha (2 mg/cm² ± 10 %). The evenness of the spray deposits were checked visually and the amount was weighed with three replicates using a precision balance. The droplets uniformly covered the surface of plates and the deposits were within the required 2 ± 0.2 mg fluid/cm². The water control treatment was sprayed first, then the test item in increased rate order and finally the test reference.

3. Observations:

Larval mortality was assessed from the same day of each exposure (approx. 2 hours after the exposures) and at least every working day to the completion of the adult stage or last evaluation of larval or pupal mortality, 17 days after the exposure. Small quantities of fresh aphids were supplied on each of the evaluating days. Any larva that escaped from the test chambers or was killed by mechanical manipulation during the study was excluded from the mortality calculations (Schmuck R., et al., 2000).

Pupation and hatching of the adults were also recorded. As soon as pupae had formed, exposure units were closed with gauze covered lids to prevent escaping of beetles after emergence. Once the adults hatched, they were transferred to maintenance plastic containers (32.5 x 26.5 x 20 cm), separated by treatment. Latecomers (emerged adults after 90% had emerged) were discarded and they were not placed in the containers

The reproduction performance was assessed for treatment groups with a corrected mortality ≤ 50 %. Therefore, in this study, the reproduction test was conducted for the control and all the test item treatments (mortality ≤ 50 %). Reproduction was not studied for the reference item group.

The reproduction test was started seven days after the first egg batch had been observed in the control group. Sex was determined and the beetles were transferred to reproduction units. Beetles with deformities were excluded. Food and egg laying substrate was provided.

Egg laying substrate was checked daily to reduce possible cannibalism of the eggs by the adults.

During the fecundity assessment, adults were fed every working day with fresh aphids (Acyrthosiphon pisum). Water with honey and pollen were provided continuously.

Egg production (fecundity) was assessed every day (24 hours period) except weekends during a two weeks period and each assessment covered a twenty-four hour period (8 assessments). At each assessment, all the eggs on the container walls, on the sprouts of broad bean, on the black plastic cylinder and on the paper, were counted and recorded. All clusters of eggs, with exception of those laid on the container walls, were removed to assess the hatching rate. The number of damaged eggs due to handling or by the adults (nibbled or broken) was registered.

At the start and end of each egg-laying period, the number of adults was counted and sexed and the mean

number of eggs produced per female was calculated. The mean number of eggs per female and day was determined by dividing the total number of eggs laid, by the mean number of viable females in that group, corrected for mortality during egg laying.

For determination of the hatching rate (fertility), the egg batches were clipped out from the egg laying substrate, bean sprouts or paper, and transferred individually into cell plates. The egg batches on the wall of the containers were not taken into account to assess the hatching rate because they could be damaged when removed. Those eggs damaged by handing or by adults were also not taken into account to assess the hatching rate. The eggs were kept at test conditions until hatch. After 3 to 6 days of starting each egg-laying period, as soon as the majority had hatched, the number of "not emerged" eggs was recorded. The percentage of fertile eggs was determined by assessing the number of eggs that did not hatch from the removed eggs into cell plates.

The number of fertile eggs per female per day was calculated from the number of eggs/female/day and the hatching rate.

At the end of bioassays, any individual was eliminated according to internal SOP's.

Prolongation of the reproduction period was not necessary since control egg production was above the threshold value of ≥ 2 fertile eggs/female/day for each assessment date.

4. Statistics:

The Chi2 2×2 table test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used to determine a significant increase in the mortality of the test item groups compared to the control group. The LR50 could not be calculated and it was estimated empirically from the results.

For evaluation, Microsoft® Excel 2010 version 14.0 and the statistical program ToxRat® Professional 3.2.1 was used.

The reproduction test was evaluated only qualitatively due to the very high species-inherent variability in egg laying performance (Schmuck R., et al., 2000). Statistical evaluations were therefore not conducted.

II. RESULTS AND DISCUSSION

A MORTALITY

The following table presents the mortality of *Coccinella septempunctata* after exposure to treated maize leaves.

Table 2.3.2.2.-5 Mortality of Coccinella septempunctata after exposure to PP-113H (Clopyralid 100 g/L SL)

Treatment group	Application rate [L FP/ha] [a]	Mortality [%]	Corrected mortality [b] [%]
Control (deionised water)	(0)	5.00	--
Test item (PP-113H (Clopyralid 100 g/L SL))	0.15625	2.50	-2.63
	0.3125	0.00	-5.26
	0.625	5.00	0.00
	1.25	7.50	2.63
	2.50	7.50	2.63

Reference item (Dimethoate 40 %w/v EC)	0.03	100.00	100.00

[a] Rate of the test and reference items in L of formulated product (FP) per ha
[b] Corrected mortality according to Abbott (1925), modified by Schneider-Orelli (1947). Negative values indicate a decrease in mortality compared to the control group

The test item (PP-113H (Clopyralid 100 g/L SL) did not cause statistically significant effect in mortality at any of the studied test item rates compared to the control group (Chi2 2×2 table test with Bonferroni correction, one-sided, α = 0.05). Accordingly, the NOER (No Observed Effect Rate) for mortality was determined to be equal to or greater than 2.50 L test item/ha.

Since the corrected mortality in the test item rates studied was always below 50 % compared to control, the LR50 was assumed as higher than the maximum tested rate; 2.50 L test item/ha.

No behavioural abnormalities, malformations or any pathological symptoms of the test organisms were observed in the control group and in any of the test item groups during the mortality phase.

The main response to the test item was observed at the beginning of the exposure period, when individuals were still as larval stage (see figure below and Table 8 in Appendix C). After, almost all surviving larvae got to adult stage.

Endpoint	Rate [L test item/ha] [a]	Rate [g clopyralid/ha] [b]
$NOER_{mortality}$	≥ 2.50	≥ 255.00
LR_{50}	> 2.50	> 255.00

[a] Rate in L of formulated product /ha
[b] Active ingredient content according to the certificate of analysis (102 g/L)

B REPRODUCTION

The results of the reproduction test are shown in the following table.

Table 2.3.2.2.-5 Reproduction of Coccinella septempunctata after exposure to PP-113H (Clopyralid 100 g/L SL)

Treatment group	Application rate [L test item/ha]	Fecundity [Mean eggs/female/day]	Mean hatching rate [%]	[Mean fertile eggs/female/day]
Control (deionised water)	(0)	25.90	99.41	25.80
Test item PP-113H (Clopyralid 100 g/L SL)	0.15625	17.78	98.11	17.57
	0.3125	17.13	100.00	17.13
	0.625	21.62	99.18	21.38
	1.25	13.53	99.74	13.50
	2.50	18.63	99.70	18.55

The mean fecundity in the test item groups (0.15625 to 2.50 L test item/ha) was between 13.53 and 21.62 eggs per female per day compared to 25.90 eggs per female per day in the control group.
The mean hatching rate was close to or equal to 100 % in all test item groups, between 98.11 and 100 %, compared to 99.41 % in the control group.
The mean fertility in the test item groups was between 13.50 and 21.38 fertile eggs per female per day compared to 25.80 fertile eggs per female per day in the control group.
Reproduction values with the test item rates were always greater than the control validity criterion of 2 fertile eggs per female per day.

III. CONCLUSIONS

All validity criteria were met and the sensitivity of the test organisms was confirmed: 5.00 % mortality in the control group and 25.80 fertile eggs per female per day. Accordingly, the study was deemed valid.

Under the conditions of this study, PP-113H (Clopyralid 100 g/L SL) applied to fragments of leaf of Aspidistra elatior, the No Observed Effect Rate (NOER) for mortality was determined to be equal to or greater than

2.50 L test item/ha, the highest tested rate, equivalent to 255.00 g active ingredient (a.i.)/ha according to the certificate of analysis. The rate producing 50 % mortality (LR50) was assumed as greater than 2.50 L test item/ha (255.00 g a.i./ha).

It can be assumed that there are no adverse effects on the reproductive performance of the test organism at the tested rates of the test item up to and including 2.50 L test item/ha, since the mean fertility was above the control validity criterion of 2 fertile eggs per female per day.

Endpoint	Rate	
	[L test item/ha] [a]	[g clopyralid/ha] [b]
NOER mortality	≥ 2.50	≥ 255.00

LR_{50}	> 2.50	> 255.00

[a] Rate in L of formulated product /ha
[b] Active ingredient content according to the certificate of analysis (102 g/L)

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1.1 Study 1: Acute toxicity to Earthworms

Comments of zRMS:	According to the latest guidelines, testing is not required. It is treated only as an additional source of information.

Reference: KCP 10.4/01

Report Tediosi E.,Dini R., 2011
PP-113H (clopyralid 10 % w/v sl):acute toxicity to earthworm determined in an artificial soil study.
Report Nº DR-CH60511

Guideline(s): OECD N 23

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No - not appropriate

Executive Summary
The acute toxicity of the test PP-113H (Clopyralid 10 % w/v SL) to the earthworm *Eisenia fetida* was determined in a 14-days artificial soil study according to the OECD Guideline for Testing of Chemicals, No. 207, 1984.

For this purpose, adult earthworms were exposed, under defined conditions, to an artificial soil containing the test item at the only nominal concentration of 1000.0 mg/Kg dry weight, corresponding to 95.0 mg/kg as active ingredient Clopyralid (corrected for its purity, 9.5% w/w, determined in ChemService GLP study CH-397/2011).
Moreover, an artificial soil without test item was also tested as negative control.

The exposed organisms were checked for mortality 7 and 14 days after test initiation.
At the test start, moisture mean content of artificial soil after hydration was 37.9 % (measured mean value in negative control and treated soil) while at the test end the mean water content was 32.7 %.

The pH of artificial soil after preparation was 3.09, therefore it was corrected by adding 0.3 % of $CaCO_3$ according to mentioned guideline. The pH value of artificial soil corrected at the test start was equal to 5.86 for negative control soil and 5.94 for treated soil. After 14 days, the pH value was respectively equal to 5.95 and 6.02.

Temperature was in the range 19.9 – 20.8 °C and the light intensity was in the range 605 - 616 Lux.

In the negative control 0% mortality was observed after 7 and 14 days. This value complies with the validity criterion of the test providing a maximum mortality of 10 % in the negative control at the end of the test (OECD 207, 1984).

The obtained experimental results allowed determine the LC_0, LC_{50} and the LC_{100} values at 7 and 14 days. The results, based on the nominal concentration of test item, were as follows:

Time	LC_0 (mg/Kg)	LC_{50} (mg/Kg)	LC_{100} (mg/Kg)
7 days	≥ 1000.0	> 1000.0	> 1000.0
14 days	≥ 1000.0	> 1000.0	> 1000.0

Corresponding to the following values in terms of nominal concentration of active ingredient Clopyralid:

Time	LC_0 (mg a.i./Kg)	LC_{50} (mg a.i./Kg)	LC_{100} (mg a.i./Kg)
7 days	≥ 95.0	> 95.0	> 95.0
14 days	≥ 95.0	> 95.0	> 95.0

The LC_{50} values were determined from the Raw Data by the statistical analysis in comparison with the negative control (Linear interpolation analysis), while LC_0 and LC_{100} values at each observation time were directly extracted from the Raw Data.

Furthermore, the difference in the survived earthworm's body weight between the start and the end of the test for the only test item tested concentration was not statistically significant (Equal Variance Test).

The NOEC and LOEC values for this sublethal effect after 14 days of exposure, assessed both in terms of nominal test item and active ingredient concentration, were as follows:

Concentration	14-d NOEC (mg/Kg)	14-d LOEC (mg/Kg)
PP-113H	1000.0	> 1000.0
Clopyralid	95.0	> 95.0

A 2.4.1.1.2 Study 2: Sub-lethal toxicity to Earthworms

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. ☑ Criterion: ≥ 30 juveniles by the end of the experiment, in this study 149-318, ☑ The coefficient of variation of reproduction was 21.74 % (criterion: ≤ 30%), ☑ Adult mortality over the initial 4 weeks of the experiment was 0% (criterion: ≤ 10%). **Agreed endpoints:**

Endpoints	[mg t.i./kg soil dry weight]	[mg active ingredient/kg sdw] [a]
LOEC body weight change	164.86	16.06
NOEC body weight change	91.59	8.92
LOEC mortality	> 164.86	> 16.06
NOEC mortality	164.86	16.06
LOEC reproduction	> 164.86	> 16.06
NOEC reproduction	164.86	16.06
$EC_{10, 20, 50}$	> 164.86	> 16.06

t.i.: test item; sdw: soil dry weight.
- Concentrations in terms of active ingredient are based on the active ingredient content and density from the certificate of analysis: clopyralid: 102 g/L; density 1.047 g/cm^3.

Reference: KCP 10.4/02

Report Antón, B. 2020.
PP-113H (Clopyralid 100 g/L SL):
Sublethal Toxicity to the Earthworm Eisenia fetida (Oligochaeta, Lumbricidae) in Artificial Soil with 10 % Peat
Report Nº S20-02714

Guideline(s): OECD 222, ISO 11268-2 (2012)

Deviations: None

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No - not appropriate

Executive Summary

This study was conducted to evaluate the effects of PP-113H (Clopyralid 100 g/L SL):on mortality and reproductive output of the earthworm *Eisenia fetida*.

Adult earthworms of the species Eisenia fetida between two months and one year old, with clitellum and a wet mass between 300 mg and 600 mg were used. The study was conducted as a dose-response test with eight different test item concentrations including four replicates per concentration as well as a control (without test item) with eight replicates. Each replicate contained ten adult worms. Application of the test item was performed by preparing a test item solution and mixing it into the artificial test soil. The total duration of the exposure was 56 days. After 28 days, mortality and body weight change of the adult earthworms was assessed. After 56 days, effects on reproduction were assessed.

Eight groups were treated with the test item at 2.00 (control), 2.69, 4.85, 8.73, 15.71, 28.27, 50.88, 91.59, and 164.86 mg test item/kg soil dry weight, corresponding to 0.26, 0.47, 0.85, 1.53, 2.75, 4.96, 8.92 and 16.06 mg of active ingredient/kg soil dry weight (based on the analysed content and density).. The test duration was 55 days.

After 28 days, mortality and body weight change of the adult earthworms was assessed. After 56 days, effects on reproduction were assessed. The toxic reference item carbendazim (applied as formulation 'Sigma-Aldrich 97 %') was tested in a separate study to confirm the sensitivity of the test organism against compounds with known effects under the applied test conditions

The 28-day LOEC of PP-113H (Clopyralid 100 g/L SL) for adult mortality of Eisenia fetida was determined as > 164.86 mg test item/kg soil dry weight. The NOEC was determined as 164.86 mg test item/kg soil dry weight, (equivalent to > 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively).
The 28-day LOEC of PP-113H (Clopyralid 100 g/L SL) for body weight change for Eisenia fetida was determined as 164.86 mg test item/kg soil dry weight. The NOEC was determined as 91.59 mg test item/kg soil dry weight, (equivalent to 16.06 and 8.92 mg active ingredient/kg soil dry weight, respectively).
The 56-day LOEC of PP-113H (Clopyralid 100 g/L SL) for reproduction of Eisenia fetida was determined as > 164.86 mg test item/kg soil dry weight. The NOEC was determined as 164.86 mg test item/kg soil dry weight, (equivalent to > 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively).
The EC10, EC20 and EC50 for reproductive output were estimated to be >164.86 mg test item/kg soil dry weight (equivalent to > 16.06 mg active ingredient/kg soil dry weight).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Faint yellow liquid
Lot/Batch #:	20190506
Purity:	Clopyralid: 102 ± 1
C.A. Number:	Clopyralid: 1702-17-6
Stability of test compound:	Stable
2. Vehicle and/or positive control:	Vehicle: Deionized water Positive Control: Carbendazim (tested in a separate study)
3. Test animals -	
Species:	*Eisenia fetida (Oligochaeta: Lumbricidae)*
Age:	Adults (at least 2 months old but not older than 1 year) with clitellum, age difference not more than 4 weeks
Body weight:	200 - 600 mg
Source:	Miquel Riera Gassó
Acclimation period:	1 day
Housing:	Glass containers with a capacity of approximately 1.5 litres
Loading:	Number of replicate per treatment group: 4 Number of replicate in control group: 8 Number of Earthworms per Replicate: 10
4. Environmental conditions -	
Temperature:	19.9 ºC to 21.2 ºC
Photoperiod:	16 hours light and 8 hours darkness (Light Intensity 472.3 to 799.8 lux.)
pH:	pH of Soil (at the start): 5.82 to 5.86 pH of Soil (at the end): 6.01 to 6.21
Moisture content (%):	At the start of the test: 30.38 to 31.03 % At the end of the test: 42.68 to 49.02 %

B. STUDY DESIGN AND METHODS:

1. In life dates: 16 Jun 2020 to 12 August 2020.

2. Animal assignment and treatment:

Artificial soil was used containing the following components (based on dry mass):
- 10% of sphagnum peat air dried, finely ground and with no visible plant remains.
- 20% of kaolin clay containing not less than 30% kaolinite.
- Approximately 70% of industrial quartz sand (fine sand dominant with more than 50% of the particles between 50 and 200 microns).
- 0.263% calcium carbonate ($CaCO_3$) analytical grade (the soil pH was adjusted to 6.0 0.5 at the start of the test before the addition of the test item). Both the sphagnum peat and the kaolin were passed through a fine-meshed sieve and left to air-dry in the laboratory. The dry components were blended and mixed thoroughly with an electric mixer. The maximum water holding capacity (WHC_{max}) 51.05% of the soil was determined. A surplus of soil was prepared in order to cover possible losses during the handling. The soil was pre-moistened with deionised water to reach approximately half of the final water content, corresponding to 25% of the WHC_{max}, one day before the application. The pre-moistened soil was stored in a closed container until use. Final moistening was achieved during the application.

The treatment solutions were prepared immediately before application. The concentrations selected were 2.69, 4.85, 8.73, 15.71, 28.27, 50.88, 91.59, and 164.86 mg test item/kg soil dry weight, corresponding to 0.26, 0.47, 0.85, 1.53, 2.75, 4.96, 8.92 and 16.06 mg of active ingredient/kg soil dry weight (based on the analysed content and density). There were four replicates per concentration with each replicate containing 10 earthworms.

For the application solutions preparation, the required amount of test item was diluted with deionised water up to a final volume proportional to 25 % of the soil WHC_{max}

The earthworms were conditioned for about 24 hours in the test substrate. On the day of application earthworms were washed, dried and individual body weight was determined. Healthy two to twelve months old test organisms with a clitellum and a body weight between 300 and 600 mg were selected. The age of the worms used for the test did not differ by more than four weeks. Earthworms were housed in glass containers (capacity approximately 1.5 litres), filled with approximately 500 g (dry weight) of artificial soil mixed with deionised water up to 50% of its WHC_{max}. After introduction of the earthworms the test units were covered with plastic lids in which small openings were made to ensure gaseous exchange and avoid excessive evaporation.

Earthworms were fed with approximately 5 g air-dried finely ground pasteurised horse manure. The food should be provided once a week during the first 4-week test period. In case the food will not be fully consumed, the remaining food will be removed before each new feeding. After that (at day 28), a further 5 g of food is then supplied to each test container but no further feeding takes place during the remaining 4 weeks of the test.. Offspring were not fed further during the remainder of the study.

The test duration was 56 days. After 28 days, mortality and body weight change of the adult earthworms was assessed. After 56 days, effects on reproduction were assessed..

On day 56 the total number offspring per test container were counted. Extraction of the juveniles from the soil was performed by heating the containers in water at 50 – 60 ºC

3. Observations:

Mortality - number of live earthworms present after 28 days.
Sublethal effects - Any observed behavioural or pathological symptoms after 28 days
Body weight- changes occurring in the 28 Day exposure period.
Reproduction - number of juveniles present 56 days after application.

Food consumption - assessed weekly throughout the mortality phase of the study

4. Statistics:

Adult body weight variation data were analysed with the Shapiro-Wilk's test for normality of data distribution and with the Levene's test for homoscedasticity. The parametric Step-down Williams t-test Procedure, α = 0.05, two-sided) was used for hypothesis testing since a monotone response was observed.

Mortality data were analysed by means of Fisher´s Exact Binomial Test with Bonferroni Correction (α = 0.05, one-sided).

Reproduction data were analysed with the Shapiro-Wilk's test for normality of data distribution and with the Levene's test for homoscedasticity. Dunnett's t-test procedure (α = 0.05, one-sided) was used for hypothesis testing, since data did not show a trend.

The EC10, 20, 50 calculation was not possible, since offspring reduction for the higher test item concentrations was below 10 %.Therefore, these values were estimated according to the results.

Statistical analysis was performed using the software ToxRat® professional 3.3.0 and Microsoft© Office Excel 2013 (v. 15.0).

II. RESULTS AND DISCUSSION

A. MORTALITY

No dead individuals were observed in the control group. Mean mortality of the earthworms exposed to the test item ranged between 0 % and 5.00 %.

No statistical significant differences in percent mortality were observed between any of the treatments with the test item and the control (Fisher´s Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided).

Therefore, under the conditions of this study, the LOEC for adult mortality was determined as > 164.86 mg test item/kg soil dry weight and the NOEC was determined as 164.86 mg test item/kg soil dry weight, equivalent to
> 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively.

Adult mortality of *Eisenia fetida* after 28 days of exposure

Concentration (Treatment group) [mg t.i./kg sdw]		Total number of adult earthworms introduced	Total number of dead/not recovered adult earthworms after 28 days of exposure	Mean mortality [%]
0.00	(C)	80	0	0.00
2.69	(T1)	40	0	0.00
4.85	(T2)	40	0	0.00
8.73	(T3)	40	0	0.00
15.71	(T4)	40	0	0.00
28.27	(T5)	40	0	0.00
50.88	(T6)	40	1	2.50
91.59	(T7)	40	2	5.00
164.86	(T8)	40	2	5.00

t.i.: test item; sdw: soil dry weight; C: control; T: treatment.

B. BODY WEIGHT CHANGES

Average weight variation at 28 days after the application, with respect to the initial weight, was positive (body mass gain) for the control group and for all the treatments with the test item.
Statistically significant differences in body weight change of Eisenia fetida (Williams t-test Procedure, α = 0.05, two-sided) were found for the test item concentration 164.86 mg/kg soil dry weight, compared to the control after the exposure period. Accordingly, under the conditions for this study, the LOEC for body weight change was determined as 164.68 mg test item/kg soil dry weight and the NOEC was determined as 91.59 mg test item/kg soil dry weight, equivalent to 16.06 and 8.92 mg active ingredient/kg soil dry weight, respectively.

Average body weight and weight change of adult earthworms at test start and after 28 days of exposure

Concentration (Treatment group) [mg t.i./kg sdw]		at test start		after 28 days			
		Mean weight [mg/worm]	±SD	Mean weight [mg/worm]	±SD	Mean weight change [mg/worm]	[%]
0.00	(C)	344.81	2.15	553.18	14.46	208.36	60.44
2.69	(T1)	345.38	2.76	568.00	17.64	222.63	64.49
4.85	(T2)	344.25	3.46	568.03	19.59	223.78	65.02
8.73	(T3)	346.13	2.52	572.90	38.85	226.78	65.58
15.71	(T4)	345.65	2.92	582.85	31.37	237.20	68.62
28.27	(T5)	345.83	3.04	558.23	21.79	212.40	61.40
50.88	(T6)	345.48	2.19	573.10	35.97	227.63	65.86
91.59	(T7)	346.48	1.60	560.18	23.33	213.71	61.69
164.86	(T8)	345.58	3.75	606.33	27.22	260.75	75.51 [a]

t.i.: test item; sdw: soil dry weight; SD: standard deviation; C: control; T: treatment.
[a] statistically significantly different compared to the control (Williams Test Procedure, α = 0.05, two-sided).

C. OTHER EFFECTS (FOOD CONSUMPTION and BEHAVIOURAL OBSERVATION)

Behavioural abnormalities and pathological symptoms: All worms in both the control and test item treatment groups burrowed into the soil shortly after placement on the soil surface on Day 0.
One ulcerated individual was observed in replicate T2-4 after 28 days of exposure. There were no other pathological symptoms of the adult earthworms observed during the first four weeks of exposure to the test item.

Food consumption of the adult earthworms was estimated to be similar in all test item groups compared to the control group during the first four weeks of the study. Food was completely consumed within the 7-day period between each food replacement by the control earthworms and the earthworms exposed to all treatments with the test item

D. REPRODUCTIVE OUTPUT

Mean offspring production in the control treatment was 246.38 ± 53.55 individuals (mean ± SD). Mean offspring production in the treatments with the test item ranged from 205.25 ± 46.24 individuals at T3 to 272.50 ± 54.83 individuals at the treatment T5.

No statistically significant differences for the reproductive output of Eisenia fetida were found between the control and the test item treatments.

Accordingly, under the conditions of this study, the LOEC for reproduction was determined as > 164.86 mg test item/kg soil dry weight, and the NOEC was determined as 164.86 mg test item/kg soil dry weight, equivalent to > 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively.

The EC10, 20 and 50 could not be calculated, since offspring reductions below 10 % were observed for the higher test item concentrations. Therefore, these values were estimated according to the results.

Mean number of juveniles of *Eisenia fetida* after 56 days of exposure

Concentration (Treatment group) [mg t.i./kg sdw]		Mean number of juveniles per replicate	±SD	CV [%]	Variation in reproduction [a] [%]
0.00	(C)	246.38	53.55	21.74	-
2.69	(T1)	258.50	45.97	17.78	4.92
4.85	(T2)	251.50	53.98	21.46	2.08
8.73	(T3)	205.25	46.24	22.53	-16.69
15.71	(T4)	208.25	67.03	32.19	-15.47
28.27	(T5)	272.50	54.83	20.12	10.60
50.88	(T6)	272.25	32.07	11.78	10.50
91.59	(T7)	252.00	40.44	16.05	2.28
164.86	(T8)	251.25	48.73	19.39	1.98

t.i.: test item; sdw: soil dry weight; SD: standard deviation; CV: Coefficient of variation; C: control; T: treatment.
[a] negative values indicate lower reproduction compared to the control group, positive values higher reproduction.

Results of the $EC_{10, 20, 50}$ for reproductive output

Endpoints	[mg t.i./kg soil dry weight]	[mg active ingredient/kg sdw] [a]

$EC_{10,20,50}$	> 164.86	> 16.06

t.i.: test item; sdw: soil dry weight;

[a] Concentrations in terms of active ingredient are based on the active ingredient content and density from the certificate of analysis: clopyralid: 102 g/L; density 1.047 g/cm3.

E. DEFICIENCIES

None

III. CONCLUSIONS

All validity criteria were met and sensitivity of the test organisms could be confirmed. Accordingly, the study was deemed valid.
All validity criteria were met and sensitivity of the test organisms could be confirmed. Accordingly, the study was deemed valid.
The 28-day LOEC of PP-113H (Clopyralid 100 g/L SL) for adult mortality of Eisenia fetida was determined as
> 164.86 mg test item/kg soil dry weight. The NOEC was determined as 164.86 mg test item/kg soil dry weight, (equivalent to > 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively).
The 28-day LOEC of PP-113H (Clopyralid 100 g/L SL) for body weight change for Eisenia fetida was determined as 164.86 mg test item/kg soil dry weight. The NOEC was determined as 91.59 mg test item/kg soil dry weight, (equivalent to 16.06 and 8.92 mg active ingredient/kg soil dry weight, respectively).
The 56-day LOEC of PP-113H (Clopyralid 100 g/L SL) for reproduction of Eisenia fetida was determined as >
164.86 mg test item/kg soil dry weight. The NOEC was determined as 164.86 mg test item/kg soil dry weight, (equivalent to > 16.06 and 16.06 mg active ingredient/kg soil dry weight, respectively).
The EC10, EC20 and EC50 for reproductive output were estimated to be >164.86 mg test item/kg soil dry weight (equivalent to > 16.06 mg active ingredient/kg soil dry weight).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1: Toxicity to *Folsomia candida*

Comments of zRMS:

The study is considered as acceptable . All validity criteria were met.
- Mean adult mortality: 3.75% (criterion: ≤ 20%)
- Mean number of juveniles per vessel at the end of the test: 591.63 (criterion: ≥ 100 juveniles at the end of the test)
- The coefficient of variation calculated for the number of juveniles: 7.48% (criterion: ≤ 30%)

Data requirement - study limitation:

Abbott correction was performed for control mortality in the *Folsomia* study. However, this approach is not accepted for *Folsomia* and it is also not referred to in OECD GL 232. According to OECD Statistical Guidance Document No 54, quantal data are not suitable for the correction with Abbott. Instead, more-parametric not-linear models should be used and therefore considered for the evaluation of studies on soil organisms showing mortality in the control. In opinion zRMS – in this case - this not have an impact on the toxicity endpoint.

Agreed endpoints:

Endpoints	[mg test item/kg soil dry weight]	[mg active ingredient/kg soil dry weight] [c]	NW 95% CL [d]
LOEC mortality	> 1000.00	> 97.42	--
NOEC mortality	≥ 1000.00	≥ 97.42	--
LC_{50}	> 1000.00	> 97.42	--
LOEC reproductive output	555.56	54.12	--
NOEC reproductive output	308.64	30.07	--
EC_{10} (lower / upper CL)	335.43 (263.66 / 431.36)	32.68 (25.69 / 42.02)	0.50
EC_{20} (lower / upper CL)	590.35 (511.44 / 680.08)	57.51 (49.82 / 66.25)	0.29
EC_{50} (lower / upper CL)	> 1000.00	> 97.42	--

[c] endpoints in terms of active ingredient concentrations are indicated according to the analysed content and density: 102 g/L, 1.0470 g/cm³.

[d] 95 % confidence limits normalised width (NW)

Reference	KCP 10.4/03
Report:	Antón, B., 2020 PP-113H (Clopyralid 100 g/L SL): Effects on the Reproductive Output of the Springtail Folsomia candida Willem (Collembola, Isotomidae) in Artificial Soil Report Nº S20-02712
Guidelines	OECD 232 (2016)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The study was conducted to determine the effect of PP-113H (Clopyralid 100 g/L SL), on survival and the reproductive output of the springtail *Folsomia candida* Willem (Collembola, Isotomidae) under worst-case exposure conditions.
A total eight test item groups plus an untreated control were selected for the study. The eight test item groups were treated at concentrations of 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.00 mg test item/kg soil dry weight, equivalent to 1.59, 2.86, 5.16, 9.28, 16.70, 30.07, 54.12 and 97.42 mg active ingredient/kg soil dry weight.. The control group was treated with deionised water. Each test group was replicated four times and the control group was replicated eight times. The test organisms were exposed in treated artificial soil for 28 days. After 28 days of exposure adult mortality and effects on reproduction (offspring number) were assessed.

In the control group, 3.75 % mortality (≤ 20 %) and an acceptable reproductive capacity with a mean of 591.63 juveniles per replicate (≥ 100 juveniles per vessel) were observed at the end of the test. Furthermore, the coefficient of variation for the number of juveniles was 7.48 %, less than 30 %. Therefore, the validity criteria were fulfilled and the study is considered valid.
The NOEC and LOEC for adult mortality were determined as ≥ 1000.00 mg test item/kg soil dry weight and > 1000.00 mg test item/kg soil dry weight, respectively (equivalent to ≥ 97.42 and > 97.42 mg active ingredient/kg soil dry weight, respectively).

The LC50 for mortality was estimated as > 1000.00 mg test item/kg soil dry weight, (equivalent to > 97.42 mg active ingredient/kg soil dry weight).

The NOEC and LOEC regarding reproductive output of Folsomia candida were determined as 308.64 mg test item/kg soil dry weight and as 555.56 mg test item/kg soil dry weight, respectively (equivalent to 30.07 and 54.12 mg active ingredient/kg soil dry weight, respectively).

The EC10 was determined as 335.43 mg test item/kg soil dry weight (equivalent to 32.68 mg active ingredient/kg soil dry weight, 95 % confidence limits normalised width: 0.50).

The EC20 was determined as 590.35 mg test item/kg soil dry weight (equivalent to 57.51 mg active ingredient/kg soil dry weight, 95 % confidence limits normalised width: 0.29).

The EC50 was estimated as > 1000.00 mg test item/kg soil dry weight (equivalent to > 97.42 mg active ingredient/kg soil dry weight).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Faint yellow liquid
Lot/Batch :	20190506
Purity:	Clopyralid: 102 g/l ± 1
CAS #:	Clopyralid: 1702-17-6
Stability of test compound:	Stable
2. Control/Positive Control	Control: Deionised water Positive control: boric acid (tested in a separate study)
3. Test animals:	
Species:	*Fosomia candida* Willem (Collembola, Isotomidae)
Age:	10 to 11 days old
Source:	In-house culture maintained at the Test Facility
Acclimation period:	N/A
Housing:	Glass vessels of 100 mL capacity containing 30 ± 0.01g (dry

	mass) of test substrate
Loading:	Number of *F. candida* per replicate: 10

4. Environmental conditions

Temperature:	19.86 - 20.71 °C
pH:	pH of Soil (at the start): 6.13 - 6.40 pH of Soil (at the end): 6.24 to 6.53
Light intensity	472.2 to 607.5 lux
Photoperiod:	16 hours light and 8 hours darkness
Moisture content (%):	17.22 % of soil dry weight (45% of WHC)

B. STUDY DESIGN AND METHODS:

In life dates: August 19 2020 to September 16 2020

Animal assignment and treatment:

Artificial soil was used containing the following components (based on dry mass): 5% sphagnum peat (air-dried and finely ground); 20% kaolin clay, air dried; 74.879% air-dried industrial quartz sand (predominantly fine sand with particle size 0.2-0.05 mm; 0.1212% calcium carbonate ($CaCO_3$). The dry components were blended and mixed thoroughly in an electric mixer. The pH value of the artificial soil was 5.94. The maximum water holding capacity (WHC_{max}) of the soil was 38.27 % of dry mass.

Five days before starting the test, the dry artificial soil was pre-moistened by adding enough deionised water to obtain approximately half of the finally desired water content of 45 % of the WHCmax (164.81 g water for 1914.00 g dry soil). The moistened soil was then stored in a sealed container until application. The final water content was achieved by adding deionised water or test item solution during application.

The concentrations selected were 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.00 mg test item/kg soil dry weight, equivalent to 1.59, 2.86, 5.16, 9.28, 16.70, 30.07, 54.12 and 97.42 mg active ingredient/kg soil dry weight. A stock solution of was prepared by dissolving 0.0100 g of test item in 10 mL of deionised water. The application solutions for the treatments 16.33, 29.40, 52.92 and 95.26, mg/kg soil dry weight were prepared by diluting the corresponding volume of the stock solution to reach the required concentration in deionised water.

The application solutions for the treatments were prepared immediately before application. The test item and prepared solutions were managed following the appropriate SOPs of Trialcamp. Application was performed in the laboratory at ambient conditions.
A stock solution was prepared with the test item. Application solutions for T1-T4 treatments were prepared by diluting the required quantities of the stock solution in the calculated quantity of deionised water to complete the 45 % of WHC. Application solutions for treatments T5-T8 were prepared by dissolving the required quantities of test item in the calculated quantity of deionised water to complete the 45% of WHC.
The application solutions were mixed thoroughly with a magnetic stirrer and thereafter were mixed thoroughly with the previously pre-moistened substrate using a stainless steel hand-mixer until the soil appeared homogeneous in color, texture and moisture content (for approximately 3 minutes).
The control treatment was prepared first, and then the test item was applied in increasing concentration.
Once treatments had been fully mixed in, the soil had a natural crumbly structure, which allowed the springtails to migrate into the substrate. Care was taken to avoid compressing the soil following its treatment. Immediately after mixing, the final test soil was distributed into four vessels (eight vessels for the control group) for springtail exposure plus two additional vessels for pH check at the start and at the end

of the test. The final amount of treated test soil per vessel was approximately 30 g (dry mass).

Four replicates per test item concentration and eight replicates for control were prepared. Each replicate contained 10 *F. candida* juveniles. Following test organism and food addition the replicates were closed with the lid of vessels. At the beginning of the test, approximately 4-8 mg of granulated yeast was added to each exposure unit. This food source was added again after 14 days of the exposure (approx. 3-6 mg).Test containers were covered but they were briefly opened three times a week to allow aeration.

The number of springtails present four weeks after introducing the parental individuals onto the test and control substrates was determined (28 DAA). Water coloured with dark ink was added to containers and the collembolans floated to the surface. The number of adults and juveniles, if present, were counted under a binocular microscope and the numbers were reported. Regarding the number of adults, it was assumed that all individuals not found had died, since the dead bodies tend to decay in the soil relatively quickly. Periodical tests for validation of the evaluation method are carried out in our laboratory to ensure that the average error does not exceed 5%; a fixed and known number of juveniles and adults are introduced into test substrate simulating one test container, and then it is evaluated and the average error is calculated.

For water content control, the test vessels (extra replicate) were weighed at the beginning (0 DAA), in the middle (14 DAA) and at the end of the test (28 DAA). Weight loss was < 2%, so it was not necessary the addition of water.

3. Observations:

Test organisms were assessed for mortality and reproduction after 28 days of exposure.

The test vessels (extra replicate) were weighed at the beginning (day 0), in the middle (day 14) and at the end of the test. pH was measured at the initiation and at the end of the test.

Substrate samples were collected on days 0, 14 and 28 after the application and analysed for Clopyralid

4. Statistics:

Calculation of treatment means and standard deviations. Fisher's exact test after Bonferroni Holm (sig. 95%, one-sided) was used for comparison of mortality data of the test item treatments and the control group. The LC50 was estimated according to the observed mortalities. Reproductive output data were tested for normality and homoscedasticity using Shapiro-Wilk test and Levene's test. The Williams t-test (sig. 95%, one-sided) was used for hypothesis testing. The EC10, and the EC20 values were calculated by interpolation using a normal sigmoid dose-response function (3-param. normal CDF), with acceptable 95 % confidence limits. The EC50 was estimated according to the observed offspring reductions.

For data evaluation the statistical programme ToxRat Professional 3.2.1 was used.

II. RESULTS AND DISCUSSION

A MORTALITY

The results of the assessments of the mortality of springtails during the bioassay are given in Appendix D.

Adult mortality of Folsomia candida after 28 days of exposure

Code	Treatment	Concentration [a] [mg TI/kg sdw]	Mortality [%]	Std. Deviation	Std. Error	Corrected mortality [%] [b]

C	CONTROL (deionised water)	0	3.75	5.18	1.83	
T 1	PP-113H (Clopyralid 100 g/L SL)	16.33	5.00	5.77	2.89	1.30
T 2		29.40	2.50	5.00	2.50	-1.30
T 3		52.92	7.50	9.57	4.79	3.90
T 4		95.26	5.00	5.77	2.89	1.30
T 5		171.47	5.00	5.77	2.89	1.30
T 6		308.64	2.50	5.00	2.50	-1.30
T 7		555.56	0.00	0.00	0.00	-3.90
T 8		1000.00	10.00	14.14	7.07	6.49

[a] nominal concentration: mg of the test item (TI) per kilogram soil dry weight mass of the soil.
[b] corrected Mortality after applying the Abott's formula. Negative values indicate lower mortality compared to the control group.

Exposure to the test concentrations of "PP-113H (Clopyralid 100 g/L SL)" resulted in adult mortality lower than 15 % at concentrations up to 1000.00 mg test item/ kg soil dry weight. Corrected Adult mortality observed for the highest test item concentration 1000.00 mg/kg soil dry weight was 6.49 %.

When mortalities were compared to control, no statistically significant differences (Fisher's exact binomial test after Bonferroni-Holm, sig. 95 %, one-sided) were found for any of the test item concentrations.

Therefore, the parameters LOEC and NOEC for adult mortality were:

- NOEC ≥ 1000.00 mg test item/kg soil dry weight, (equivalent to ≥ 97.42 mg active ingredient/kg soil dry weight).
- LOEC > 1000.00 mg test item/kg soil dry weight, (equivalent to > 97.42 mg active ingredient/kg soil dry weight).

The LC50 value estimated as > 1000.00 mg test item/kg soil dry weight (equivalent to > 97.42 mg active ingredient/kg soil dry weight), according to the results.

B REPRODUCTION

The results on the reproduction capacity of springtails during the bioassay are given in the Appendix D and have been summarised in the following tables. Regarding physiological symptoms, no behavioural abnormalities or pathological symptoms were registered.

The reproductive output of the test organisms was not affected by the exposure to the test item at concentrations from 16.33 up to 308.64 mg/kg soil dry weight. Statistical procedures were performed and significant differences compared to the control treatment were found with the test item concentrations 555.56 and 1000.00 mg test item/kg soil dry weight (Williams t-test, sig. 95%, one-sided).

Therefore, the parameters LOEC and NOEC for the reproduction capacity were:

- NOEC = 308.64 mg test item/kg soil dry weight, (equivalent to 30.07 mg active ingredient/kg soil dry weight).
- LOEC = 555.56 mg test item/kg soil dry weight, (equivalent to 54.12 mg active ingredient /kg soil dry weight).

Mean number of juvenile *Folsomia candida* after 28 days of exposure

Code	Treatment	Concentration [mg TI/kg sdw][a]	Mean of progeny per replicate	Coefficient of variation [%]	Std. Dev.	Std. Error	Reduction relative to control [%]	Offspring output [%] (relative to control)
C	CONTROL (deionised water)	0	591.63	7.48	44.26	15.65	--	100.00
T 1	PP-113H (Clopyralid 100 g/L SL)	16.33	593.25	10.28	61.00	30.50	-0.27	100.27
T 2		29.40	612.75	4.82	29.55	14.77	-3.57	103.57
T 3		52.92	524.00	3.03	15.85	7.93	11.43	88.57
T 4		95.26	545.00	20.63	112.41	56.21	7.88	92.12
T 5		171.47	571.00	6.82	38.94	19.47	3.49	96.51
T 6		308.64	547.00	10.92	59.74	29.87	7.54	92.46
T 7		555.56	457.25 [b]	13.53	61.88	30.94	22.71	77.29
T 8		1000.00	388.75 [b]	16.45	63.97	31.98	34.29	65.71

[a]: nominal concentration: mg of the test item (TI) per kilogram soil dry weight mass of the soil.

[b]: significant differences compared to control (Williams t-test, sig. 95%, one-sided).

EC10 and EC20 values were determined by interpolation in a normal sigmoid dose/response curve. Since no reduction on reproduction above 50 % was observed, the EC50 calculation was not possible and this value was estimated according to the results.

Results of the $EC_{10, 20, 50}$ determinations for reproductive output

ECx		95% LCL		95% UCL		NW
EC10 = 335.43	mg TI/kg sdw	263.66	mg TI/kg sdw	431.36	mg TI/kg sdw	**0.50**
EC20 = 590.35	mg TI/kg sdw	511.44	mg TI/kg sdw	680.08	mg TI/kg sdw	**0.29**
EC50 > 1000.00	mg TI/kg sdw	--	mg TI/kg sdw	--	mg TI/kg sdw	--

TI: test item; sdw: soil dry weight; LCL: Lower 95% confidence limit; UCL: Upper 95% confidence limit; NW: confidence limits normalized width

C DEFICIENCIES

None

III. CONCLUSIONS

In the control group, 3.75 % mortality (≤ 20 %) and an acceptable reproductive capacity with a mean of 591.63 juveniles per replicate (≥ 100 juveniles per vessel) were observed at the end of the test. Further-

more, the coefficient of variation for the number of juveniles was 7.48 %, less than 30 %. Therefore, the validity criteria were fulfilled and the study is considered valid.
The NOEC and LOEC for adult mortality were determined as ≥ 1000.00 mg test item/kg soil dry weight and > 1000.00 mg test item/kg soil dry weight, respectively (equivalent to ≥ 97.42 and > 97.42 mg active ingredient/kg soil dry weight, respectively).
The LC50 for mortality was estimated as > 1000.00 mg test item/kg soil dry weight, (equivalent to > 97.42 mg active ingredient/kg soil dry weight).
The NOEC and LOEC regarding reproductive output of Folsomia candida were determined as 308.64 mg test item/kg soil dry weight and as 555.56 mg test item/kg soil dry weight, respectively (equivalent to 30.07 and
54.12 mg active ingredient/kg soil dry weight, respectively).
The EC10 was determined as 335.43 mg test item/kg soil dry weight (equivalent to 32.68 mg active ingredient/kg soil dry weight, 95 % confidence limits normalised width: 0.50).
The EC20 was determined as 590.35 mg test item/kg soil dry weight (equivalent to 57.51 mg active ingredient/kg soil dry weight, 95 % confidence limits normalised width: 0.29).
The EC50 was estimated as > 1000.00 mg test item/kg soil dry weight (equivalent to > 97.42 mg active ingredient/kg soil dry weight).

A 2.4.2.1.2 Study 2: Toxicity to *Hypoaspis aculeifer*

Comments of zRMS:

The study is considered as acceptable . All validity criteria were met.
- Mean adult mortality: 7.5% (criterion: ≤ 20%);
- Mean number of juveniles per vessel at the end of the test: 307.13 (criterion: ≥ 50 juveniles at the end of the test);
- The coefficient of variation for the number of juveniles: 13.06% (criterion: ≤ 30%).

Agreed endpoints:

Endpoints	Concentration [mg/kg soil dry weight]	
	Test item [a]	clopyralid [b]
$LOEC_{mortality}$	> 2250.00	> 219.20
$NOEC_{mortality}$	≥ 2250.00	≥ 219.20
$LOEC_{reproductive\ output}$	> 2250.00	> 219.20
$NOEC_{reproductive\ output}$	≥ 2250.00	≥ 219.20
EC_{10}	1500.00 < EC_{10} < 2250.00	146.13 < EC_{10} < 219.20
(95 %-confidence interval)	n.d.	n.d.
EC_{20}	> 2250.00	> 219.20
(95 %-confidence interval)	n.d.	n.d.
EC_{50}	> 2250.00	> 219.20
(95 %-confidence interval)	n.d.	n.d.

n.d.: not determined.
[a] Test item: PP-113H (Clopyralid 100 g/L SL).
[b] Based on the analysed content of active ingredient and density from the certificate of analysis (clopyralid: 102 g/L; density: 1.047 g/mL).

Reference: KCP 10.4/04

Report Lozano J. 2020.

	PP-113H (Clopyralid 100 g/L SL): Effects on the Reproductive Output of the Predatory Soil Mite Hypoaspis (Geolaelaps) aculeifer Canestrini (Acari: Laelapidae) in Artificial Soil. Report Nº S20-02713
Guideline(s):	OECD 226
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No - not appropriate

Executive Summary
The study was conducted to determine the effects of the test item PP-113H (Clopyralid 100 g/L SL) in soil on the reproductive output and, additionally, on the mortality of the predatory soil mite species Hypoaspis (Geolaelaps) aculeifer Canestrini (Acari: Laelapidae) under worst-case exposure conditions. For this purpose, the Lowest Observed Effect Concentration (LOEC) for mortality and reproductive output, the No Observed Effect Concentration (NOEC) for mortality and reproductive output, and the Effect Concentration (EC10, 20, 50) for reproductive output were determined, where possible.

Eight groups were treated at the test item concentrations 16131.69, 197.53, 296.30, 444.44, 666.67, 1000.00, 1500.00 and 2250.00 mg test item/kg soil dry weight. Equivalent to: 0 (control), 12.83, 19.24, 28.87, 43.30, 64.95, 97.42, 146.13 and 219.20 mg clopyralid/kg soil dry weight.. The toxic reference item BAS 152 65 I (Dimethoate 400 g/L, EC), a.i. dimethoate, was tested in a separate study (S19-23139, issued: 09 Jan 2020). Each test item group was replicated four times and the control group was replicated eight times. The test organisms were exposed in treated artificial soil for 14 days. Mites were extracted on day 14 after exposure and evaluated for mortality and reproduction.

Under the conditions of this Hypoaspis aculeifer 14-day reproduction test in soil with the test item PP-113H (Clopyralid 100 g/L SL); the resulting endpoints are as presented below.
The LOEC for mortality could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The NOEC for mortality was determined as equal or greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content.
The LOEC for reproductive output could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The NOEC for reproductive output was determined as equal or greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content.
The EC10 for reproductive output is assumed to be between 1500.00 and 2250.00 mg test item/kg soil dry weight; equivalent to 146.13 and 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The EC20 and EC50 for reproductive output are assumed as greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content the control group or in any of the test item groups.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	PP-113H (Clopyralid 100 g/L SL)
Description:	Faint yellow liquid
Lot/Batch #:	20190506
Purity:	Clopyralid: 102 ± 1 g/L
C.A.S. Number:	Clopyralid: 1702-17-6
Stability of test compound:	Stable
2. Vehicle and/or positive control:	Vehicle : Deionised water Positive Control : BAS 152 11 I (dimethoate) (tested in a separate study)
3. Test organism:	
Species:	*Hypoaspis aculeifer* Canestrini (Acari: Laelapidae)
Age:	32 day old (after the start of the egg-laying for synchronisation)
Body weight:	
Source:	A healthy laboratory rearing stock maintained at the laboratory of Trialcamp S.L.U.
Acclimation period:	NA
Housing:	Transparent glass vessels (Ø: 45 mm, height: 70 mm, volume: approx. 100 mL) closed with a silicone lid. For gas exchange the lid had a little hole in the centre which was sealed by a mite-impermeable cotton wool ball. Each test unit was filled with approximately 23.85 g of test soil (equivalent to 20 g of dry mass) reaching a soil depth greater than 1.5 cm.
Test duration:	14 days
Soil	Formulated according to OECD 226 containing 5% finely ground sphagnum peat, 20% kaolin clay, 74.87% fine quartz sand, 0.13% calcium carbonate to adjust the pH to 6.0 ± 0.5, water. Water holding capacity of the artificial soil was approximately 38.24 %. of the dry weight of the artificial soil.
4. Environmental conditions -	
Temperature:	19.9 °C - 20.4 °C
Photoperiod:	16 hours light:8 hours dark (Light Intensity 723 - 761 lux)
pH:	pH of Soil (at the start): 5.47 - 5.72 pH of Soil (at the end): 5.67 - 5.87
Moisture content (%):	At the start of the test: 17.95 % - 18.62 % (corresponding to 46.93 – 48.70 % of the WHCmax), At the end of the test: 17.44 – 18.27 % (corresponding to 45.61 – 47.79 % of the WHCmax)

B. STUDY DESIGN AND METHODS:

1. In life dates:	June, 2020 to July 2020
2. Animal assignment and treatment:	

Already mated females which have passed the pre-oviposition stage were used for the study. Adult females from the in-house culture were transferred to new rearing units filled with a plaster of Paris/charcoal mixture. Synchronisation units with 100 females in each, were prepared and food was added. After a period of 3 days of egg-laying, females were removed from the synchronisation containers. The eggs and later on the emerged mites, were kept in rearing units. During their development the mites were fed with cheese mites (Tyrophagus putrescentiae) 2 to 3 times a week. The developed females were introduced into the test units 32 days after the start of the egg-laying period for synchronisation

Artificial soil was prepared containing the following components (based on dry mass): 5% sphagnum peat (air-dried and finely ground); 20% kaolin clay (kaolinite content preferably > 30%); 74.87% air-dried industrial sand (predominantly fine sand with more than 50% of the particles between 50 and 200 microns); 0.13% calcium carbonate - precipitated extra pure was added to adjust the soil pH (target pH 6.0 ± 0.5) before the test. A surplus of soil was prepared in order to cover possible losses. The dry components were blended and mixed thoroughly with an electric laboratory mixer. The maximum water holding capacity (WHC_{max}) of the soil was determined gravimetrically and was calculated to be 38.24 %.. The pH was 6.07.

Seven days before starting the test, the dry artificial soil was pre-moistened by adding enough deionised water to obtain approximately half of the final desired water content of 40 to 60 % of the WHCmax (target: 45 % of the WHCmax). The moistened soil was then stored in a sealed container until application. The final water content was achieved by adding deionised water or test item solution during application

The concentrations selected were 0 (control – deionised water), 16131.69, 197.53, 296.30, 444.44, 666.67, 1000.00, 1500.00 and 2250.00 mg test item/kg soil dry weight. A toxic reference BAS 152 11 I (dimethoate) was tested in a separate study. All dilutions of the test item were diluted with deionised water up to a final volume proportional to 22.5% of the soil's total water holding capacity. Therefore the final water content was equal to 45% of its water holding capacity.

The final test soil was distributed into four vessels per test item treatment (eight vessels for the control group) for mite exposure; one additional vessel per treatment for pH and water content check at the end of the test and one additional vessel in the water control treatment to control the temperature during the mite extraction. The amount of test soil per vessel was approximately 23.44 g (equivalent to 20 g dry mass).

The test organisms were transferred to the test units within 41 minutes after the application of the test soil with a fine brush. The duration of the exposure was 14 days. On day 14 of the test the surviving adult females and juveniles were extracted from the soil using a high temperature gradient extractor (Berlese-Tullgren funnel).

3. Observations:

Mortality - number of live adult female mites present after 14 days.

Sublethal effects - number of morphology abnormal animals after 14 days

Reproduction - number of juveniles mites present 14 days after application.

4. Statistics:

Calculation of treatment means and standard deviations. Level of significance $\alpha = 0.05$ for the statistical final comparison tests. After performing a qualitative trend analysis by contrasts using proportions (monotonicity of concentration/response), the mortality data was analysed using a Chi2 2x2 table test with Bonferroni correction ($\alpha = 0.05$, one-sided greater). The repro-

ductive output was pre-tested for normality of data distribution with Shapiro-Wilk's test and for homoscedasticity with Levene's test. After performing a trend analysis by contrasts (monotonicity of concentration/response), the reproductive output was analysed using Dunnett's multiple t-test procedure ($\alpha = 0.05$, one-sided smaller). Since reduction in reproductive output compared to the control group was always below 20 % and no statistically significant concentration/response was found; the EC10, EC20 and EC50 for reproductive output could not be calculated and they were estimated empirically from the results.
Statistical calculations were performed with ToxRat Professional 3.3.0 and Microsoft Office Excel-2013® v.15.0.

II. RESULTS AND DISCUSSION

A. MORTALITY

No statistically significant increase in mortality of *Hypoaspis aculeifer* was detected any of the test item concentrations as compared to the control group after 14 days of exposure. Accordingly the LOEC for mortality could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight. The NOEC for mortality was determined as equal or greater than 2250.00 mg test item/kg soil dry weight.
No behavioural abnormalities or any pathological symptoms of the test organisms could be observed in the control group and in any of the test item groups.

B. OTHER EFFECTS (BEHAVIOURAL OBSERVATION)

No abnormal behaviour/appearance was observed with the surviving *Hypoapis aculeifer*.

C DIMETHOATE VALUES

Study Number S19-23139

D REPRODUCTION

No statistically significant reduction in the number of juveniles was detected at any of the test item concentrations as compared to the control group after 14 days of exposure. Accordingly, the LOEC for reproductive output could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight. The NOEC for reproductive output was determined as equal or greater than 2250.00 mg test item/kg soil dry weight.
No statistically significant concentration/response was found, therefore ECx for reproductive output could not be calculated. The greatest reduction in reproductive output compared to the control group, 13.55 %, was observed at the highest concentration tested (2250.00 mg test item/kg soil dry weight). The EC10 for reproductive output is assumed to be between 1500.00 and 2250.00 mg test item/kg soil dry weight. The EC20 and EC50 for reproductive output are assumed as greater than 2250.00 mg test item/kg soil dry weight..

E VALIDITY CRITERIA

Control Mortality: Mean mortality was 7.5 %, validity criterion was met.

Control Reproduction: Mean number of juvenile per replicate was 307.13 juveniles/unit, validity criterion was met.
Coefficient of Variation of the Control Reproduction: 13.06 % validity criterion was met.

D. DEFICIENCIES

None.

III. CONCLUSIONS

All validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.
Under the conditions of this Hypoaspis aculeifer 14-day reproduction test in soil with the test item PP-113H (Clopyralid 100 g/L SL); the resulting endpoints are as presented below.
The LOEC for mortality could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The NOEC for mortality was determined as equal or greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content.
The LOEC for reproductive output could not be determined and was estimated to be greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The NOEC for reproductive output was determined as equal or greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content.
The EC10 for reproductive output is assumed to be between 1500.00 and 2250.00 mg test item/kg soil dry weight; equivalent to 146.13 and 219.20 mg clopyralid/kg soil dry weight according to the analysed content. The EC20 and EC50 for reproductive output are assumed as greater than 2250.00 mg test item/kg soil dry weight; equivalent to 219.20 mg clopyralid/kg soil dry weight according to the analysed content.

Endpoints	**Concentration [mg/kg soil dry weight]**	
	Test item [a]	**clopyralid [b]**
LOEC mortality	> 2250.00	> 219.20
NOEC mortality	≥ 2250.00	≥ 219.20
LOEC reproductive output	> 2250.00	> 219.20
NOEC reproductive output	≥ 2250.00	≥ 219.20
EC10 (95 %-confidence interval)	1500.00 < EC10 < 2250.00 n.d.	146.13 < EC10 < 219.20 n.d.
EC20 (95 %-confidence interval)	> 2250.00 n.d.	> 219.20 n.d.
EC50 (95 %-confidence interval)	> 2250.00 n.d.	> 219.20 n.d.

n.d.: not determined.
[a] Test item: PP-113H (Clopyralid 100 g/L SL).
[b] Based on the analysed content of active ingredient and density from the certificate of analysis (clopyralid: 102 g/L; density: 1.047 g/mL).

Data requirement
zRMS add the respective table with the data that show mortality and reproductive success to the study report for the sake of transparency.

Mortality and reproductive output of *Hypoaspis aculeifer* after 14 days exposure to artificial soil treated with PP-113H (Clopyralid 100 g/L SL).

Treatment group	Test item concentration [mg TI/kg sdw]	Mean mortality [%]	Corrected mortality [a] [%]	Mean number of juveniles per replicate	CV [%]	Reduction in reproductive output [%]
C	0	7.50	–	307.13	13.06	–
T1	131.69	7.50	0.00	296.50	0.44	3.46
T2	197.53	5.00	-2.70	315.25	19.91	-2.65
T3	296.30	5.00	-2.70	279.50	8.06	8.99
T4	444.44	12.50	5.41	300.50	5.91	2.16
T5	666.67	15.00	8.11	305.50	10.53	0.53
T6	1000.00	7.50	0.00	284.25	16.77	7.45
T7	1500.00	10.00	2.70	303.00	6.47	1.34
T8	2250.00	10.00	2.70	265.50	13.15	13.55

C: control group; T: test item group; TI: test item (PP-113H); sdw: soil dry weight; CV: Coefficient of variation.
[a] Mortality corrected for control group according to Abbott's formula (1925) modified by Schneider-Orelli (1947). Negative values indicate lower mortality compared to control group.
[b] Reduction in reproductive output compared to control group. Negative values indicate higher reproduction compared to control group.

Abbott correction was performed for control mortality in the *Hypoaspis (Geolaelaps) aculeifer* study. However, this approach is not accepted for *Hypoaspis*. According to OECD Statistical Guidance Document No 54, quantal data are not suitable for the correction with Abbott. Instead, more-parametric not-linear models should be used and therefore considered for the evaluation of studies on soil organisms showing mortality in the control. In opinion zRMS, in this case, this will likely not have an impact on the endpoint.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1: Toxicity to the soil microflora

Comments of zRMS:	All validity criteria were met. - The variation between replicate control samples should not be greater than ±15% However, the study needs to be completed.

	RMS pointed out that The Applicant should provide the following data: ☑ calculation of soil nitrate-N transformation rate. ~~**DATA GAP: Calculation of soil nitrate-N transformation rate in this study should be provided. After providing complementary information to this study, the study will be reassessed by RMS.**~~ As the second on nitrogen transformation has been accepted in RA. Therefore, the 1st study does not need to be reassessed.

Reference	KCP 10.5/01
Report:	Dottorini F., 2011 Assessment of the effects of PP-113H (CLOPIRALIDE 10% w/v SL) on soil microorganism respiration and nitrification Report Nº BT154/11
Guidelines	OECD Guideline 217; OECD Guideline 216)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of the Test item *PP-113H (CLOPIRALIDE 10% w/v SL)* on soil microbial nitrification (*nitrogen turn-over*) and carbon transformation (*short-term respiration*) processes were studied according to OECD Guidelines 216 and 217, adopted on the 21st January 2000.

The Test item was mixed into a sandy loam agricultural soil, at the application rates provided by the Sponsor: **4.6 µL** *of PP-113H (CLOPIRALIDE 10% w/v SL)*/Kg soil dry weight for the Low dose and **23.2 µL** of *PP-113H (CLOPIRALIDE 10% w/v SL)*/Kg soil dry weight for the High dose. The control consisted of soil treated with tap water and incubated at the same condition of the treated soil, in the dark at 20 ± 2°C, for 28 days.

The Toxic reference item (Dinoseb acetate) was tested, for this soil batch, in a previous study to confirm the normal reaction of the soil against herbicides.

The influence of the Test item on the nitrification of lucerne meal was investigated and the results were compared to untreated samples.

The CO_2 produced by microbial soil microflora during short-term respiration trials, after addition of glucose, was measured and the results were compared to untreated samples.

*The Test item, **PP-113H (CLOPIRALIDE 10% w/v SL)**, at the Lowest dose applied and at the Highest dose applied did not affect the microbial short-term respiration in soil since the treated samples deviated less than 25% from the control after 28 days (**+1.9%** for the Low and **+3.7%** for the High dose of the Test item).*

*The Test item, **PP-113H (CLOPIRALIDE 10% w/v SL)**, at the Lowest dose applied and at the Highest dose applied did not affect the microbial nitrogen turn-over in soil since the treated samples deviated less than 25% from the control after 28 days (**+2.3%** for the Low and **-9.0%** for the High dose respectively).*

*According to the results of the present study, the Test item, **PP-113H (CLOPIRALIDE 10% w/v SL)**, has no effects neither on carbon transformation nor on soil nitrification at the rates:* **4.6 µL** *of PP-113H (CLOPIRALIDE 10% w/v SL)*/Kg soil dry weight (Low dose) and **23.2 µL** *of PP-113H (CLOPIRALIDE 10% w/v SL* /Kg soil dry weight (High dose).

Study 2: Effects on soil nitrogen transformation

Comments of zRMS:	The study was accepted in dRR B9 for plant product protection Faworyt 300 SL in 07.2022 by PL zRMS. Agreed endpoint: After 28 days of experiment, a statistically insignificant influence of the tested material on the nitrate production rates at 0.743 and 3.7152 mg /kg of dry weight of soil, respectively, was found in comparison to the control in the tested concentrations. The tested material does not show long-term effects on nitrogen transformation in soil.

Reference:	KCP 10.5
Report	Study of impact of test item Faworyt 300 SL on soil microorganisms – nitrogen transformation test according to guideline OECD 216, Agnieszka Woźniak, 2021 Study code 0016/0138/E, SORBOLAB Research Laboratory LLC, Poland
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 216
Deviations	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

1. Test material: Faworyt 300 SL
   Batch number: 01/2020
   Concentration of clopyralid 310 g/L

2. Soil: Was bought in Fraunhofer Institute for Molecular Biology and Applied Ecology in Germany. The soil was obtained from agricultural areas not ploughed for the last six months, which were not treated with plant protection products for five years before being used for testing, nor fertilized with any fertilizer for at least six months. Soil samples were taken from a depth of 0-20 cm and transported to the laboratory in dark containers, which guaranteed that the initial parameters were maintained.

3. Test design:

   Test duration: 28 days. On the 28th day, differences between the rates of nitrate formation in treated and untreated soils samples were smaller than 25%, therefore measurements were not continued.

Two parts of soil were spiked with test material solutions and mixed. The third part of soil was mixed with distilled water without test material (control).

4. The study was carried out in two concentrations of the test item:

C1 = 0.743 mg/kg dw and C2 = 3.7152 mg/kg dw

In addition, control (without test item addition) was used in the test. Each concentration and control was prepared in three replicates.

A study of the test item Faworyt 300 SL effects on the activity of soil microorganisms responsible for nitrogen transformations occurring in aerobic soils in accordance to the guideline OECD 216.
The study consisted of comparing the rate of nitrate production in the soil exposed to the test item with the rate of nitrate production in the control soil.

Test conditions:

Conditions of the test were as recommended by OECD TG 216 (2000). The test was conducted in darkened plastic containers made of chemically inert material, with perforated covers. Soil samples of 500 grams took up ¼ of the test vessel to prevent the development of anaerobic conditions. The soil was modified by the addition of powdered lucerne meal in an amount of 5 g/kg of soil dry matter, as recommended by the OECD 216 guidance.
Once a week the water content in the soil (in control and test concentrations) was checked, after which the appropriate quantity of deionized water was added.

The following soil characteristic and environmental conditions were achieved during the test:

Soil characteristic and environmental conditions during the study:

pH of soil	6.32
Water content of soil	40±5%% of WHC
Sand content in the soil	69.8%
Organic carbon content in the soil	0.95%
Microbial biomass	1.83% of total organic carbon content
Temperature	19.7-20.0°C
Photoperiod	24 hours darkness

Results and discussion:

Faworyt 300 SL at the concentration of 1PEC and 5PEC (0.743 and 3.7152 mg /kg of dry weight of soil, respectively) did not have a statistically significant effect on the production rate of nitrates after 28 days of the experiment.
At this sampling period deviation from the control was < 25% for both concentrations of the test item. In addition, influence of both tested concentrations of the material on the nitrate production rate in compare to the control was statistically insignificant.

Calculated using the ToxRat Professional statistical program							
Time of observation	Control	1PEC*)			5PEC**)		
	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)
after 7 days	8.537	6.726	21.210	+	6.319	25.978	+
after 14 days	16.670	16.527	0.856	-	16.134	3.211	+
after 28 days	11.417	11.698	2.461	-	11.655	2.082	-

*) (Predicted Environmental Concentration): maximum predicted effective concentration in soil (0.743 mg of test item/kg dry weight of soil)
**) (Predicted Environmental Concentration): 5 times the maximum expected effective concentration in soil (3.7152 mg of test item/ kg dry weight of soil
***) significance calculated by ToxRat Professional using the Student's t test at the significance level of p≤0.05
- statistically insignificant
+ statistically significant

Validity criteria:

The study satisfied the OECD 216 validity criterion that the variation between replicate control samples should not be greater than ±15%.

- Day 0: 3.08%
- Day 7: 1.13%
- Day 14: 1.79%
- Day 28: 0.72%

Conclusions:

After 28 days of experiment, a statistically insignificant influence of the tested material on the nitrate production rate was found in comparison to the control in the tested concentrations. The tested material does not show long-term effects on nitrogen transformation in soil.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1: Toxicity to non-target plants (vegetative vigour)

Comments of zRMS:	Although the validity criteria are met, the study cannot be accepted by RMS. Due to an inadequately selected dose range, in this case, ER_{50} based on phytotoxicity effect cannot be determined. Even at the lowest tested concentration at 1 L **BARILOCHE**/ha, the phytotoxicity effect was above 75% (chlorosis). *All phytotoxicity endpoint should be considered in the risk assessment, in line with EFSA Technical Report (2019), i.e. all effects and endpoints will be reported in the study summary and the lowest endpoint should be used by the zRMS ensuring a harmonized risk assessment at zonal level. However, in this case, ER_{50} based on phytotoxicity effect cannot be determined because even at the lowest tested concentration, the phytotoxicity effect was above 75% (chlorosis).* Therefore, the *new study to determine a potential phytotoxic effect of the product **BARILOCHE** for non-target plant species in terms of vegetative vigour should be performed.* Peer review of the pesticide risk assessment of the active substance clopyralid in 2018 also confirmed that a data gap was identified for a new study with non-target plants for the formulation which

should be addressed at Member States level.

DATA GAP:
1. The new study to determine a potential phytotoxic effect of the product **BARILOCHE** for non-target plant species in terms of vegetative vigour should be performed including phytotoxicity effect.
2. Risk assessment for non-target plants has been not performed (insufficient data set - data gap).

The study is considered as additional source of information.

All validity criteria were met.

- The seedling emergence was at least 70% in each species (control and teatred)
- In the control group the plants showed no visible phytotoxicity effects or variation in growth
- In the control group the mean plants survival was at least 90% at the end of the test

Toxicity endpoints as additional source of information:

EC_{50} value for mortality and biomass (fresh shoot weight)

Species	Mortality (L of test item/ha)		Fresh shoot weight (L of test item/ha)	
	EC_{50}	CL*	EC_{50}	CL*
Brassica napus	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sinapis alba	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Daucus carota	0.65	Lw: 0.33 Up: 0.80	0.89	Lw: 0.71 Up: 0.98
Medicago sativa	0.65	Lw: 0.26 Up: 0.81	1.22	Lw: 1.15 Up: 1.32
Glycine max	0.59	Lw: 0.08 Up: 0.79	1.15	Lw: 1.07 Up: 1.23
Solanum lycopersicum	0.74	Lw: 0.46 Up: 0.87	0.75	Lw: 0.51 Up: 0.87
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.

* CL 95% = Confidence Limit 95% Lw = Lower Up= Upper

NOEC values for mortality and biomass (fresh shoot weight)

Species	Mortality - **NOEC** (L of test item/ha)	Fresh shoot weight - **NOEC** (L of test item/ha)
Brassica napus	3.00	3.00
Sinapis alba	3.00	3.00
Daucus carota	<1.00	<1.00
Medicago sativa	<1.00	<1.00
Glycine max	<1.00	<1.00
Solanum lycopersicum	<1.00	<1.00
Hordeum vulgare	3.00	3.00
Triticum aestivum	3.00	3.00
Sorghum halepense	3.00	1.50
Zea mays	3.00	3.00

Phytotoxicity effect:
After 21/28 days of treatment the product **BARILOCHE** caused chlorosis at the rate of 1.0 and 1.2 L/ha in the *Daucus carota, Medicago sativa, Glycine max and Solanum lycopersicum* and necrosis in *Medicago sativa and Glycine max*. The **BARILOCHE** at 3.00 L/ha caused necrosis n the *Hordeum vulgare*. *Brassica napus, Sinapis alba, Sorghum halepense, Triticum aestivum, Zea mays* - no phytotoxicity effects.

The ER_{50} (*Glycine max*) > 0.59 L/ha – the lowest toxicity endpoint based on mortality

Reference:	KCP 10.6/02
Report	Corbolli M, 2011 Vegetative vigour rate response test for non-target plants following application of the product PP-113H (Clopyralid 10% w/v SL) Report No. BT 100/11
Guideline(s):	OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No - not appropriate

Executive Summary

The purpose of this study was the to determine a potential phytotoxic effect of the product PP-113H (Clopyralid 10% w/v SL) on 10 non-target plant species after deposition of the test item on the leaves and on above-ground portion of the plants.

Plants were grown from seeds until the 2 - 4 true leaf stage; then the test item was sprayed on the plants in order to evaluate the effects on vigour and growth at different times (7, 14, 21 and 28 days after the treatment).

The measured endpoints were mortality, biomass (fresh shoot weight) and visual detrimental effects (chlorosis and necrosis) at 28 days after the treatment. The height of plants was also measured.

In the test the following species were treated: six dicotyledonous species (*Brassica napus, Sinapis alba, Daucus carota, Medicago sativa, Glycine max* and *Solanum lycopersicum*) and four monocotyledonous species (*Hordeum vulgare, Triticum aestivum, Sorghum halepense* and *Zea mays*). A single control group, treated with deionised water was set up in the test.

The following application rates were used in the test:

L product/ha	L a.i./ha
0	0
1.00	Clopyralid: 0.10
1.20	Clopyralid: 0.12
1.50	Clopyralid: 0.15
2.00	Clopyralid: 0.20
3.00	Clopyralid: 0.30

I. MATERIALS AND METHODS

A. MATERIALS

1.Test Item:	PP-113H (Clopyralid 10% w/v SL) (see Annex III)
Active ingredient:	Clopyralid
Batch N°:	20110713
Purity:	Clopyralid 9.9 ± 0.1% w/v
2. Vehicle and/or positive control:	Water
3. Reference item:	Boric Acid (H_3BO_3) (see Annex V)
4. Experimental conditions:	
Test soil:	Field soil contains 1.87 ± 0.20 % of organic carbon and the particle size is < 2 mm (see Annex V)
Temperature:	16.50 – 29.00 °C (see Annex VI)
Humidity:	55.00 – 86.50 % (see Annex VI)
Photoperiod:	Minimum 16 h light (see Annex VI)

Light intensity:	15500-20000 Lux (see Annex VI)

B. STUDY DESIGN AND METHODS

1. Experimental Period:	21st December 2011 – 18th January 2012
2. Test system:	Each group comprised at least 20 plants (monocotyledonous and dicotyledonous) for each concentration; the number of planted seeds (two, three or five) per pot depended on the species.
3. Statistics:	In order to estimate the EC_{50} and its confidence limits, statistical Probit analysis was used. NOEC was estimated with statistical test (Dunnett's test).

II. RESULTS AND DISCUSSION

A. Effects on non-target plants

The EC_{50} values for the mortality and biomass (fresh shoot weight) are hereunder reported:

EC_{50} value for mortality and biomass (fresh shoot weight)

Species	Mortality (L of test item/ha)		Fresh shoot weight (L of test item/ha)	
	EC_{50}	CL*	**EC_{50}**	CL*
Brassica napus	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sinapis alba	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Daucus carota	0.65	Lw: 0.33 Up: 0.80	0.89	Lw: 0.71 Up: 0.98
Medicago sativa	0.65	Lw: 0.26 Up: 0.81	1.22	Lw: 1.15 Up: 1.32
Glycine max	0.59	Lw: 0.08	1.15	Lw: 1.07

		Up: 0.79		Up: 1.23
Solanum lycopersicum	0.74	Lw: 0.46 Up: 0.87	0.75	Lw: 0.51 Up: 0.87
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.

* CL 95% = Confidence Limit 95% Lw = Lowest Up= Upper

The NOEC for the mortality and biomass (fresh shoot weight) was calculated with Dunnett's test and the results are hereunder reported:

NOEC values for mortality and biomass (fresh shoot weight)

Species	Mortality - **NOEC** (L of test item/ha)	Fresh shoot weight - **NOEC** (L of test item/ha)
Brassica napus	3.00	3.00
Sinapis alba	3.00	3.00
Daucus carota	<1.00	<1.00
Medicago sativa	<1.00	<1.00
Glycine max	<1.00	<1.00
Solanum lycopersicum	<1.00	<1.00
Hordeum vulgare	3.00	3.00
Triticum aestivum	3.00	3.00
Sorghum halepense	3.00	1.50
Zea mays	3.00	3.00

III. CONCLUSIONS

Mortality: After 28 days, the treatment with the product PP-113H (Clopyralid 10% w/v SL) caused significative effects on mortality in Daucus carota (EC50 value = 0.65 L of test item/ha), Medicago sativa (EC50 value = 0.65 L of test item/ha), Glycine max (EC50 value = 0.59 L of test item/ha) and Solanum lycopersicum (EC50 value = 0.74 L of test item/ha).

The test item did not cause effects on mortality for the other species (Brassica napus, Sinapis alba, Hordeum vulgare, Triticum aestivum, Sorghum halepense and Zea mays), if compared to the control groups. The EC50 value for these species is >3.00 L of test item/ha.

The NOEC value was <1.00 L of test item/ha for Daucus carota, Medicago sativa, Glycine max and Solanum lycopersicum. For the other species (Brassica napus, Sinapis alba, Hordeum vulgare, Triticum aestivum, Sorghum halepense and Zea mays) the NOEC value was 3.00 L of test item/ha.

Fresh shoot weight: After 28 days, the treatment with the product PP-113H (Clopyralid 10% w/v SL) caused significative effects on fresh shoot weight in Daucus carota (EC50 value = 0.89 L of test item/ha), Medicago sativa (EC50 value = 1.22 L of test item/ha), Glycine max (EC50 value = 1.15 L of test item/ha) and Solanum lycopersicum (EC50 value = 0.75 L of test item/ha).

The test item did not cause inhibition effects on fresh shoot weight for the other species (Brassica napus, Sinapis alba, Hordeum vulgare, Triticum aestivum, Sorghum halepense and Zea mays), if compared to the control groups. The EC50 value for these species is >3.00 L of test item/ha.

The NOEC value was <1.00 L of test item/ha for Daucus carota, Medicago sativa, Glycine max and Solanum lycopersicum; for Sorghum halepense the NOEC value was 1.50 L of test item/ha. For the other species (Brassica napus, Sinapis alba, Hordeum vulgare, Triticum aestivum and Zea mays) the NOEC value was 3.00 L of test item/ha.

Phytotoxicity: After 28 days of treatment the product PP-113H (Clopyralid 10% w/v SL) caused chlorosis at the rate of 1.00 and 1.20 L of test item/ha in the Daucus carota, Medicago sativa, Glycine max and Solanum lycopersicum and necrosis in Medicago sativa and Glycine max.

The PP-113H(Clopyralid 10% w/v SL) at 3.00 L of test item/ha caused necrosis in the Hordeum vulgare

Comments of zRMS:	The study was accepted in dRR B9 for plant product protection Faworyt 300 SL in 07.2022 by PL zRMS. Conclusion: **The ER_{50} (lowest endpoint) = 0.151 L/ha (Mung bean)** Currently, it is assumed that the formulation Faworyt 300 SL is worst case in comparison to Bariloche and, thus, can be taken for the risk assessment of the later.

Study 2: Toxicity to non-target plants (vegetative vigour)

Reference:	KCP 10.6.2/02
Report	Vegetative Vigour Test according to OECD 227, Aleksandra Kamińska, 2019, Study code 0016/0060/E, Sorbolab Research Laboratory, Poland Annex No. 1 to the Final report: Vegetative Vigour Test according to OECD 227, Agnieszka Woźniak 2021, Sorbolab Research Laboratory Poland

Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 227 "Terrestrial Plant Test: Vegetative Vigour Test".
Deviations:	OECD 227 guideline recommends CO2 concentration at the level 350±50 ppm during the course of the experiment. In the test this parameter was exceeded. However literature data show , that in year 2017 average CO_2 level in atmosphere exceeded 400 ppm. This deviation had no effect on the course of the study and obtained results.
GLP:	Yes
Acceptability:	Yes

Materials and methods:

1. Test material: Faworyt 300 SL
   Batch number: 201805002
   Concentration of clopyralid 302.7 g/L

1. Test organism: Six plant species were tested, four dicotyledonous and two monocotyledonous: *Phaseolus aureus*, *Cucumis dativus*, *Brassica oleracea L. var. italica Plenck*, *Raphanus sativus*, *Dacus carota*, *Avena sativa*, *Zea mays.*

3. Test design:

Definitive test with dicotyledonous species was performed using five doses of the test item. In the test doses: 0.4 L/ha; 0.22 L/ha; 0.12 L/ha; 0.069 L/ha 0.038 L/ha and control were used.
In case monocotyledonous species only the limit test was performed with the dosage of 0.4 L/ha.

All tested doses were chosen based on the results of range finding test.

Soil used in the study

Artificial soil was used in the study containing 20% kaolin clay and approx. 80% quartz sand.

The basic indigents were calcined in temperature of 105±5˚C and then mixed together in an appropriate amounts. Dry soil was sieved by a sieve of 2 mm mesh. Before the start of the test, artificial soil was moisten with deionized water, to not let it became too wet. After placing soil in the pots, the deionized water (with addition of fertilizer) was poured into stands. During the experiment regular watering was carried out, to prevent drying of the upper parts of the soil. The pH of the soil was 6.0 and contained 0.1% total dissolved solids as a measure of salinity.

Environmental conditions

Temperature	19.4- 27.5°C
Air relative humidity:	average relative air humidity 77%
Lightning	light cycle (16 h day / 8 h night); average light intensity 26654.59 lux
CO2 concentration:	average CO_2 concentration 379.1 ppm

Results and discussions:

Based on the performed experiment, executed observations and obtained results, it is stated that the tested item exhibits ecotoxic effects on mung bean and carrot in relation to fresh and dry weigh. Statistical difference compared to control were determined at highest tested dose on mung bean.
Strong stimulation of momentum (exuberance) was observed in all cucumber doses tested. In the study, no statistically significant differences were found for plant mortality in the doses tested.
It does not cause ecotoxic effects on oats, corn and broccoli.

The final results of the study were determined using the ToxRat Professional statistical software.
In the presented study the LR10/ER10, LR25/ER25, LR/ER50, LOER and NOER value was determined for the individual parameters: fresh weight, dry weight, shoot length and survival.

Dicotyledons					
Species	**Parameter**	**Fresh weight**	**Dry weight**	**Shoot's length**	**Survival**
Mung Bean *Phaseolus aureus*	**ER_{10}/LR_{10} [L/ha]**	0.054 (0.028 – 0.101)*	0.019 (0.010 – 0.039)*	0.276 (0.215 – 0.353)*	n.d.** (n.d. – n.d.)*
	ER_{25}/LR_{25} [L/ha]	0.113 (0.076 – 0.169)*	0.051 (0.032 – 0.081)*	0.406 (0.360 – 0.458)*	n.d.** (n.d. – n.d.)*
	ER_{50}/LR_{50} [L/ha]	0.260 (0.165 – 0.418)*	0.151 (0.091 – 0.259)*	0.625 (0.473 – 0.816)*	n.d.** (n.d. – n.d.)*
	LOER [L/ha]	≤0.038	≤0.038	0.4	>0.4
	NOER [L/ha]	<0.038	<0.038	0.22	≥0.4
Dicotyledons					
Species	**Parameter**	**Fresh weight**	**Dry weight**	**Shoot's length**	**Survival**
Cucumber *Cucumis sativus*	**ER_{10}/LR_{10} [L/ha]**	n.d.** (n.d. – n.d.)*	0.582 (n.d. – n.d.)*	0.197 (n.d. – n.d.)	n.d.** (n.d. – n.d.)*
	ER_{25}/LR_{25} [L/ha]	n.d.** (n.d. – n.d.)*	n.d.** (n.d. - ∞)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	ER_{50}/LR_{50} [L/ha]	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	LOER [L/ha]	>0.4	>0.4	>0.4	n.d.**
	NOER [L/ha]	≥0.4	≥0.4	≥0.4	n.d.**
Carrot *Daucus carota*	**ER_{10}/LR_{10} [L/ha]**	0.035 (0.009 – 0.131)*	0.016 (0.004 – 0.062)*	0.287 (n.d. – n.d.)	n.d.** (n.d. – n.d.)*
	ER_{25}/LR_{25} [L/ha]	0.082 (0.035 – 0.192)*	0.047 (0.019 – 0.113)*	0.541 (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	ER_{50}/LR_{50} [L/ha]	0.211 (0.084 – 0.549)*	0.155 (0.060 – 0.421)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	LOER [L/ha]	0.120	≤0.038	>0.4	n.d.**
	NOER [L/ha]	0.069	<0.038	≥0.4	n.d.**

Dicotyledons					
Species	**Parameter**	**Fresh weight**	**Dry weight**	**Shoot's length**	**Survival**
Broccoli *Brassica oleracea L. va. italica Plenck*	**ER_{10}/LR_{10} [L/ha]**	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	ER_{25}/LR_{25} [L/ha]	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	ER_{50}/LR_{50} [L/ha]	nd.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
	LOER [L/ha]	>0.4	>0.4	>0.4	n.d.**
	NOER [L/ha]	≥0.4	≥0.4	≥0.4	n.d.**
Monocots - limit test					
Species	**Parameter**	**Fresh weight**	**Dry weight**	**Shoot's length**	**Survival**
Oats *Avena sativa*	**Reduction [%]**	- 2.0	- 3.9	- 0.6	
	Statistical significance	statistically insignificant	statistically insignificant	statistically insignificant	statistically insignificant
Corn *Zea mays*	**Reduction [%]**	2.7	12.7	- 0.5	
	Statistical significance	statistically insignificant	statistically insignificant	statistically insignificant	statistically insignificant

ER_x dose of the material that exhibits intoxication effects in x% of population
LR_x dose of the material that exhibits intoxication causes mortality in x% of population
NOER highest non observe effective concentration cause no statistically significant differences in comparison to the control
LOER lowest observe effective concentration cause statistically significant differences in comparison to the control
n.d. impossible to calculate due to mathematical reasons
*) the lower and upper 95% confidence intervals
) **based on the analysis of the results, this value was defined as > 0.4 L/ha

Additionally, ER_{50} for phytotoxicity effects were also determined.

Parameter / Species	Average phytotoxicity [%]						ER_{10} [L/ha]	ER_{25} [L/ha]	ER_{50} [L/ha]
	Control	**0.038 [L/ha]**	**0.069 [L/ha]**	**0.12 [L/ha]**	**0.22 [L/ha]**	**0.4 [L/ha]**			
Mung Beans *Phaseolus aureus*	0.0	0.0	0.0	10.0	20.0	100.0	0.150 (0.078 – 0.192)*	0.188 (0.122 – 0.233)*	0.240 (0.186 – 0.314)*
Cucumber *Cucumis sativus*	0.0	5.0	10.0	15.0	20.0	35.0	0.076 (n.d. – n.d.)*	0.251 (n.d. – n.d.)*	n.d.** (n.d. – n.d.)*
Broccoli *Brassica oleracea L. var. italica Plenck*	0.0	0.0	0.0	0.0	0.0	0.0	n.d.**	n.d.**	n.d.**
Carrot *Daucus carota*	0.0	10.0	20.0	30.0	50.0	90.0	0.048 (0.012 – 0.078)*	0.087 (0.040 – 0.129)*	0.170 (0.113 – 0.296)*
Oat *Avena sativa*						0.0	n.d.**	n.d.**	n.d.**
Corn *Zea mays*						0.0	n.d.**	n.d.**	n.d.**

ER_x dose of test item causing symptoms of intoxication in x% of the population
n.d. impossible to determine for mathematical reasons
*) the lower and upper 95% confidence intervals
) **based on the analysis of the results, the value was determined as > 0.4 L/ha

Validity criteria:

All OECD 227 validity criteria were met:

1. Seedling emergence in control was at least 70%:
- bean mung *Phaseolus aureus* – 100%
- cucumber *Cucumis dativus* – 100%
- carrot *Daucus carota* – 100%
- broccoli *Brassica oleracea L. var. italica Plenck* – 100%
- oats *Avena sativa* – 100% (Table 23)
- corn *Zea mays* – 100% (Table 24)

2. In none of the control replications of any plants species there were any signs of intoxications visible (i.e. chlorosis, necrosis, wilting, leaf/stalk deformation) for every species.

3. Mean survival of plants in control was 100% (required at least 90%) for every species

4. Environmental conditions and soil were identical for all used in the experiment plants species.

Conclusions:

The lowest ER_{50} was 0.151 L of test item/ha for Mung bean dry weight.

A 2.6.2.2 Study 3: Toxicity to non-target plants (Seedling Emergence and Growth)

Comments of zRMS:	The study is considered as acceptable . All validity criteria were met. **Agreed endpoints:**

EC_{50}/LC_{50} values for the emergence and mortality

Species	Emergence (L of test item/ha)		Mortality (L of test item/ha)	
	ER_{50}	CL*	LC_{50}	CL*
Brassica napus	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sinapis alba	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Daucus carota	0.98	Lw: 0 .74 Up: 1.14	1.53	Lw: 1.50 Up: 1.58
Medicago sativa	1.43	Lw: 0.54 Up: 2.87	0.19	Lw: n.a. Up: n.a.
Glycine max	1.08	Lw: 0.58 Up: 1.38	1.11	Lw: n.a. Up: n.a.
Solanum lycopersicum	1.96	Lw: 1.31 Up: 3.59	1.55	Lw: 0.80 Up: 3.28
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.

ER_{50} values for the fresh shoot weight

Species	Fresh shoot weight (L of test item/ha)	
	ER_{50}	CL*
Brassica napus	2.72	Lw: 2.19 Up: 4.51
Sinapis alba	2.62	Lw: 2.49 Up: 2.78
Daucus carota	1.56	Lw: 1.42 Up: 1.78
Medicago sativa	0.36	Lw: 0.23 Up: 0.89
Glycine max	1.01	Lw: 0.99 Up: 1.03
Solanum lycopersicum	1.40	Lw: 1.15 Up: 1.77
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.

* CL = Confidence Limit 95% Lw = Lower Up= Upper

Phytotoxicity effect:
The ER_{50} (*Daucus carota*) > 1.2 L/ha
The ER_{50} (*Medicago sativa*) > 0.1 L/ha – the lowest toxicity endpoint
The ER_{50} (*Glycine max*) > 1 L/ha
The ER_{50} (*Solanum lycopersicum*) > 1 L/ha
Brassica napus, Sinapis alba, Hordeum vulgare, Sorghum halepense, Triticum aestivum, Zea mays - no phytotoxicity effects

NOEC values for the emergence, mortality and biomass (fresh shoot weight)

Species	Emergence NOEC (L of test item/ha)	Mortality NOEC (L of test item/ha)	Fresh shoot weight NOEC (L of test item/ha)
Brassica napus	3.00	3.00	1.20
Medicago sativa	0.10	0.05	0.05
Glycine max	<1.00	<1.00	<1.00
Daucus carota	<1.00	1.20	1.00
Solanum lycopersicum	<1.00	1.00	1.00
Sinapis alba	3.00	3.00	1.20
Hordeum vulgare	3.00	3.00	1.00
Triticum aestivum	3.00	3.00	2.00
Sorghum halepense	3.00	3.00	1.20
Zea mays	3.00	3.00	1.00

The ER_{50} (*Medicago sativa*) > 0.1 L/ha – the lowest toxicity endpoint based on phytotoxicity effect should be used in risk assessment

Reference:	KCP 10.6/01
Report	Corboli Massimiliano,2012 Seedling emergence rate response test for non-target plants following application of the product PP-113H (Clopyralid 10% w/v SL)" Report No. BT 101/2011
Guideline(s):	OECD 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No - not appropriate

Executive Summary

The purpose of this study was to determine potential phytotoxic effects of the product PP-113H (Clopyralid 10% w/v SL) on seedling emergence, mortality and early growth of 10 non-target plants species following exposure to the test item distributed on the soil surface.
Seeds were placed into the pots and then the soil surface was treated with the test item solutions. The effects (visual assessments of seedling emergence and detrimental effects like chlorosis, necrosis etc.) were evaluated after 7, 14 and 21 days from the emergence of 50% of the seedlings in the control group. The measured endpoints were visual assessments of seedling emergence, mortality, biomass (fresh shoot weight) and the assessment of visible detrimental effects (chlorosis, necrosis etc.). These measurements and observations were compared to those of untreated control plants
Six dicotyledonous species (*Brassica napus, Medicago sativa, Glycine max, Daucus carota, Solanum lycopersicum,* and *Sinapis alba*) and four monocotyledonous species (*Hordeum vulgare, Triticum aestivum, Sorghum halepense and Zea mays*) were used in the test. Each species comprised at least twenty seeds for each application rate; the number of seeds planted (two, three or five) per pot depended on the species. The control group was treated with deionised water.
The following application rates were used in the test:

	L product/ha	**Kg a.i./ha**

Control group	0	0
	1.00	Clopyralid:0.10
	1.20	Clopyralid:0.12
	1.50	Clopyralid:0.15
	2.00	Clopyralid:0.20
	3.00	Clopyralid:0.30

For the species *Medicago sativa* two further application rates were used:

	L product/ha	**Kg a.i./ha**
Control group	0	0
Treated	0.05	Clopyralid:0.005
	0.10	Clopyralid:0.01

I. MATERIALS AND METHODS

A. MATERIALS

1.Test Item:	PP-113H (Clopyralid 10% w/v SL) (see Annex III)
Active ingredient:	Clopyralid
Batch No:	20110713
Purity:	Clopyralid: 9.9 ± 0.1 % w/v
Water solubility:	miscible with water
2. Vehicle and/or positive control:	Water
3. Reference item:	Boric Acid (H_3BO_3) (see Annex V)
4. Experimental conditions:	
Test soil:	Field soil contains 1.87 ± 0.20 % of organic carbon and with particle size < 2 mm (see Annex V)
Temperature:	14.67 - 29.33 °C (see Annex VI)
Humidity:	41.50 – 90.00 % (see Annex VI)
Photoperiod:	Minimum 16 h of light (see Annex VI)
Light intensity:	15500-20000 Lux (see Annex VI)

B. STUDY DESIGN AND METHODS

1. Experimental period:	21st December 2011– 09th July 2012
2. Test system:	Each group comprised at least 20 seeds for each application rate; the number of seeds planted per pot (two, three or five) depended on the species.
3. Statistics:	In order to estimate the ER_{50} and its confidence limits, statistical Probit analysis was used. NOEC was estimated with statistical test (Dunnett's test).

II. RESULTS AND DISCUSSION

A. Effects on non-target plants

The EC_{50}/LC_{50} values for the emergence, mortality and biomass (fresh shoot weight) are following reported:

EC_{50}/LC_{50} values for the emergence and mortality

Species	**Emergence** (L of test item/ha)		**Mortality** (L of test item/ha)	
	ER_{50}	CL*	**LC_{50}**	CL*
Brassica napus	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sinapis alba	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Daucus carota	0.98	Lw: 0 .74 Up: 1.14	1.53	Lw: 1.50 Up: 1.58
Medicago sativa	1.43	Lw: 0.54 Up: 2.87	0.19	Lw: n.a. Up: n.a.
Glycine max	1.08	Lw: 0.58 Up: 1.38	1.11	Lw: n.a. Up: n.a.
Solanum lycopersicum	1.96	Lw: 1.31 Up: 3.59	1.55	Lw: 0.80 Up: 3.28
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.	>3.00	Lw: n.a. Up: n.a.

ER_{50} values for the fresh shoot weight

Species	**Fresh shoot weight** (L of test item/ha)	
	ER_{50}	CL*
Brassica napus	2.72	Lw: 2.19 Up: 4.51
Sinapis alba	2.62	Lw: 2.49 Up: 2.78
Daucus carota	1.56	Lw: 1.42 Up: 1.78
Medicago sativa	0.36	Lw: 0.23 Up: 0.89
Glycine max	1.01	Lw: 0.99 Up: 1.03
Solanum lycopersicum	1.40	Lw: 1.15 Up: 1.77
Hordeum vulgare	>3.00	Lw: n.a. Up: n.a.
Triticum aestivum	>3.00	Lw: n.a. Up: n.a.
Sorghum halepense	>3.00	Lw: n.a. Up: n.a.
Zea mays	>3.00	Lw: n.a. Up: n.a.

* CL = Confidence Limit 95% Lw = Lower Up= Upper

The NOEC for the emergence, mortality after emergence and biomass was calculated with Dunnett's test and the results are hereunder reported:

NOEC values for the emergence, mortality and biomass (fresh shoot weight)

Species	**Emergence NOEC** (L of test item/ha)	**Mortality NOEC** (L of test item/ha)	**Fresh shoot weight NOEC** (L of test item/ha)
Brassica napus	3.00	3.00	1.20
Medicago sativa	0.10	0.05	0.05
Glycine max	<1.00	<1.00	<1.00
Daucus carota	<1.00	1.20	1.00
Solanum lycopersicum	<1.00	1.00	1.00
Sinapis alba	3.00	3.00	1.20
Hordeum vulgare	3.00	3.00	1.00
Triticum aestivum	3.00	3.00	2.00
Sorghum halepense	3.00	3.00	1.20
Zea mays	3.00	3.00	1.00

III. CONCLUSIONS

Emergence: at the end of the test, the treatment with PP-113H (Clopyralid 10% w/v SL) caused inhibition in the emergence in *Medicago sativa* (ER_{50} value was 1.43 L of test item/ha), *Glycine max* (ER_{50} value was 1.08 L of test item/ha), *Daucus carota* (ER_{50} value was 0.98 L of test item/ha) and in *Solanum lycopersicum* (ER_{50} value was 1.96 L of test item/ha) if compared with the control group.
The test item did not cause emergence inhibition in the other species (the ER_{50} value was greater than 3.00 L of test item/ha).

The NOEC value was 0.10 L of test item/ha for *Medicago sativa*; <1.00 L of test item/ha for *Daucus carota*, *Glycine max* and *Solanum lycopersicum;* 3.00 L of test item/ha for,the other species .

Mortality: at the end of the test, the treatment with PP-113H (Clopyralid 10% w/v SL) caused mortality, if compared to the control groups, in *Medicago sativa* (LC_{50} value was 0.19 L of test item/ha), *Glycine max* (LC_{50} value was 1.11 L of test item/ha), *Daucus carota* (LC_{50} value was 1.53 L of test item/ha) and in *Solanum lycopersicum* (LC_{50} value was 1.55 L of test item/ha).The test item did not cause mortality in the other species and then the LC_{50} value was higher than 3.00 L of test item/ha.

The NOEC value was 0.05 L of test item/ha for *Medicago sativa*,; <1.00 L of test item/ha *Glycine max*, 1.00 L of test item/ha *Solanum lycopersicum* and 1.20 L of test item/ha *Daucus carota*; the NOEC value was 3.00 L of test item/ha for the *Brassica napus*, *Sinapis alba*, *Hordeum vulgare*, *Triticum aestivum*, *Sorghum halepense* and *Zea mays*.

Fresh shoot weight: at the end of the test, the treatment with PP-113H (C lopyralid 10% w/v SL) caused inhibition of the shoot weight, if compared to the control groups, in *Medicago sativ*a (ER_{50} value was 0.36 L of test item/ha), *Daucus carota* (ER_{50} value was 1.56 L of test item/ha), *Solanum lycopersicum* (ER_{50} = 1.40 L of test item/ha), *Glycine max* (EC_{50} value was 1.01 L of test item/ha), *Sinapis alba* (ER_{50} = 2.62 L of test item/ha)and *Brassica napus* (ER_{50} = 2.72 L of test item/ha). The ER_{50} value was higher than 3.00 L of test item/ha in *Hordeum vulgare*, *Triticum aestivum*, *Sorghum halepense* and *Zea mays*.

The NOEC value was : 0.05 L of test item/ha for *Medicago sativa;* <1.00 L of test item/ha *Glycine max*; 1.00 L of test item/ha for *Solanum lycopersicum*, *Daucus carota*, *Hordeum vulgare* and *Zea mays;* 1.20 L of test item/ha for *Brassica napus*, *Sinapis alba* and *Sorghum halepense*; 2.00 L of test item/ha for the *Triticum aestivum*.

A 2.6.3 KCP 10.6.1 Summary of screening data

A 2.6.4 KCP 10.6.2 Testing on non-target plants

A 2.6.5 KCP 10.6.3 Extended laboratory studies on non-target plants

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

A 2.8 KCP 10.8 Monitoring data