FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: CHR/H/CPD 300SL

Product name(s): Major 300SL, Cloe 300SL, ProSto 300SL

Chemical active substance(s):

Clopyralid, 300 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

Core Assessment

(renewal of authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 12.2021

MS Finalisation date: 09.2022; 02.2023

Version history

When	What
December 2021	New data for CHR/H/CPD based on the renewal of active substance - clopyralid. New data is highlighted in yellow.
September 2022	zRMS first evaluation for commenting
March 2023	zRMS evaluation after commenting

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9 Ecotoxicology (KCP 10)

In the following document, data for active substance clopyralid was described during its renewal. Data matching and equivalent matching studies have been evaluated by RMS. Therefore, Applicant is allowed to refer to the endpoints and input parameters determined at the European level.

9.1 Critical GAP and overall conclusions

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15							
Use-No. (e)	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg as/hL a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																					
1	PL, CZ, RO, SLO, HU, SK	Winter oilseed rape Brassica napus, (BRSNW)	F	broadleaf weeds	Spray, medium sprayer	Spring BBCH 30–51	a) 1 b) 1	n/a	a) 0.06 b) 0.06	a) 0.12 b) 0.12	200-300	n/a									
2	PL, CZ, RO	Winter oilseed rape Brassica napus, (BRSNW)	F	annual and perennial broadleaf weeds	Spray, medium sprayer	Spring BBCH 30–50	a) 1 b) 1	n/a	a) 0.0.0117+0.045 b) 0.0.0117+0.045 CHR/H/PCR* + CHR/H/CPD*	a) 0.0234+0.09 b) 0.0234 +0.09 CHR/H/PCR* + CHR/H/CPD*	200-300	n/a									
3	PL, CZ, RO	Winter oilseed rape Brassica	F	broadleaf weeds	Spray, medium spray-	Autumn BBCH 20–21	a) 1 a) 1	n/a	a) 0.03 0.03	a) 0.06 0.06	200-300	n/a									

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15							
Use-No. (e)	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg as/hL a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
		napus, (BRSNW)			er																
4	PL, CZ, RO	Winter oilseed rape Brassica napus, (BRSNW)	F	annual and perennial broadleaf weeds	Spray, medium sprayer	Autumn BBCH 13-14 20–21	a) 1 b) 1	n/a	c) 0.0.0117+0.045 d) 0.0.0117+0.045 CHR/H/PCR* + a) CHR/H/CPD*	c) 0.0234+0.09 d) 0.0234 +0.09 CHR/H/PCR* + a) CHR/H/CPD*	200-300	n/a									
5	PL, CZ, RO	Winter oilseed rape Brassica napus, (BRSNW)	F	annual and perennial broadleaf weeds	Spray medium sprayer	Autumn BBCH 13-14	b) 1 a) 1	n/a	a) 0,0117+0,045+0,375 b) 0,0117+0,045+0,375 CHR/H/PCR* + CHR/H/CPD* + a) CHR/H/MTC*	a) 0.0234+0.09+0.750 b) 0.0234+0.09+0.750 CHR/H/PCR* + CHR/H/CPD* + a) CHR/H/MTC*	200-300	n/a									
6	PL, CZ, RO, SLO	Winter wheat Triticum aestivum (TRZAW);	F	broadleaf weeds	Spray medium sprayer	Spring BBCH 20-29 CZ: BBCH 21-29	a) 1 a) 1	n/a	b) 0.06 a) 0.06	b) 0.12 a) 0.12	200-300	n/a									
7	PL, CZ, RO, SLO	Sugar beet Beta vulgaris (BEAVP)	F	broadleaf weeds	Spray medium sprayer	BBCH 12 - 14	b) 1 b) 1	n/a	b) 0.06 b) 0.06	b) 0.12 b) 0.12	200-300	n/a									
8	PL, CZ, RO, SLO	Sugar beet Beta	F	broadleaf weeds	Spray medium	BBCH 12-14	c) 3 d) 3	6-10	c) 0.03 d) 0.09	c) 0.06 d) 0.18	200-300	n/a									

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15							
Use-No. (e)	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg as/hL a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
		vulgaris (BEAVP)			sprayer															
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
7																				
8																				
Minor uses according to Article 51 (zonal uses)																				
9																				
10																				
Minor uses according to Article 51 (interzonal uses)																				
11																				
12																				

Remarks table heading:

(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c) g/kg or g/l
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use.

Remarks columns:

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

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

*The risk assessment for CHR/H/PCR and CHR/H/MTC was covered by the individual registration process. CHR/H/PCR and CHR/H/MTC was evaluated and registered in Poland.

zRMS comment:

The zRMS has reviewed this document as part of the application for registration of the product **CHR/H/CPD 300SL** in Poland.
Clarifications and conclusions of the zRMS are presented in grey in the commenting boxes.
Not agreed or not relevant information is struck through and shaded for transparency. Clarifications and conclusions of the zRMS after commenting are presented in brown.

9.1.1 Overall conclusions

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

CHR/H/CPD 300 SL pose no unacceptable risk to birds and mammals used according to the label.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

CHR/H/CPD 300 SL pose no unacceptable risk to non-target terrestrial plants according to the label with appropriate buffer zone.

9.1.1.3 Effects on bees (KCP 10.3.1)

CHR/H/CPD 300 SL pose no unacceptable risk to bees according to the label

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

CHR/H/CPD 300 SL pose no unacceptable risk to bees according to the label.
In addition, the chronic study for adult bees and chronic study for larvae were submitted according to EU Reg. 284/2009 and should be considered further at MSs level.

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

CHR/H/CPD 300 SL pose no unacceptable risk to non-target soil meso- and macrofauna and microbial activity according to the label.

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

CHR/H/CPD 300SL pose no unacceptable risk to non-target terrestrial plants according to the label with appropriate buffer zone and drift reducing techniques.

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

Not relevant

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-1: Critical use pattern of CHR/H/CPD 300 SL grouped according to crop, application rate, number of applications, timing criterion

Grouping according to crop, application rate, number of applications, timing criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	Winter oilseed rape BBCH 30-50 459.2g [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,
2	Winter oilseed rape BBCH 13-14 344.4g [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,
3	Winter wheat BBCH 20-29 459.2g [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,
4	Sugar beet BBCH 12-14 229.6g [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,
5	Sugar beet BBCH 12-14 459.2g [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,

9.1.3 Consideration of metabolites

CHR/H/CPD has no metabolites.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with clopyralid. Full details of these studies are provided in the respective EU RAR and related documents. Effects on birds of CHR/H/CPD were not evaluated as part of the EU assessment of clopyralid. However, the provision of further data on the CHR/H/CPD is not considered essential, because studies from Annex I inclusion can be used in Annex I inclusion

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

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

Species	Substance	Exposure System	Results	Reference
Mallard duck	Clopyralid	Acute	LD_{50} =1465 mg/kg	EFSA Journal 2018;16(7):5389

Species	Substance	Exposure System	Results	Reference
			bw per day	
Mallard duck	Clopyralid	Long term	NOEC=118 mg/kg bw per day	EFSA Journal 2018;16(7):5389

9.2.2 Risk assessment for spray applications

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

Birds and mammals risk assessment were performed using worst case scenario application in sugar beets 3 x 60 g a.s./ha per season, sugar beets 120g a.s./ha, winter oilseed rape 120g a.s./ha, winter oilseed rape 90g a.s/ha, winter wheat 120g/ha which are the risk envelope for other uses of CHR/H/CPD 300 SL according to the GAP presented in table 9.1.-1.

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CPD 300 SL

Intended use	**Winter oilseed rape(spring)**				
Active substance/product	Clopyralid				
Application rate (g/ha)	120				
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**
Screening step	Small omnivorous bird	158.8	1.0	19.06	76.9
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}**
Screening step	Small omnivorous bird	64.8	1.0	4.12	28.6

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

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CPD 300 SL

Intended use	**Winter oilseed rape**
Active substance/product	Clopyralid

Application rate (g/ha)	90 g a.s/ha				
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**
Screening step	Small omnivorous bird	158.8	1.0	14.29	102.5
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}**
Screening step	Small omnivorous bird	64.8	0.53	3.09	38.2

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CPD 300 SL

Intended use	**Winter wheat**				
Active substance/product	Clopyralid				
Application rate (g/ha)	120 g a.s/ha				
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**
Screening step	Small omnivorous bird	158.8	1.0	19.06	76.9
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}**
Screening step	Small omnivorous bird	64.8	1.0	4.12	28.6

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CPD 300 SL

Intended use	**Sugar beets**				
Active substance/product	Clopyralid				
Application rate (g/ha)	120 g a.s/ha				
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**
Screening step	Small omnivorous bird	158.8	1.0	19.06	76.9
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}**
Screening step	Small omnivorous bird	64.8	1.0	4.12	28.6

Table 9.2-6: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CPD 300 SL

Intended use	**Sugar beets**				
Active substance/product	Clopyralid				
Application rate (g/ha)	3x60 g a.s/ha				
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**
Screening step	Small omnivorous bird	158.8	1.7	16.20	90.4
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}**
Screening step	Small omnivorous bird	64.8	2.1	4.33	27.3

zRMS comment:

The risk assessment at first-tier performed according **to** Document on Risk Assessment for Birds and Mammals EFSA (EFSA Journal 2009; 7(12): 1438 was accepted.
Safe uses 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 CHR/H /CPD 300 SL no unacceptable risk for birds is expected from acute or long-term exposure.

9.2.2.2 Higher-tier risk assessment

Since for clopyralid acute TER is above 10 and TER long term is above 5, no higher-tier risk assessment is required.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since CHR/H/CPD 300SL is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not

have to be considered.

Puddle scenario

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

With a K(f)oc of 1.41mL/g, Clopyralid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on sugar beet which is covered all other intended uses from GAP.

Effective application rate (g/ha)	=	180		
Acute toxicity (mg/kg bw)	=	1465	quotient =	0.123
Reprod. toxicity (mg/kg bw/d)	=	118	quotient =	1.52

Hazard quotient for Puddle scenario for Clopyralid are below trigger value 50, so no specific calculations of exposure and TER are necessary.

zRMS comment:

We agree that since 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.

9.2.2.4 Effects of secondary poisoning

The log Pow of Clopyralid is below 3 and thus do not exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment:

The risk assessment for earthworm-eating birds via secondary poisoning is not required as active substance has log P_{ow}<3.
The risk assessment for fish-eating birds via secondary poisoning is not required as active substance has log P_{ow}<3.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

In conclusion, the acute, short term risk and long term to birds from the proposed uses of clopyralid was found acceptable.

zRMS comment:

The acute and chronic risks of CHR/H /CPD 300 SL 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 CHR/H /CPD 300 SL does not pose an unacceptable risk to birds following applications according to recommended use pattern.
Evaluation 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.

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. Full details of these studies are provided in the respective EU RAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report.

However, the provision of further data on the CHR/H/CPD 300 SL is not considered essential, because the selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Rat	Clopyralid	Acute	LD_{50} > 5000 mg/kg bw per day	EFSA Journal 2018;16(7):5389
Rabbit	Clopyralid	Long-term Developmental study	LOAEL=50	EFSA Journal 2018;16(7):5389
Rat	Clopyralid	Long-term Developmental study	NOAEL=75	EFSA Journal 2018;16(7):5389
Rat	Clopyralid	Long-term 2-gen study	NOAEL=275	EFSA Journal 2018;16(7):5389
Rat	Clopyralid	Long-term 2-year study	NOAEL=50	EFSA Journal 2018;16(7):5389

9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on the sugar beets crop.

Birds and mammals risk assessment were perfomed using worst case scenario application in sugar beets 3 x 60 g a.s./ha per season, sugar beets 120g a.s./ha, winter oilseed rape 120g a.s./ha, winter oilseed rape 90g a.s/ha, winter wheat 120g/ha which are the risk envelope for other uses of CHR/H/CPD 300 SL according to the GAP presented in table 9.1.-1.

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

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

Table 9.3-1: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CPD 300 SL

Intended use		**Winter oilseed rape(spring)**			
Active substance/product		Clopyralid			
Application rate (g/ha)		120			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	118.4	1.0	14.21	351.9
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}**
Screening step	Small herbivorous mammal	48.3	0.53	3.07	16.28

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

Table 9.3 -2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CPD 300 SL

Intended use		**Winter oilseed rape**			
Active substance/product		Clopyralid			
Application rate (g/ha)		90 g a.s/ha			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small Small herbivorous mammal	118.4	1.0	10.66	469.2
Reprod. toxicity (mg/kg bw/d)		50			
TER criterion		5			
Crop scenario	**Indicator/generic focal species**	**SV_m**	**MAF_m ×**	**DDD_m**	**TER_{lt}**

Growth stage			**TWA**	**(mg/kg bw/d)**	
Screening step	Small herbivorous mammal	48.3	0.53	2.30	21.70

Table 9.33 -3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/ECPD 300 SL

Intended use		**Winter wheat**			
Active substance/product		Clopyralid			
Application rate (g/ha)		120 g a.s/ha			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario Growth stage	Indicator/generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg/kg bw/d)	TER_a
Screening step	Small herbivorous mammal	118.4	1.0	14.21	351.9
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}
Screening step	Small herbivorous mammal	48.3	1.0×0.53	3.07	16.28

Table 9.33 -4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CPD SL

Intended use		**Sugar beets**			
Active substance/product		Clopyralid			
Application rate (g/ha)		120 g a.s/ha			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	118.4	1.0	14.21	351.9
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}**
Screening step	Small omnivorous bird	48.3	1.0×0.53	3.07	16.28

Table 9.33 -5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CPD SL

Intended use	**Sugar beets**
Active substance/product	Clopyralid

Application rate (g/ha)	3x60 g a.s/ha				
Acute toxicity (mg/kg bw)	>5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	118.4	1.7	12.08	414.0
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}**
Screening step	Small herbivorous mammal	48.3	2.1×0.53	3.23	15.50

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

zRMS comment:

The risk assessment at first-tier performed according to Document on Risk Assessment for Birds and Mammals EFSA (EFSA Journal 2009; 7(12): 1438 was accepted.
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 for CHR/H /CPD 300 SL no unacceptable risk for mammals is expected from acute or long-term exposure.
Based on the intended use on for CHR/H/ECPD 300 SL no unacceptable risk for mammals is expected.

9.3.2.2 Higher-tier risk assessment

No further risk refinement is required.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc of ~~118~~, 1.41mL/g Clopyralid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on sugar beet which covered all other intended uses from GAP. Sugar beet is covered all other intended uses from GAP table

Sugar beet 3x60g /ha				
Effective application rate (g/ha)	=	180		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.036

Reprod. toxicity (mg/kg bw/d)	=	50	quotient	=	3.6

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of clopyralid is below 3 and thus do not exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment:

The risk assessment for earthworm - eating mammals via secondary poisoning is not required as active substance has log P_{ow}<3.
The risk assessment for fish - eating mammals via secondary poisoning is not required as active substance has log P_{ow}<3.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

In conclusion, the acute, short term risk and long term to mammals from the proposed uses of clopyralid was found acceptable.

zRMS comment:

The acute and chronic risks to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active substance, and maximum residues occurring on food items. For active substance all TER values exceed the relevant triggers indicating that CHR/H/ECPD 300 SL does not pose an unacceptable risk to mammals following applications according to recommended use pattern. Evaluation 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.

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

N/A

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. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on aquatic organisms of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment of clopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results mg/L	Reference
Oncorhynchus mykiss	Clopyralid	96h	LC50>99.9 mg a.s./L	EFSA Journal 2018;16(7):5389
Pimephales promelas	Clopyralid	ELS	NOEC= 10.8 mg as/L	EFSA Journal 2018;16(7):5389
Daphnia magna	Clopyralid	48h	EC50>99.0 mg as/L	EFSA Journal 2018;16(7):5389
Daphnia magna	Clopyralid	21d	NOEC=17.0 mg as/L	EFSA Journal 2018;16(7):5389
Chironomus riparius	Clopyralid	28d (static)	NOEC= 50 mg a.s/L	EFSA Journal 2018;16(7):5389
Selenastrum capricornutum	Clopyralid	72h	ErC50= 30mg a.s/L	EFSA Journal 2018;16(7):5389
Myriophyllum spicatum	Clopyralid	14 day	ErC50 > 3.0 mg a.s./L	EFSA Journal 2018;16(7):5389
Higher-tier studies (micro- or mesocosm studies)				
No further tests submitted				

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/H/CPD 300 SL

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	CHR/H/CPD 300 SL	96h	LC50=55.104 mg [formulation]/L	A.Evans, Study code: 3200612, 2014
Daphnia magna	CHR/H/CPD 300 SL	48h	EC_{50} =104.47 mg [formulation]/L	A.Evans, Study code: 3200608, 2014
Pseudokirchneriella subcapitata	CHR/H/CPD 300 SL	72h	EyC50 = 29.45 mg [formulation]/L ErC50 = 95.67 mg[formulation]/L	A.Evans, Study code: 3200609, 2014

Species	Substance	Exposure System	Results	Reference
Anabaena flos-aquae	CHR/H/CPD 300 SL	72h	ErC50>382.67 mg [formulation]/L EyC50=371.19 mg [formulation]/L	A.Evans, Study code: 3200610, 2014
Lemna gibba	CHR/H/CPD 300 SL	7 d	ErC50>382.67 mg/L EyC50>382.67 mg/L	A.Evans, Study code: 3200611, 2014
Myriophyllum spicatum	CHR/H/CPD 300 SL	14 d	ErC50 = 34.095 mg/L EyC50 = 12.711 mg/L	A. Woźniak, Study code: 0038/0030/E, 2021
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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

The endpoint ErC50 (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 in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/CPD 300 SL

Group		**Fish acute**	**Fish pro-longed**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Test species		***Oncorhyn-chus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Selenastrum capricornu-tum***	***Chirono-mus riparius***	***Myriophyl-lum spicatum***
End-point (µg/L)		LC_{50} 99 900	NOEC 10 800	EC_{50} 99 000	NOEC 17 000	ErC50 30 000	NOEC 50 000	ErC50 3000
AF		100	10	100	10	10	10	10
RAC (µg/L)		999	1080	990	1700	3000	5000	300
Expo-sure	**PEC $_{gl-max}$(µg/L)**	**Sugar beets 3x60 g/ha**						
Step 1	61.54	0.0616	0.05698	0.06216	0.0362	0.020513	0.012308	0.20513
Step 2								
	2.71	0.0027	0.0025	0.002737	0.00159	0.000903	0.000542	0.00903
Step 3	-	-	-	-	-	-	-	-
	Sugar beets 120 g/ha							
Step 1	41.03	0.041	0.038	0.0414	0.024	0.0137	0.00821	0.1368
Step 2								
	2.72	0.0027	0.0025	0.0027	0.0016	0.00091	0.00054	0.0091
Step 3	-	-	-	-	-	-	-	-
Winter oilseed rape (spring)								
Step 1	41.03	0.041	0.038	0.0414	0.024	0.0137	0.00821	0.1368
Step 2								
	2.45	0.00245	0.00227	0.00247	0.00144	0.00082	0.00049	0.0082
Step 3	-	-	-	-	-	-	-	-
Winter oilseed rape (autumn)								
Step 1	30.77	0.03080	0.02849	0.03108	0.01810	0.01026	0.00615	0.1026
Step 2								
	1.83	0.00183	0.00169	0.00185	0.00108	0.00061	0.00037	0.0061
Step 3	-	-	-	-	-	-	-	-
Winter wheat								
Step 1	41.03	0.041	0.038	0.0414	0.024	0.0137	0.00821	0.1368

Group		Fish acute	Fish pro-longed	Inver-teb. acute	Inverteb. pro-longed	Algae	Sed. dwell. prolonged	Aquatic plants
Step 2								
	2.72	0.0027	0.0025	0.0027	0.0016	0.00091	0.00054	0.0091
Step 3	-	-	-	-	-	-	-	-

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

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) CHR/H/CPD SL 300 for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CPD 300SL in sugar beets 1x459.2g/ha

Intended use	Sugar beets
Formulation	CHR/H/CPD 300 SL
Application rate (g[prod]/ha)	1 X 459.2
Entry into surface water via spray drift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	2.4392
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PEC/RAC ratio** **Oncorhynchus mykiss =EC50 55104µg/L** **RAC=551.04 (AF=100)**
1	0.004426
Buffer zone (m)	**PEC/RAC ratio** **Daphnia magna =EC50 104 470 µg/L** **RAC=1044.7 (AF=100)**
1	0.00233
Buffer zone (m)	**PEC/RAC ratio** **Pseudokirchneriella subcapitata = EyC50 29 450 µg/L** **RAC=2945 (AF=10)**
1	0.00083
Buffer zone (m)	**PEC/RAC ratio** **Anabaena flos-aque =ErC50 382670µg/L** **RAC=38267 (AF=10)**
1	0.00006

Buffer zone (m)	**PEC/RAC ratio** **Lemna Gibba =ErC50 382670µg/L** **RAC= 382670 (AF=10)**
1	0.00006
Buffer zone (m)	**PEC/RAC ratio** **Myriophyllum spicatum =EyC50 12 711 µg/L** **RAC= 1 271.1 (AF=10)**
1	0.00192

Table 9.5-3:**Aquatic organisms: acceptability of risk (PEC/RAC < 1) CHR/H/CPD SL 300 for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CPD 300 SL in sugar beets 3x229.6g/ha**

Intended use **Formulation** **Application rate (g[prod]/ha)**	Sugar beets CHR/H/CPD 300 SL 1 X 229.6
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	6.4246
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Oncorhynchus mykiss =EC50 55104µg/L** **RAC=551.04 (AF=100)**
1	0.01166
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC50 104 470 µg/L** **RAC=1044.7 (AF=100)**
1	0.00615
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchneriella subcapitata = EyC50 29 450 µg/L** **RAC=2945 (AF=10)**
1	0.00218
Buffer zone (m)	**RAC/PEC ratio** **Anabaena flos-aque =ErC50 382670µg/L** **RAC=38267 (AF=10)**
1	0.000168
Buffer zone (m)	**RAC/PEC ratio** **Lemna Gibba =ErC50 382670µg/L** **RAC= 38267 (AF=10)**

1	0.000168
Buffer zone (m)	**PEC/RAC ratio** **Myriophyllum spicatum =EyC50 12 711 µg/L*** **RAC= 1 271.1 (AF=10)**
1	0.00505

Table 9.5-6: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) CHR/H/CPD SL 300 for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CPD 300 SL in winter oilseed rape 1x459.2g/ha**

Intended use	Winter oilseed rape (spring)
Formulation	CHR/H/CPD 300 SL
Application rate (g[prod]/ha)	459.2
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	2.9502
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Oncorhynchus mykiss =EC50 55104µg/L** **RAC=551.04 (AF=100)**
1	0.00535
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC50 104 470 µg/L** **RAC=1044.7 (AF=100)**
1	0.0028240
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchneriella subcapitata = EyC50 29 450 µg/L** **RAC=2945 (AF=10)**
1	0.00100
Buffer zone (m)	**RAC/PEC ratio** **Anabaena flos-aque =ErC50 382670µg/L** **RAC=38267 (AF=10)**
1	0.000077
Buffer zone (m)	**RAC/PEC ratio** **Lemna Gibba =ErC50 382670µg/L** **RAC= 382670 (AF=10)**
1	0.000077
Buffer zone (m)	**PEC/RAC ratio** **Myriophyllum spicatum =EyC50 12 711 µg/L** **RAC= 1 271.1 (AF=10)**
1	0.00232

Table 9.5-7: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) CHR/H/CPD SL 300 for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CPD 300 SL in winter oilseed rape 1x344.4g/ha**

Intended use	Winter oilseed rape (autumn)
Formulation	CHR/H/CPD 300 SL
Application rate (g[prod]/ha)	344.4
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	2.2126
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Oncorhynchus mykiss =EC50 55104µg/L** **RAC=551.04 (AF=100)**
1	0.004015
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC50 104 470 µg/L** **RAC=1044.7 (AF=100)**
1	0.0021179
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchneriella subcapitata = EyC50 29 450 µg/L** **RAC=2945 (AF=10)**
1	0.00075
Buffer zone (m)	**RAC/PEC ratio** **Anabaena flos-aque =ErC50 382670µg/L** **RAC=38267 (AF=10)**
1	0.000058
Buffer zone (m)	**RAC/PEC ratio** **Lemna Gibba =ErC50 382670µg/L** **RAC= 382670 (AF=10)**
1	0.000058
Buffer zone (m)	**PEC/RAC ratio** **Myriophyllum spicatum =EyC50 12 711 µg/L** **RAC= 1 271.1 (AF=10)**
1	0.00174

Table 9.5-8: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) CHR/H/CPD SL 300 for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CPD 300 SL in winter wheat 1x459.2g/ha**

Intended use	Winter wheat
Formulation	CHR/H/CPD 300 SL
Application rate (g[prod]/ha)	459.2
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	2.9502
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Oncorhynchus mykiss =EC50 55104µg/L** **RAC=551.04 (AF=100)**
1	0.005354
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC50 104 470 µg/L** **RAC=1044.7 (AF=100)**
1	0.0000237
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchneriella subcapitata = EyC50 29 450 µg/L** **RAC=2945 (AF=10)**
1	0.00001
Buffer zone (m)	**RAC/PEC ratio** **Anabaena flos-aque =ErC50 382670µg/L** **RAC=38267 (AF=10)**
1	0.000001
Buffer zone (m)	**RAC/PEC ratio** **Lemna Gibba =ErC50 382670µg/L** **RAC= 382670 (AF=10)**
1	0.000001
Buffer zone (m)	**PEC/RAC ratio** **Myriophyllum spicatum =EyC50 12 711 µg/L** **RAC= 1 271.1 (AF=10)**
1	0.00232

9.5.3 Overall conclusions

Based on the predicted rates of CHR/H/CPD 300 SL in aquatic system, the TER values describing the risk for aquatic organisms following exposure to CHR/H/CPD 300 SL according to the GAP of the formulation CHR/H/CPD 300 SL achieve the acceptability criteria without applying buffer zone.

zRMS comment:

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 CHR/H/CPD 300 SL based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organism without applying buffer zone.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

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

Effects on bees of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment of clopyralid. Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration, and were presented in the core dossier. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Clopyralid	Acute; oral	LD_{50} > 100 µg a.s/bee	EFSA Journal 2018;16(7):5389
Apis mellifera	Clopyralid	Acute; Contact	LD_{50} >98.1 µg a.s/bee	EFSA Journal 2018;16(7):5389
Apis mellifera	Clopyralid	Chronic; oral	LDD50>71.2 µg a.s/bee/day	EFSA Journal 2018;16(7):5389
Apis mellifera	Clopyralid	Chronic; oral	LD10=12.5 µg a.s./larva	EFSA Journal 2018;16(7):5389
Apis mellifera	CHR/H/CPD 300 SL	Oral	LD_{50} > 100 µg [product]/bee	Grzebisz E, Study code: 3200616, 2014
Apis mellifera	CHR/H/CPD 300 SL	Contact	LD50 > 100 µg [product]/bee	Grzebisz E, Study code: 3200616, 2014

Species	Substance	Exposure System	Results	Reference
Apis mellifera	CHR/H/CPD 300 SL	Larval	EC_{50}/ 22d > 100 µg prod/larva NOEC ≥ 100 µg prod/larva	Grzesica M., Study code: EMI/4/15/2021, 2021
Apis mellifera	CHR/H/CPD 300 SL	Chronic Oral	LC_{50}/10d > 3333.3 mg t.i./kg diet LDD_{50}/10d ≥ 100 µg/bee/day NOEC > 100 µg/bee/day NOEDD ≥ 3333.3 mg t.i./kg diet	E. Myrczek, Sudy code: EMI/4/16/2021, 2021
Higher-tier studies (tunnel test, field studies)				
Not required				

9.6.2 Risk assessment

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

Risk assessment have been provided for worst case situation (sugar beets, 1x 120 g/ha), which covered a risk envelopment for all uses of GAP table.

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of CHR/H/CPD 300 SL in all intended uses (risk envelope)

Intended use **Active substance** **Application rate (g/ha)**	Sugar beets Clopyralid 1 x 120		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	120	1.2
Contact toxicity	98.1		1.223
Product **Application rate (g/ha)**	CHR/H/CPD 300 SL 1 x 459.2		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	459.2	4.592
Contact toxicity	100		4.592

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

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

Not relevant.

9.6.3 Effects on bumble bees

Not available

9.6.4 Effects on solitary bees

Not available

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that CHR/H/CPD 300 SL applied at the maximum use rate in cereals winter poses low risk to bees.

zRMS comment:

The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of CHR/H/CPD 300 SL. 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.

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. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on non-target arthropods of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment of clopyralid. Toxicity data for formulation CHR/H/CPD on T.pyri and Aphidius were evaluated during first registration, and were presented in the core dossier.

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	CHR/H/CPD 300 SL	Tier II, 2D	LR_{50} >460 mL/ha, which is equal to ~~528.08~~ 534 g /ha	E. Kulec-Płoszczyca, Study code: B/184/16, 2016
Aphidius	CHR/H/CPD 300 SL	Tier II, 2D	LR_{50} > 540 mL/ha,	Grzebisz E, Study

Species	Substance	Exposure System	Results	Reference
rhopalosiphi (adults)			which is equal to 619.92 g prod. /ha	code: 3200618; 2015
Coccinella septempunctata	CHR/H/CPD 300 SL	Tier II, 2D	LR50 >460 mL/ha 138 g a.s./ha which is equal to 528.08	Moll M., Study code: 125681012, 2017
Chrysoperla carnea	CHR/H/CPD 300 SL	TIER II, 2D	LR50 >460 mL/ha 138 g a.s./ha which is equal to 528.08	Moll M., Study code: 125681047, 2017
Field or semi-field tests				
Not required				

9.7.2 Risk assessment

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

Risk assessment have been provided for worst case situation (sugar beets, 1x 120 g prod/ha, sugar beets 3x 60 g prod/ha), which covered a risk envelopment for all uses of GAP table.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CHR/H/CPD 300 SL in sugar beets 1 × 459.2

Intended use	Sugar beets		
Active substance/product	CHR/H/CPD 300 SL		
Application rate (g/ha)	1 × 459.2		
MAF	1		
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	~~> 528.08~~ 534	459.2	~~< 0.8695~~ 0.85993
Aphidius rhopalosiphi	> 619.92		< 0.7407
Coccinella septempunctata	> 528.08		< 0.8695
Chrysoperla carnea	> 528.08		< 0.8695

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

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CHR/H/CPD 300 SL in sugar beets 3×229.6g

Intended use	Sugar beets		
Active substance/product	CHR/H/CPD 300 SL		
Application rate (g/ha)	3×229.6g		
MAF	2.3		
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	> ~~528.08~~ 534g	528.08	<~~1~~ 0.9889
Aphidius rhopalosiphi	>619.92		<0.85185
Coccinella septempunctata	> 528.08		<1
Chrysoperla carnea	>528.08		<1

zRMS comment:

zRMS agrees with the Applicant's assessment with the in-field risk to non-target arthropods from the proposed use of CHR/H/CPD 300 SL, above. A low risk is demonstrated to the 2 standard first tier and additional test species.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CHR/H/CPD 300 SL in sugar beets

Intended use	Sugar beets				
Active substance/product	CHR/H/CPD 300 SL				
Application rate (g/ha)	1 ×459.2				
MAF	1.0				
VDF	10 for 2D study and 1 for 3D				
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	> ~~528.08~~ 534	2.77	1.27	10	~~0.01382~~ 0.002381
Aphidius rhopalosiphi	>619.92		1.27		0.1024
Coccinella septempunctata	>528.08		1.27		0.01382
Chrysoperla carnea	>528.08		1.27		0.01382

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

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

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CHR/H/CPD 300 SL in sugar beets

Intended use	Sugar beets				
Active substance/product	CHR/H/CPD 300 SL				
Application rate (g/ha)	3 × 229.6g				
MAF	2.3				
VDF	10				
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	> 534	2.01	11.109	10	~~0.0105~~ 0,06554
Aphidius rhopalosiphi	>619.92		11.09		0.089
Coccinella septempunctata	>528.08		11.109		0.0105
Chrysoperla carnea	>528.08		11.109		0.0105

zRMS comment:

zRMS agrees with the Applicant's assessment with the of-field risk to non-target arthropods from the proposed use of CHR/H/CPD 300 SL above, however additionale the VDF is set to 5 in the Central zone instead of 10.

Intended use	**Sugar beets**				
Active substance/product	**CHR/H/CPD 300 SL**				
Application rate (g/ha)	**3 × 229.6g**				
MAF	**2.3**				
VDF	**5 for 2D study and 1 for 3D**				
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	> 534	2.01	11.109	5	0.02077
Aphidius rhopalosiphi	>619.92		11.109		0.01792
Coccinella septempunctata	>528.08		11.109		0.02104
Chrysoperla carnea	>528.08		11.109		0.02104

Intended use	**Sugar beets**				
Active substance/product	**CHR/H/CPD 300 SL**				
Application rate (g/ha)	**1 ×459.2**				
MAF	**1.0**				
VDF	**5 for 2D study and 1 for 3D**				
Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**

Typhlodromus pyri	> 534	2.77	12.72	5	0,02382
Aphidius rhopalosiphi	>619.92		12.72		0.02052
Coccinella septempunctata	>528.08		12.72		0,02409
Chrysoperla carnea	>528.08		12.72		0,02409

A low risk is demonstrated to the 2 standard first tier and additional test species.
All hazard quotients (HQ) are less than 1, indicating that CHR/H/CPD 300 SL applied at the maximum use rate in sugar beets poses no risk to non-target arthropods. No risk mitigation needed.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

RMS comment:

All hazard quotients (HQ) are less than 1, indicating that CHR/H/CPD 300 SL applied at the maximum use rate in sugar beets poses no risk to non-target arthropods. The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002),and in consideration of the recommendations of the guidance document ESCORT 2**.** The calculations of the risk assessment for in – field for two indicator species Typhlodromus pyri and Aphidius rhopalosiphi based on laboratory studies were accepted by zRMS as HQ values were below 2 for these species. In addition, based on the results from extended laboratory tests for Chrysoperla carnea and Aphidius rhopalosiphi the $PER_{in\text{-}field}$ of Cloe 300 SL is considered to be acceptable ~~as $PER_{in\text{-}field}$ was below rate with ≤50 % effect.~~
Finally, the risk in - field for NTA is considered acceptable.

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

9.8.1 Toxicity data

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment of clopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Eisenia foetida	Clopyralid	Overspray / 10% OM; chronic	NOEC = 1.97 mg a.s/kg dry soil	EFSA Journal 2018;16(7):5389
Eisenia fetida Sav.	CHR/H/CPD 300 SL	Acute (7, 14 d)	LD50>1000mg /kg dry soil	Evans A., Study code: 3200613 3200613, 2014
Eisenia andrei	CHR/H/CPD 300 SL	56 d	NOEC=125mg test item/ kg	Straube D., Study code:125681022
Folsomia candida	CHR/H/CPD 300 SL	Chronic 28 d	NOEC≥1000mg test item/ kg	Straube D., Study code: 125681016, 2017
Hypoaspis aculeifer	CHR/H/CPD 300 SL	Chronic 14 d	NOEC≥1000mg test item/kg	Straube D., Study code: 125681089, 2017
Field studies				
Not required				
Litter bag test				
Not required				

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

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", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

Risk assessment have been provided for worst case situation (sugar beets, 3x60 g/ha), which covered a risk envelopment for all uses of GAP table.

9.8.2.1 First-tier risk assessment

The relevant PEC soil for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for clopyralid.

Table 9.8-1: 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 CHR/H/CPD 300SL in sugar beets 3x60g/ha

Intended use	Sugar beets		
Acute effects on earthworms			
Product/active sub-stance/metabolite	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**

Clopyralid	**Not required**		
CHR/H/CPD 300 SL			
Chronic effects on earthworms			
Product/active sub-stance/metabolite	**NOEC (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Clopyralid	1.97	0.1628	12.1
CHR/H/CPD 300 SL	125	0.735	170.07
Chronic effects on other soil macro- and mesofauna *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Clopyralid	No data available	-	-
CHR/H/CPD 300 SL	1000	0.735	1360.54
Chronic effects on other soil macro- and mesofauna *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Clopyralid	No data available	-	-
CHR/H/CPD 300 SL	1000	0.735	1360.54

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
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 and long term risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for CHR/H/CPD 300 SL in a first-tier risk assessment.

zRMS comment:

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). The TER_{LT} values for active substance and for product are above trigger value of 5, indicating an acceptable risk for earthworm and soil macro-organism for proposed use of the product CHR/H /CPD 300 SL.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with clopyralid. Full details of these studies are provided in the respective EU RAR and related documents.
Effects on soil microorganisms of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment

of clopyralid. Toxicity data for formulation CHR/H/CPD on soil microorganisms were evaluated during first registration, and were presented in the core dossier.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Clopyralid	56d	< 25 % deviation in nitrate formation to the control	EFSA Journal 2018;16(7):5389
N-mineralisation	CHR/H/CPD 300 SL	56 days	CHR/H/CPD had no significant effect on soil micro-organisms at 1.0605 mg [a.s.]/kg.	Soil Nitrogen Transformation Test; E. Grzebisz; study No: 3200615
C-mineralisation	Clopyralid	28d	<25 % deviation to the control	EFSA Journal 2018;16(7):5389

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CHR/H/CPD 300 SL in sugar beets 120g- single application

Intended use	Sugar beets		
N-mineralisation			
Product/active substance	Max. conc. With effects≤25% (mg/kg dw)	PEC_{soil}(mg/kg dw)	Risk acceptable?
Clopyralid	209 mg a.s./kg dw soil	0.1280 mg a.s./kg	YES
CHR/H/CPD 300SL	CHR/H/CPD had no significant effect on soil micro-organisms at 1.0605 mg [a.s.]/kg.	0.4898 mg product/kg	YES
C-mineralisation			
Not required			

Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of CHR/H/CPD 300 SL in sugar beets 3x60g- multiple application

Intended use	Sugar beets		
N-mineralisation			
Product/active substance	Max. conc. With effects≤25% (mg/kg dw)	PEC_{soil}(mg/kg dw)	Risk acceptable?
Clopyralid	209 mg a.s./kg dw soil	0.1628mg a.s./kg	YES
CHR/H/CPD 300SL	CHR/H/CPD had no significant effect on soil micro-organisms at 1.0605 mg [a.s.]/kg.	0.735mg product/kg	YES
C-mineralisation			
Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/H/CPD 300 SL and its active substance Clopyralid in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 56 days or more of exposure, indicating that the proposed use of CHR/H/CPD 300 SL poses an acceptable risk to soil microorganisms.

zRMS comments:

The risk assessment for soil micro-organism after exposure of CHR/H /CPD 300 SL has been accepted by the 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 CHR/H /CPD 300 SL. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of CHR/H /CPD 300 SL**.**

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with clopyralid. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on non-target terrestrial plants of CHR/H/CPD 300 SL were not evaluated as part of the EU assessment of clopyralid. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Sunflower Helianthus annuus	CHR/H/CPD 300 SL	21 d Seedling emergence	ER50= 305.0 g prod/ha	CLOPYRALID 300SL (CHR/H/CPD) Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test ;P. Pieczka; study code: G/205/18
Cabbage Brassica oleracea var.capitata	CHR/H/CPD 300 SL	21 d Seedling emergence	ER50 > 695.5 g prod/ha	
Pea Pisum sativum	CHR/H/CPD 300 SL	21 d Seedling emergence	ER50= 67.2 g prod /ha	
Carrot Daucus carota	CHR/H/CPD 300 SL	21 d Seedling emergence	**ER50= 10.6 g prod/ha**	
Perennial ryegrass	CHR/H/CPD 300 SL	21 d Seedling emergence	ER50 > 695.5 g prod/ha	
Oats Avena sativa	CHR/H/CPD 300 SL	21 d Seedling emergence	ER50= 695.5 g prod/ha	
Sunflower Helianthus annuus	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER50=120.7 g prod/ha	CLOPYRALID 300SL (CHR/H/CPD) Terrestrial Plant Test: Vegetative Vigour Test; P. Pieczka; Study code: G/204/18
Cabbage Brassica oleracea var.capitata	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER50 > 695.5 g prod/ha	
Pea Pisum sativum	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER50 =25.0 g prod/ha	
Carrot Daucus carota	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER50 = 369.7 g prod/ha	
Perennial ryegrass	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER50 > 695.5 g prod/ha	
Oats Avena sativa	CHR/H/CPD 300 SL	21 d Vegetative vigour	ER 50> 695.5 g prod/ha	

m: monocotyledonous; d: dicotyledonous

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Risk assessment have been provided for worst case situation (sugar beets, 3x 229.6 g/ha), which covered a risk envelopment for all uses of GAP table.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CHR/H/CPD 300 SL in sugar beets

Intended use	Sugar beets
Active substance/product	CHR/H/CPD 300 SL
Application rate (g/ha)	3 x 229.6
MAF	2.3

Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Sunflower Helianthus annuus	305.0	0.0201	10.61	28.74
Cabbage Brassica oleracea var.capitata	695.5	0.0201	10.61	65.55
Pea Pisum sativum	67.2	0.0201	10.61	6.33
Carrot Daucus carota	**10.6**	0.0201	10.61	**0.99**
Perennial ryegrass	695.5	0.0201	10.61	65.55
Oats Avena sativa	695.5	0.0201	10.61	65.55
Sunflower Helianthus annuus	120.7	0.0201	10.61	11.37
Cabbage Brassica oleracea var.capitata	695.5	0.0201	10.61	65.55
Pea Pisum sativum	25.0	0.0201	10.61	**2.35**
Carrot Daucus carota	369.7	0.0201	10.61	34.84
Perennial ryegrass	695.5	0.0201	10.61	65.55
Oats Avena sativa	695.5	0.0201	10.61	65.55

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of CHR/H/CPD 300 SL in sugar beets considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Sugar beets			
Active substance/product		CHR/H/CPD 300 SL			
Application rate (g/ha)		3x 229.6			
MAF		2.3			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.01	10.61	5.305	2.65	1.06
5	0.41	2.165	1.08	0.54	0.21
10	0.20	1.056	0.53	0.26	0.106
Toxicity value		**TER**			
ER_{50} = 10.6 g/ha		**criterion: TER ≥ 5**			
1		**0.99**	**1.99**	**4.0**	10
5		**4.9**	9.81	19.62	50.47
10		10.03	20.0	40.77	100.0

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

The data from following studies were added by zRMS after commenting

Study 1

Clopyralid 300 SL (CHR/H/CPD)

Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test

Table 1: Visual injury as % of the untreated control (0 = no injury)

Treatment (g formulation/ha)	**Sunflower**	**Cabbage**	**Pea**	**Carrot**	**Perennial**	**Oats**
0.4	0	0	0	0	0	0
0.8	0	0	0	0	0	0
2.7	0	0	0	0	0	0
8.5	47.1	0	0	5	0	0
25.9	**54.3**	0	0	10	0	0
77.3	**77.9**	0	**60**	43.8	0	0
231.8	**95.7**	0	**100**	**100**	0	0
695.5	**100**	0	**	**	0	0
EC_{50} visual effect, estimated by RMS (g formulation/ha)	12.19*	> 695.5	> 25.9	> 77.3	>695.5	>695.5

* In this case, the EC_{50} value for sunflower was calculated by RMS using CurveExpert Professional 2.6.5. program. The EC_{50} is 12.19 g formulation/ha based on Weibull Model (Correlation Coeff. (r) = 0.98; standard error = 8.09).

** lack of plants

Note: In bold, the visual effects - more than 50% affected plants

Comments RMS: Based on visual EC50 the most sensitive species is *Sunflower* with EC50 = 12.19 g formulation/ha. Therefore, the risk assessment based on plant dry weight – EC_{50} = 10.6 g formulation/ha covers the visual effect from exposure of formulation Clopyralid 300 SL to non-target plants.

Study 2
Clopyralid 300 SL (CHR/H/CPD)
Terrestrial Plant Test: Vegetative Vigour Test

Table 2: Visual injury as % of the untreated control (0 = no injury)

Treatment (g formulation/ha)	Sunflower	Cabbage	Pea	Carrot	Perennial	Oats
0.4	0	0	0	0	0	0
0.8	0	0	0	0	0	0
2.7	30	0	0	5	0	0
8.5	45	0	15	15	0	0
25.9	50	0	20	30	0	0
77.3	**55**	10	**75**	50	0	0
231.8	**91.4**	25	**85**	**85**	0	0
695.5	**100**	35	**100**	**87.5**	0	0
EC_{50} visual effect, estimated by RMS (g formulation/ha)	25.9	> 695.5	> 25.9	77.3	> 695.5	> 695.5

Note: In bold, the visual effects - more than 50% affected plants

Comments RMS: Based on visual EC50 the most sensitive species is *Sunflower* with EC50 = 25.9 g formulation/ha. Therefore, the risk assessment based on plant dry weight – EC_{50} = 25.0 g formulation/ha for Pea covers the visual effect from exposure of formulation Clopyralid 300 SL to non-target plants.

General comment: The lowest toxicity endpoint for sunflower based on plant dry weight – EC_{50} = 10.6 g formulation/ha (seedling emergence parameter) should be used for risk assessment for non-target plants.

9.10.3 Overall conclusions

Based on the predicted rates of CHR/H/CPD 300 SL in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/CPD 300 SL according to the GAP of the formulation CHR/H/CPD 300SL achieve the acceptability criteria TER ≥ 5 with applying:

- 10 m buffer zone
- 5 m and use of 90%, 75% or 50% drift reducing nozzles
- 1 m and use of 90% drift reducing nozzles

zRMS comment:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. The deterministic risk based on the ER_{50} = 10.6 g/ha value from

seedling emergence test and $PER_{off\text{-}field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: CHR/H/CPD 300SL achieve the acceptability criteria TER ≥ 5 with applying:
- 10 m buffer zone
- 5 m and use of 90%, 75% or 50% drift reducing nozzles
- 1 m and use of 90% drift reducing nozzles

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

Not relevant

9.12 Monitoring data (KCP 10.8)

Not available

9.13 Classification and Labelling

Not required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1	M.Zielińska	2021	**Clopyralid - TER Calculations for Terrestial Verterbrates** Chemirol GLP No Unpublished	N	Chemirol
KCP 10.1.2	M.Zielińska	2021	**Clopyralid - TER Calculations for Terrestial Verterbrates** Chemirol GLP No Unpublished	N	Chemirol
KCP 10.1.2	[redacted]	2014	CHR/H/CPD - an acute oral toxicity study to rats – Fixed dose method [redacted] Study Code: PO-15/14 GLP Yes Unpublished	Y	Chemirol
KCP 10.2	[redacted]	2014	CHR/H/CPD - acute toxicity to rainbow trout (oncorhynchus mykiss) [redacted] Study Code: 3200612 GLP Yes		

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.2	A.Evans	2014	CHR/H/CPD - acute toxicity to water fleas (daphnia magna) under static conditions Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200608 GLP Yes **Unpublished**	N	Chemirol
KCP 10.2	A.Evans	2014	CHR/H/CPD – 72h acute toxicity test with freshwater green alga, Pseudokirchneriella subcapitata. Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200609 GLP Yes **Unpublished**	N	Chemirol
KCP 10.2	A.Evans	2014	CHR/H/CPD- 72h acute toxicity test with freshwater green alga, Anabaena flos-aguae. Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200610 GLP Yes **Unpublished**	N	Chemirol
KCP 10.2	A.Evans	2014	CHR/H/CPD – 7day growth inhibition test with duckweed, Lemna gibba Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200611 GLP Yes **Unpublished**	N	Chemirol

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	Agnieszka Woźniak	2021	Water-sediment Myriophyllum spicatum toxicity test of the test item Major 300 SL (CHR/H/CPD 300 SL) according to guideline OECD 239 SORBOLAB Research Laboratory LLC Zaniemyska 11 Street 61-029 Poznań Study Code: 0038/0030/E GLP Yes Unpublished	N	Chemirol
KCP 10.3.1	E. Grzebisz	2014	CHR/H/CPD - acute contact and oral toxicity to honeybees Apis mellifera. Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200616 GLP Yes Unpublished	N	Chemirol
KCP 10.3.1	E. Grzebisz	2014	CHR/H/CPD - acute contact and oral toxicity to honeybees Apis mellifera. Smithers Viscient (ESG) Limited Harrogate, UK Study Code: 3200616 GLP Yes Unpublished	N	Chemirol
KCP 10.3.1	M. Grzesica	2021	Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure EMI/4/15/2021 Ecomelius Institute Sp. z o.o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	Chemirol

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1	E. Myrczek	2021	Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test EMI/4/16/2021 Ecomelius Institute Sp. z o. o. Kalinowa 2, Zaborze 43-520 Chybie, Poland GLP Unpublished	N	Chemirol
KCP 10.3.1.6	E.Kulesz-Ploszyca	2016	An extended laboratory test for evaluating the effects of CHR/H/CPD 300 SL on the predatory mite, Typhlodromus pyri (Sch.) Institute of Organic Industry, Pszczyna, Poland Study Code B/184/16 GLP Yes Unpublished Dossier Documents-Reports	N	Chemirol
KCP 10.3.1.6	E.Grzebisz	2014	Extended Acute Toxicity to Aphidius rhopalosiphi in the Laboratory Smithers Viscient Ltd., UK Study Code 3200618 GLP Yes Unpublished Dossier Documents-Reports	N	Chemirol

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.6	M. Moll	2017	CHR/H/CPD: Effects on the Ladybird Beetle Coccinella septempunc-tata under Extended Laboratory Conditions,; Ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681012 GLP No Unpublished	N	Chemirol
KCP 10.3.1.6	M. Moll	2017	CHR/H/CPD: Effects on the Lacewing Chrysoperla carnea under Extended Laboratory Conditions ,; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681047 GLP Yes Published No	N	Chemirol
KCP 10.4.1/01	D. Straube	2017	CHR/H/CPD 300SL: Effects on Reproduction and Growth of Earthworms Eisenia andrei in Artificial Soil,; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany; Study No: 125681022 GLP Yes Published No	N	Chemirol
KCP 10.4.2/01	D. Straube	2017	CHR/H/CPD 300SL: Effects on Reproduction of the Collembola Folsomia candida in Artificial Soil,; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681016 GLP Unpublished	N	Chemirol
KCP 10.4.2/02	D.Straube	2017	CHR/H/CPD 300SL: Effects on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil,; ibacon GmbH Study Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681089 GLP Yes	N	Chemirol

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
			Published No		
KCP 10.5	E. Grzebisz	2014	Soil Nitrogen Transformation Test Smithers Viscient (ESG) Ltd., UK, Study Code 3200615 GLP Yes Unpublished	N	Chemirol
KCP 10.6/01	P. Pieczka	2019	Clopyralid 300 SL (CHR/H/CPD) Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Łukasiewicz Research Network – Institute Of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/205/18 GLP Yes Published No	N	Chemirol
KCP 10.6/02	P. Pieczka	2019	Clopyralid 300 SL (CHR/H/CPD)Terrestrial Plant Test: Vegetative Vigour Test;; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/204/18 GLP Yes Published No	N	Chemirol

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 10.1/01		1980	[redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	DAS
KCP 10.1/02		1985	Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos) - Final Report. [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.1/03		1987	Lontrel T Herbicidal Chemical (Penta Process): Acute Oral Toxicity Study in Fischer 344 Rats. Report No.:K-038252-033A GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.1/04		1987	Lontrel T Herbicidal Chemical (Penta Process): Acute Dermal Toxicity Study in New Zealand White Rabbits. [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.1/05		1985	Report No A2A-052	Y	DAS
KCP 10.2/01		2000	Clopyralid: An Acute Toxicity Study with the Rainbow Trout, Oncorhynchus mykiss [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.2/02		2000	Clopyralid: Toxicity to the Early Life Stages of the Fathead Minnow, Pimephales Promelas Rafinesque. [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.2/03	Marino, T. A., McClymont, E. L.	2000	Clopyralid: An Acute Toxicity Study with the Daphnia, Daphnia magna Straus DAS report no. 001025, Ref. J52	N	DAS

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
	& Staley, J. L.		Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.2/04	Douglas, M. T., Bell, G. & Macdonald, I. A.	1992	An Assessment of the Effects of Lontrel T on the Reproduction of Daphnia magna DAS report no. DWC 615/911087, Ref. J35 Huntingdon Research Center Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2/05	Barrett, K.	2001	Clopyralid Technical Toxicity to the Sediment Dwelling Phase of the Midge Chironomus riparius Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2/06	Kirk, H. D.; Gilles, M. M.; McClymont, E. L. ; McFadden, L.G.,	2000	Clopyralid: Growth Inhibition Test with the Freshwater Green Alga, Selenastrum capricornutum Printz Dow AgroSciences LLC, Midland, Michigan, United States Raport No: 001040, Ref. J51 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2/07	Aufderheide, J.	2014	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, Navicula pelliculosa DAS Report No. 140515 ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2/08	Cowgill, U. M. ; Milazzo, D. P. ; Potter, R. B.	1990	The Fourteen Day Toxicity of Lontrel T to Lemna gibba L G-3 (Duckweed) - ES-DR-0197-3428-4 DAS Report No. ES-2243 Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes	N	DAS

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
			Published (Y/N): No		
KCP 10.2/09	Banman, C. S., Moore, S.	2015	Clopyralid: Toxicity to the Aquatic Macrophyte, Myriophyllum spicatum DAS Report No. 140735 SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2/10	Banman, C. S. & Moore, S.,	2015	Clopyralid: Toxicity to the Aquatic Macrophyte, Myriophyllum spicatum SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104 DAS report no. 140735 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.3/01	Wainwright, M.	2001a	Clopyralid Technical Acute Toxicity To Honey Bees DAS Report No. GHE T-1091 Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.3/02	Wainwright, M.	2001b	Clopyralid Technical Acute Toxicity To Honey Bees DAS Report No. GHE T-1091 Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
Kcp 10.3/03	Leonard, J. and Moore, S.	2017a	Clopyralid: A laboratory study to determine the chronic oral toxicity to the adult worker honey bee Apis mellifera L. (Hymenoptera: Apidae) 170098	N	DAS
KCP 10.3/04	Leonard, J. and Moore, S.	2017b	Clopyralid: A repeated-exposure laboratory toxicity study in larvae, pupae and emergent adults of the honey bee Apis mellifera Linnaeus. (Hymenoptera: Apidae) 170099	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3/05	Sankanu, A.	2000a	A Laboratory Study To Evaluate The Effects of Clopyralid (EF 1136, An SL Formulation Containing 100 G/L Clopyralid) on the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) DAS Report No. GHE-P-8725 Ecotox Ltd, Tavistock, Devon, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.4/01	Hayward, J. C.	2001	The Effects of EF-1136 on Reproduction and Growth in the Earthworm Eisenia fetida DAS Report No.: GHE-T-1135, Ref. J69 CEMAS Study CEMS-1637 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.5/02	Schöbinger, U.	2013	Clopyralid: Effects on the Activity of the Soil Microflora under Laboratory Conditions (Nitrogen and Carbon Transformation) DAS Report No. 130283 Eurofins Agroscience Services EcoChem GmbH Eutinger Str. 24 D-75223 Niefern-Öschelbronn Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS

List of data submitted by the applicant and not relied on

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

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
			Source GLP/non GLP/GEP/non GEP Published/Unpublished		

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No additional studies were performed.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional studies were performed.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

zRMS comment:
the study was not submitted, evaluated and used in the risk assessment.

~~Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration.~~

~~Report:~~	~~KIIIA1 10.3.2.1/01, Kupny J., 2014~~
~~Title:~~	~~CHR/H/CPD - an acute oral toxicity on rats – fixed dose method~~
~~Document No:~~	~~Institute of Organic Chemistry, Pszczyna, Poland, Study Code: PO-15/14~~
~~Guidelines:~~	~~OECD Nr 420 / Method B.1.BIS~~
~~GLP~~	~~YES~~

~~**Material and methods:**~~

- ~~CHR/H/CPD, Batch No: 22.01.2014 , production date 24.04.2014 CoA issued by the Institute of Organic Chemistry, 6 Annopol Street, 03-236 Warsaw, 2014~~
- ~~content of a.s. according to Certificate of Analysis No 130/14 issued by PESTINOVA, Jaworzno, Poland, 304.6 g/l a.s.~~
- ~~The administration amount of CHR/H/CPD was 2000 mg/kg bw.~~
- ~~A single dose of aqueous solution of test substance was administered to stomach of animals at volume 0.5 mL of solution per 100 g bw.~~
- ~~Animals – female rats Imp symbol: Wistar rat (Crl: WI(Han); outbred) obtained from a husbandry of laboratory animals of the Experimental Medicine Centre at the Medical University in Bialystok kept behind a breeding barrier~~
- ~~Time of observation – 14 day observation period~~
- ~~Necropsy – yes~~
- ~~Deviations from the study plan – No changes were introduced to the Study Plan. No deviations from the Study Plan were found..~~

~~**Table 10.3.2.1-1: Acute oral toxicity in rats of CHR/H/CPD**~~

Dose (mg/kg)	Toxicological results*	Duration of signs	Time of death	LD_{50} (mg/kg) (14 days)
female rats				
2000 mg/kg bw	0	-	-	>2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Findings:

- No clinical signs and no deaths were observed during the study.

Conclusion/endpoint:

Under the experimental conditions, the oral LD_{50} of the CHR/H/CPD is higher than 2000 mg/kg in rats.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional studies were performed.

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

No additional studies were performed.

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

zRMS comment:
The study is acceptable. The validity criteria according OECD 203 (2019) of the test were met.
$LC_{50, nom}$ (96 h) = 14.4 mg a.s./L
NOEC (96 h) = 9.7 mg a.s./L.

Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration.

Report:	KIIIA1 10.2.2.1/01, [redacted]
Title:	CHR/H/CPD – Acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions.
Document No:	[redacted]
Guidelines:	OECD, 1992. Updated 2009
GLP	Yes

Materials and methods:

- CHR/H/CPD, Batch No: 22.01.2014 production date 22.01.2014 ; CoA issued by the PESTI-

NOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s.300 ± 15%g[a.s.]/L

- trout (*Onchoryncus mykiss)* 10 fish per test:
- Details of the fish used for the definitive study taken at test termination are as follows:
- Mean wet weight = 0.9 g (range 0.5 to 1.4 g); N = 10
- Mean total length = 46 mm (range 40 to 54 mm); N = 10
- Details of the fish used for the repeat definitive study taken at test termination are as
- follows:
- Mean wet weight = 1.2 g (range 0.7 to 1.6 g); N = 10
- Mean total length = 51 mm (range 42 to 58 mm); N = 10content of a.s. under the test conditions was analized and the method validated.

Findings:

Test substance	CHR/H/CPD
Test object	Rainbow trout
Exposure	96 h, Static
96 h LC_{50} mg[a.c.]/L	14.4
96 h NOEC [a.s.]L	9.7

The validity criteria:
The following validity criteria of the definitive test were met:

- During the definitive test mortality in the negative control must not exceed one fish at termination.
- The dissolved oxygen concentration must be at least 60% of the air saturation value throughout the test.

zRMS comment:
The study is acceptable. The validity criteria according OECD 202 (2004) of the test were met.
$LC_{50,\ nom}$ (48 h) = 27.3 mg a.s./L
NOEC (48 h) = 9.4 mg a.s./L.

Report:	KIIIA1 10.2.2.1/01, A. Evans, 2014
Title:	CHR/H/CPD – Acute toxicity to Water fleas (Daphnia magna) under static conditions.
Document No:	Smithers Viscient (ESG) Limited, Study Code: 3200608
Guidelines:	OECD no. 202 (OECD, 2004)
GLP	Yes

Materials and methods:

- CHR/H/CPD, Batch No: 22.01.2014 , production date 22.01.2014 ; CoA issued by the PESTINOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s.300 ± 15%g[a.s.]/L
- first instar *Daphnia magna* (< 24 h old) in a static test system were exposed for 48 h to nominal concentration of 100 mg [a.s.]/L.

Findings:

Test substance	CHR/H/CPD
Test object	*Daphnia magna*
Exposure	48h, static
48 h EC_{50} mg [a.s.]/L	27.3
NOEC mg [a.s.]./L	9.4

The Validity criteria:
In the definitive test, the following validity criteria specified in the OECD Guideline No. 202 and the EU method C.2. were met:

- Not more than 10% of daphnids in the control should be immobilised or shown other signs of disease or stress.
- Dissolved oxygen in the control and exposure concentrations should be ≥3 mg/L at the end of the test
-

zRMS comment:
The study is acceptable. The validity criteria according OECD 201 (2006) guideline was met.
E_rC_{50} (72 h) = 25.0 mg a.s./L
E_yC_{50} (72 h) = 7.7 mg a.s./L.

Report:	KIIIA1 10.2.2.1/01, A. Evans, 2014
Title:	CHR/H/CPD – 72-hour Acute toxicity test with freshwater green alga Pseudokirchneriella subcapitata.
Document No:	Smithers Viscient (ESG) Limited, Study Code: 3200609
Guidelines:	OECD No.201
GLP	Yes

Materials and methods:

- CHR/H/CPD, Batch No: 22.01.2014 ,production date22.01.2014 ; CoA issued by the PESTINOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s.300 ± 15%g[a.s.]/L
- Algae culture of *Pseudokirchneriella subcapitata* 61.81 SAG (formerly *Selenastrum capricornutum)*
- Growth medium was prepared using prepared with sterile, reverse osmosis water with AAP medium

Findings:

Test Substance	CHR/H/CPD
Test Object	*Pseudokirchneriella subcapitata*
Exposure	72h, static
72 h E_rC_{50} (growth rate) mg (a.s.)/L	25
72 h E_yC_{50} (yield inhibition) mg (a.s.)/L	7.7
72 h NOEC mg a.s./L (growth rate)	2.5
72 h NOEC mg a.s./L (yield)	0.76

The Validity criteria:
In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 were met:

- Mean cell growth in the control must increase by >16 times after 72 hours of growth
- The mean Coefficient of Variation (CV) for section-by-section specific growth rates in the control replicates should not exceed 35%. This criterion applies to the mean value of the CV calculated for each control replicate.
- The CV for the average specific growth rate in the control for the entire test period should not exceed 7%.

zRMS comment:
The study is acceptable. The validity criteria according OECD 201 (2006) guideline was met.
E_rC_{50} (72 h) > 100 mg a.s./L
E_yC_{50} (72 h) = 97 mg a.s./L.

Report:	KIIIA1 10.2.2.1/01, A. Evans, 2014
Title:	CHR/H/CPD – 72-hour Acute toxicity test with freshwater green alga Anabaena flos-aquae.
Document No:	Smithers Viscient (ESG) Limited, Study Code: 3200610
Guidelines:	OECD No.201
GLP	Yes

Materials and methods:

- CHR/H/CPD, Batch No: CLO/19.06.12, production date19.06. 2012; CoA issued by the PESTINOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s.300 ± 15%g[a.s.]/L
- The alga used in this toxicity test was the freshwater green alga, *Anabaena flosaquae*, strain CCAP 1403/13 A. The alga was obtained from the Culture Collection of Algae and Protozoa, and was maintained in stock culture at Smithers Viscient
- culture medium used was Algal Assay Procedure (AAP) medium prepared with sterile, reverse osmosis water.

Findings:

Test Substance	CHR/H/CPD
Test Object	*Anabaena flos-aquae*
Exposure	72h, static
72 h E_rC_{50} (growth rate) mg [a.s.]/L	>100
72 h E_yC_{50} (yield inhibition) mg [a.s.]/L	97
72 h NOEC mg [a.s.]./L (growth)	32
72h NOEC mg [a.s.]./L (yield)	10

The Validity criteria:
In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 were met:

- Mean cell growth in the control must increase by >16 times after 72 hours of growth
- The mean Coefficient of Variation (CV) for section-by-section specific growth rates in the control replicates should not exceed 35%. This criterion applies to the mean value of the CV calculated for each control replicate.
- The CV for the average specific growth rate in the control for the entire test period should not exceed 10%.
-

zRMS comment:
The study is acceptable. The validity criteria according OECD 221 (2006) guideline was met.
$E_rC_{50, nom}$ (7 d) > 100 mg a.s./L
$E_yC_{50, nom}$ (7 d) = 100 mg a.s./L

Report:	KIIIA1 10.2.2.1/01, A. Evans, 2014
Title:	CHR/H/CPD – 7 day growth inhibition test with Duckweed Lemna gibba
Document No:	Smithers Viscient (ESG) Limited, Study Code: 3200611
Guidelines:	OECD No.221

GLP	Yes

Materials and methods:

- CHR/H/CPD, Batch No: 22.01.2014, production date: 22.01.2014; CoA issued by the PESTI-NOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s.300 ± 15%g[a.s.]/L
- The freshwater plant used in this toxicity test was the duckweed, *Lemna gibba*, strain G3. The Lemna was obtained from the University of Jena, Germany, and was maintained in stock culture at Smithers Viscient (ESG).
- Growth medium was prepared using prepared using sterile, reverse osmosis water
- The assay was performed according to the OECD Guideline No 221 [1], with the objective of determining the EC_{50} for yield inhibition (E_yC_{50}) and average growth rate inhibition (E_rC_{50}).
- Analytical method for the active substance: The concentration of the test item based on the signal of clopyralid was determined by high performance liquid chromatography with MS detection.

Findings:

Test Substance	CHR/H/CPD
Test Object	*Lemna gibba*
Exposure	7d, static
7day E_rC_{50} (growth rate) mg [a.s.]/L	>100
7day E_yC_{50} (yield inhibition) mg [a.s.]/L	>100
7day NOEC mg [a.s.]/L (growth)	>100
7day NOEC mg [a.s.]/L (yield)	>100

The validity criteria:

- The doubling time of frond number in the control must be less than 2.5 days. This corresponds to approximately a 7 fold increase in seven days and an average specific growth rate of 0.275 d-1

zRMS comment:
The study is acceptable. This study is in accordance with relevant guideline OECD 239 (2014). The validity criteria were met:
E_{r50} (14 days) = 34.095 mg product/L
E_yC_{50} (14 days) = 12.711 mg product/L
NOEC = 0.4 mg product/L.

Reference:	KCP 10.2
Report	Water-sediment Myriophyllum spicatum toxicity test of the test item Major 300 SL (CHR/H/CPD 300 SL) according to guideline OECD 239; 2021; Agnieszka Woźniak; SORBOLAB Research Laboratory LLC, Zaniemyska 11 Street, 61-029 Poznań, STUDY CODE: 0038/0030/E; GLP, Unpublished
Guideline(s):	OECD 239
Deviations:	The end of experimental part was in November 2021 (planned December 2021). The temperature during stability test and range-finding test was measured using thermometer with external probe. The light intensity during the stability study was measured on days 0, 7, 14 (scheduled on working days). The light intensity during definitive test was measured using photoradiometer (planned luxmeter). The above deviations did not affect the test result. The study met the validity criteria.

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

Materials and methods:
Test item: Name Major 300 SL (CHR/H/CPD 300 SL); Batch No. 02; Name of active substance clopyralid; Production date 02.2019; Expiration date 04.2022
Test organism: The study was conducted on spiked water-milfoil (Myriophyllum spicatum) bought from commercial farm. acclimated and then grown in the SORBOLAB Research Laboratory. Spiked water-milfoil is grown in laboratory conditions at temperature 20±2°C. under constant lighting of intensity 8800-11800 lux (140±20 µE · m-1s-1) as culturing medium was used Smart and Barko.
Test design test design B: one pot with tree shoots per one test vessel
Test system stability test:
- tested concentrations and control in one replicate range-finding test:
- control in four replicates, tested concentrations in two replicates
definitive test:
– control in six replicates, tested concentrations in four replicates
Exposure type static test
Test vessels glass beakers of 2 L volume Medium Smart and Barko, 1700 mL per beaker
Exposure time stability test:
- 14 days of test item exposure
range-finding test:
- establishment phase 7 days; 14 days of test item exposure
definitive test:
- establishment phase 7 days; 14 days of test item exposure
Tested concentrations stability test:
- control (0 mg/L); 1.0 mg/L; 100 mg/L
range-finding test:
- control (0 mg/L); 1.0 mg/L; 10 mg/L; 100 mg/L
definitive test:
- control (0 mg/L); 0.4 mg/L; 1.2 mg/L; 3.6 mg/L; 10.8 mg/L; 32.4 mg/L; Test conditions
stability test:
– average medium temperature 20.091°C (minimum temperature 19.7°C; maximum temperature 21.1°C)
– photoperiod 16 h light/8 h dark with light intensity 9000-11000 lux (121,5 µE · m-1s-1 – 148,5 µE · m-1s-1)
range-finding test:
– average medium temperature 20.000°C (minimum temperature 19.6°C; maximum temperature 20.5°C)
– photoperiod 16 h light/8 h dark with light intensity 9200 -11000 lux (124,2 µE · m-1s-1 – 148,5 µE · m-1s-1)
definitive test:
- average medium temperature 19.859°C (minimum temperature 18.2°C; maximum temperature 20.4°C)
- photoperiod 16 h light/8 h dark with light intensity 9421,68 lux – 11409,32 luks (127,32-154,18 µE · m-1s-1)

Results and discussions
The concentrations of the test item during the experiment differed from the determined initial concentrations in the range -1.65% to 7.31% (acceptable difference according to OECD 239: ±20%).
The test item was found to be stable under the conditions of the experiment.
Range-finding test
Test item effects statistically significantly the fresh weight yield, dry weight yield and growth rate and total shoots length growth rate after 14 days of the experiment in concentrations 10 mg/L, 100 mg/L. Test item effects statistically significantly the growth rate of fresh weight in concentration 100 mg/L and yield of total shoot length after 14 days of the experiment in all tested concentrations.

Definitive test
The tested item had a statistically significant influence on the yield and growth rate of fresh weight, yield and growth rate of dry weight, as well as the yield and growth rate of the total length of shoots after 14 days of the experiment at the tested concentrations from 1.2 mg/L to 97.2 mg/L.

Conclusions:
The tested item had a statistically significant influence on the yield and growth rate of fresh weight, yield and growth rate of dry weight, as well as the yield and growth rate of the total length of shoots after 14 days of the experiment at the tested concentrations from 1.2 mg/L to 97.2 mg/L.

Table 1. Final results

Results calculated using ToxRat Professional					
Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	NOEC [mg/L]	LOEC [mg/L]
Fresh weight yield	0.380 (0.132 – 1.093)*	1.970 (0.692 – 5.665)*	45.886 (11.423 – 172.238)*	0.4	1.2
Fresh weight growth rate	0.815 (0.304 – 2.186)*	5.149 (1.875 – 14.162)*	175.240 (40.095 – 710.176)*	0.4	1.2
Dry weight yield	0.230 (0.033 – 1.590)*	0.911 (0.143 – 5.991)*	12.711 (1.244 – 122.625)*	0.4	1.2
Dry weight growth rate	0.555 (0.095 – 3.243)*	2.282 (0.406 – 13.160)*	34.095 (3.577 – 299.035)*	0.4	1.2
Total shoots length yield after 14 days	0.051 (0.005 – 0.555)*	0.361 (0.037 – 3.699)*	15.226 (0.868 – 250.482)*	0.4	1.2
Total shoots length growth rate after 14 days	0.047 (n.d. – 16.782)*	0.557 (0.002 – 191.993)*	64.158 (0.037 – 89230.680)	0.4	1.2

EC_{10} concentration of test item causing 10% reduction
EC_{20} concentration of test item causing 20% reduction
EC_{50} concentration of test item causing 50% reduction
NOEC the highest non observe effective concentration cause no statistically significant differences in comparison to the control
LOEC the lowest observe effective concentration cause statistically significant differences in comparison to the control
n.d. impossible to determine due to mathematical reasons
* lower and upper 95% confidence limits are given in brackets

The validity criteria:

The test met the validity criteria in accordance with OECD 239 Guideline:
- fresh weight yield factor for control plants was 2.8 (required in OECD 239: ≥2.0)
- total shoots length factor for control plants was 2.5 (required in OECD 239: ≥2.0)
- variation coefficient for biomass based on the fresh weight was 5.3 % (required in OECD 239: ≤35%).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No additional studies were performed.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional studies were performed.

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 Acute oral toxicity to bees

zRMS comment:
The study is acceptable. This study was evaluated according to OECD 213. The study met the relevant validity criteria. The following endpoints are considered valid for use in the risk assessment:
1. Oral exposure: LD_{50} >100 µg product/bee; NOEL = 100 µg product/bee
2. Contact exposure: LD_{50} >100 µg product/bee; NOEL = 100 µg product/bee

Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration.

Report:	KIIIA1 10.4.2.1/01, Grzebisz E.
Title:	Acute Contact and Oral Toxicity to Honeybees Apis mellifera
Document No:	Smithers Viscient, Harrogate United Kingdom, Code : 3200616;
Guidelines:	OECD No 213
GLP	Yes

Material and methods:

- CHR/H/CPD, ; Batch No: 22.01.2014 production date: 22.01.2014, ; CoA issued by the PESTI-NOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s. 304.6 g/l
- The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bees.
- A definitive test was conducted at 100 µg product/bee for both the oral and contact exposure. There were six replicate vessels per treatment dose and the control, each with ten honeybees.

Findings:

Test substance	CHR/H/CPD
Test object	Bees (oral) (contact)
Oral LD_{50} (48 h)	> 100 µg[formulation]/bee
Contact (48 h)	> 100 µg[formulation]/bee

The Validity criteria:

- Oral and contact control mortality was < 10% at 48 hours

In the toxic reference dose levels, the effect levels indicated that the honeybees used in the study were responding normally within the test system.

- The toxic reference contact LD50 fell between 0.10 to 0.30 µg a.i./bee
- The toxic reference oral LD50 fell between 0.10 and 0.35 µg a.i./bee

Report:	KIIIA1 10.4.2.1/01, Grzebisz E.
Title:	Acute Contact and Oral Toxicity to Honeybees Apis mellifera
Document No:	Smithers Viscient, Harrogate United Kingdom, Code : 3200616;
Guidelines:	OECD No 213
GLP	Yes

Material and methods:

- CHR/H/CPD, ; Batch No: 22.01.2014 production date: 22.01.2014, ; CoA issued by the PESTI-NOVA laboratory Mozdzierzowcow Street 6a, 43-602 Jaworzno, 2014 content of a.s. 304.6 g/l
- The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bees.
- A definitive test was conducted at 100 µg product/bee for both the oral and contact exposure. There were six replicate vessels per treatment dose and the control, each with ten honeybees.

Findings:

Test substance	CHR/H/CPD
Test object	Bees (oral)
Oral LD_{50} (48 h)	> 100 µg[formulation]/bee

The Validity criteria:

- Oral and contact control mortality was < 10% at 48 hours

In the toxic reference dose levels, the effect levels indicated that the honeybees used in the study were responding normally within the test system.

- The toxic reference contact LD50 fell between 0.10 to 0.30 µg a.i./bee
- The toxic reference oral LD50 fell between 0.10 and 0.35 µg a.i./bee

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria given in OECD 245 (2017) are met.
LC_{50} > 3333.3 mg product/kg
NOEC ≥ 3333.3 mg product/kg
LDD_{50} >103.83 µg product/bee/day
NOEDD ≥ 103.83 µg product/bee/day

Reference:	KCP 10.3.1
Report	Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test, Ewelina Myrczek; Ecomelius Institute Sp. z o. o., Poland; STUDY CODE:

	EMI/4/16/2021
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	name: CHR/H/CPD 300 SL; content: clopyralid: 25.67 ± 0.49%; batch number: 02; production date: February 2019; expire date: April 2022
Test Species:	freshly emerged young worker honeybees, Apis mellifera L. (strain: carnica, line: Prima)
Test Design:	The experiment was done on one untreated control group, three test item groups. There were one replicates of each of them (10 bees/replicate). There were 1 replicate containing diet of untreated solvent control and placed in the environment alongside the test units.
Endpoints:	Median Lethal Dietary Doses (LDD50), No Observed Effect Dietary Dose (NOEDD), Median Lethal Concentrations (LC50), and the No Observed Effect Concentration (NOEC)
Test Concentration:	133.3, 666.7 and 3333.3 mg/kg (i.e. 4, 20 and 100 µg/bee/day)
Test Conditions:	temperature: 31 - 35 ºC; relative humidity: 50 - 70%

Results and discussion:

After 10 days, no dead bees in the control group were observed. Mortality of the bees exposed to the test item at the concentrations of 133.3, 666.7 and 3333.3 mg/kg of diet (i.e. 4, 20 and 100.0 µg/30 mg of diet, respectively) was 0.0, 0.0, 0.0, and 0.0%, respectively.
Average consumption of a 50% saccharose solution in the control group was 33.58 mg/bee/day. Average consumption in the groups treated with the test item at the concentrations of 133.3, 666.7 and 3333.3 mg/kg of diet (i.e. 4, 20 and 100.0 µg/30 mg of treated diet, respectively) was 35.07, 36.40 and 31.15 mg/bee/day, respectively. On the basis of average consumption of treated 50% saccharose solution in the study groups, it may be concluded that each bee treated with the test item at the concentrations of 133.3, 666.7 and 3333.3 mg/kg ingested 4.68, 24.27, and 103.83 µg/bee/day.
No symptoms of behavioural abnormality were observed.
There was no statistically significantly differences in mortality of the groups treated with the test item at the dose of 4, 20 and 100 µg/bee/day and the control group.

Endpoint values

On the basis of the obtained mortality results, the LC50 value is above to 3333.3 mg t.i./kg diet. LDD50 value is above to 103.83 µg/bee/day. The NOEC value is ≥ 3333.3 mg t.i./kg diet. The NOEDD value is ≥ 103.83 µg/bee/day

The validity criteria:

The following validity criteria were met during the experiment [1]:

- at the end of the experiment average mortality of the control groups was 0.0% (criterion: it must not exceed 15%),
- mortality of bees exposed to the reference item is ≥ 50% on day 10 of exposure.

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

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria given in OECD 239 (2016) are met.
NOEC ≥ 100 µg product/larva/day

Reference:	KCP 10.3.1
Report	Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure, M. Grzesica; Ecomelius Institute Sp. z o. o.; Kalinowa 2, Zaborze, 43-520 Chybie, Poland; STUDY CODE: EMI/4/15/2021
Guideline(s):	according to the OECD Guideline No. 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	name: CHR/H/CPD 300 SL; content: clopyralid: 25.67 ± 0.49%; batch number: 02; production date: February 2019; expire date: April 2022
Test Species:	one-day-old Apis mellifera L. (strain: carnica, line: prima) larvae (first instar)
Test Design:	one untreated control group, five test item groups, and one reference item group. There were three replicates of each of them (12 larva/replicate)
Endpoints:	EC_{50} after 22 days, NOEC
Test Concentration:	five concentrations in a geometric series, spaced by a factor of 2, i.e. 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva, plus the control and one concentration of a reference item
Test Conditions:	temperature: 34 – 35ºC; relative air humidity: 85 - 95% (D1-D8) and 75 – 85% (D8 – D15), 50-80% (D15-D22)

Results and discussion:

Preliminary non-GLP test

Test item dose [µg/larva]	Number of tested larvae	Exposure period (days)										
		(D3)		(D8)			(D15)			(D22)		
		Number of dead larvae [No.]	Mortality Total [%]	Number of dead larvae [No.]	Mortality Total [%]	Mortality Corr. [a] [%]	Number of dead pupae [No.]	Mortality Total [%]	Mortality Corr. [a]	Number of emerged adults [No.]	survivability [%]	survivability Corr. [a]
Control	12	0	0.0	1	8.33	-	2	16.7	-	10	83.3	-
1.0	12	0	0.0	2	16.7	9.1	2	16.7	0.0	10	83.3	100.0
10.0	12	0	0.0	1	8.3	0.0	2	16.7	0.0	10	83.3	100.0
100.0	12	0	0.0	2	16.7	9.1	4	33.3	20.0	8	66.7	80.0

[a]: the control response was compensated using Abbott's formula

Definitive test

Mortality of the control group at D8 was 8.3%. The percentages of mortality after 8 days, corrected using Abbott's formula, of the honeybee larvae, exposed to the test item, CHR/H/CPD 300 SL at the doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva were: 9.1, 6.1, 6.1, 9.1 and 9.1%, respectively. On D8 the larvae in the control group and the groups treated with the test item consumed the whole volume of food.
Mortality of the control group at D15 was 16.7%. The percentages of mortality pupae after 15 days, corrected using Abbott's formula, of the honeybee larvae, exposed to the test item, CHR/H/CPD 300 SL at the doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva were: 10.0, 10.0, 3.3, 6.7 and 6.7%, respectively.
The adult emergence rate on D22 in the control group was 77.8%. The adult emergence rate on D22 in the test item, CHR/H/CPD 300 SL, at the doses of 6.25, 12.5, 25.0, 50.0 and 100.00 µg/larva were: 66.7, 69.4, 80.6, 75.0 and 72.2%, respectively.
There was no statistically significantly differences in survival of the all groups treated with the test item and the control group (Chi2-2x2 Test with Bonferroni Correction, $p(z) > \alpha^*$).
The median effect concentration, after 22 days of exposure to the test item (EC_{50}/22 d) is higher than the maximum used concentration, i.e. 650 mg test item/kg of diet (100 µg test item/larva). The NOEC value is higher than or equal 650 mg/kg (100.0 µg/larva).
Mortality of the reference item group corrected using Abbott's formula was 100.0% at the end of the experiment.

The larval toxicity test – repeated exposure, of the test item, CHR/H/CPD 300 SL, on honeybees (*Apis mellifera* L.) in the laboratory test is summarized below.

Test item dose [µg/larva]	Number of tested larvae	Exposure period (days)										
		(D3)		(D8)			(D15)			(D22)		
		Number of dead larvae [No.]	Mortality	Number of dead larvae [No.]	Mortality		Number of dead pupae [No.]	Mortality		Number of emerged adults [No.]	survivability	
			Total [%]		Total [%]	Corr. [a] [%]		Total [%]	Corr. [a]		[%]	
Control	36	0	0.0	3	8.3	-	3	16.7	-	28	77.8	
6.25	36	0	0.0	6	16.7	9.1	3	25.0	10.0	24	66.7	
12.5	36	0	0.0	5	13.9	6.1	4	25.0	10.0	25	69.4	
25.0	36	0	0.0	5	13.9	6.1	2	19.4	3.3	29	80.6	
50.0	36	0	0.0	5	16.7	9.1	2	22.2	6.7	27	75.0	
100.0	36	0	0.0	5	16.7	9.1	2	22.2	6.7	26	72.2	
Reference item: dimethoat												
7.39	36	0	0.0	36	100.0		-	-	-	-	-	-

[a]: the control response was compensated using Abbott's formula.
The main experiment was performed between 15.08 - 05.09.2021.

Endpoint values
The median effect concentration, after 22 days of exposure to the test item (EC_{50}/22 d) is higher than the maximum used concentration, i.e. 650 mg test item/kg of diet (100 µg test item/larva). The NOEC value is higher than or equal 650 mg/kg (100.0 µg/larva).

The validity criteria:
The following validity criteria were met during the experiment:
- cumulative mortality of the control group from D3 to D8 was 8.3% (required ≤ 15%),
- the adult emergence rate on D22 in the control group was 77.8% (required > 70%),
- larval mortality for dimethoate on D8 across all replicates was 100% (required > 50%).

A 2.3.1.4 Lethal effects

No additional studies were performed.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional studies were performed.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional studies were performed.

A 2.3.1.7 KCP 10.3.1.6 Non target arthropods studies

zRMS comments:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.
LR_{50} = 534 g product/ha (138 g a.s./ha)

Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration.

Report:	KIIIA1 10.5.1/01, E. Kulec - Płoszyca, 2016
Title:	An extended laboratory test for evaluating the effects of CHR/H/CPD 300 SL on the predatory mite, Typhlodromus pyri (Sch.)
Document No:	IPO Pszczyna, Poland, Study code: B/184/16
Guidelines:	SETAC Guidance Documents: ESCORT I (Barrett et al., 1994); ESCORT II (Candolfi et al., 2001); IOBC, BART, EPPO Guidelines (Blümel et al., 2000)
GLP	Yes

Material and methods:

- CHR/H/CPD, Batch No: 05/14 , production date 05.2014, CoA issued by IPO Warszawa, Poland, 25.02.2016, Doc. No 023/BA/16
- content of a.s. = 25.7±0.42% (w/w) according to Certificate of Analysis No 206/14 issued by IPO Warszawa, Poland, 25.02.2016, Doc. No 023/BA/16
- In the definitive test, the one rate of the test item was applied: 534.0 g/ha (138.0 g s.a./ha). The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to rose leaf discs. The mites were fed with pine pollen (*Pinus sp.*) and the two-spotted spider mite, *Tetranychus urticae* (Carl Ludwig Koch). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and all groups treated with the test item were made after 10, 12, and 14 days of the treatment Mortality of T.pyri after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints. To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Bi 58 Nowy 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.
- The aim of the study was to determine the impact of CHR/H/CPD 300 SL on mortality and reproduction of the predatory mite, *Typhlodromus pyri*. The endpoints of this test were mortality of the mites after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment.

- **Findings:**

Test substance	CHR/H/CPD 300SL
Test object	*Typhlodromus pyri Sch.*
LR_{50} (7 d) Tier II exposure scenario mg /ha	138 g a.s/ha 534g [formulation]/ha
Pr % reproductive effect at 540ml[formulation]ha	0.1%

The validity criteria:

- mortality of the control group was 8.0% on day 7 of the exposure (criterion: a maximum of

20.0%),

- corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 89.1 % on day 7 of the exposure (criterion: a minimum of 50%),
- the mean number of eggs per female in the control group was 6.1 (required: ≥ 4 eggs per female).

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.
LR_{50} = 619.9 g product/ha (162 g a.s./ha).

Report:	KIIIA1 10.5.2/01, E.Grzebisz, 2014
Title:	Extended Acute Toxicity to Aphidius rhopalosiphi in the Laboratory
Document No:	Smithers, UK, Study no. 3200618
Guidelines:	According to the SETAC Guidance Documents: ESCORT I (Barrett et al., 1994); ESCORT II (Candolfi et al., 2001); IOBC, BART, EPPO Guidelines (Mead-Briggs et al., 2000) and Mead-Briggs and Longley, 1997
GLP	Yes

Material and methods:

- CHR/H/CPD, Batch No: 22.01.2014 , production date 22.01.2014, CoA issued by Pestinova, Jaworzno, Poland, 2014 No 12/14
- content of a.s. according to Certificate of Analysis No 206/14 issued by Pestinova, Jaworzno, Poland, Aug 11, 2014, 304.6 g a.s./L
- The test comprised of a control treatment and test substance applied at 10.125, 20.25, 40.5, 81 and 162 g a.i./ha. All treatments were applied at a volume equivalent to 400L/ha water. Prior to treatment, all barley was sprayed with a 10% fructose solution. The exposure phase was conducted using six replicates in the control and for each treatment. Test units comprised; the plant pot containing treated barley (8 – 10 plants) with the surface of the planting compost covered in sand and a Perspex cylinder which was placed over the top of the barley. Mortality was assessed at 1–3, 24 and 48 hours after introduction. Also any potential for the test formulation to repel was made immediately after wasp introduction. Wasps were observed and their location recorded. The repellency assessments were performed ca 60, 90 and 120 minutes post introduction. No repellent effect was observed to the wasps exposed to the test formulation when compared to the control. The fecundity phase was conducted with 15 females from the control, 81 and 162 g a.i./ha treatment rates. Females were individually contained on aphid infested untreated barley seedlings. All females were removed from the barley after ca 24 hours. The aphids were maintained for a further 11 days to allow for the development of the mummies (parasitized aphids), after which a count was performed.
- The objective of this study was to determine the LR50 and no observable effect rate (NOER) of the test substance on the parasitoid wasp, Aphidius rhopalosiphi DeStefani-Perez (Hymenoptera: Braconidae), exposed to residues on barley seedlings.

Findings:

Test substance	CHR/H/CPD
Test object	parasitic wasp *Aphidius rhopalosiphi*
48 h LR_{50} Tier II exposure scenario	> 162000 mg [a.s]/ha >619920 mg [form]/ha
Pr % reproductive effect at 540mlha	9.4%

The validity criteria:
The following criteria were met for the studies, therefore these data are considered to be valid.

- Control mortality was ≤ 17% after 48 hours exposure.
- The number of mummies produced per female was ≥ 5 in the control.
- There were no more than 2 replicates with 0 mummies in the control.
- Corrected mortality in the toxic reference treatment was > 50% after 48 hours exposure.

A 2.3.1.7.1 Study 1

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.
LR_{50} = 512 g product/ha (138 g a.s./ha)

Reference:	KCP 10.3.1.6/01
Report	CHR/H/CPD: Effects on the Ladybird Beetle Coccinella septempunctata under Extended Laboratory Conditions,M. Moll; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681012
Guideline(s):	GLP compliant study based on Schmuck et al. 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/CPD; batch no.: 18/08/2016
Test Species:	Ladybird beetle (Coccinella septempunctata), 4 - 5 days old larvae; source: Katz Biotech AG, Baruth, Germany
Test Design:	This study encompassed 3 treatment groups (1 dose rate of the test item, control, reference item) with 40 replicates each and each containing one C. septempunctata larva. The larvae were exposed to dried residues on treated leaf surfaces (vine leaves). Exposure time lasted until the hatching of the adults. The reproductive performance of the survivors was examined over two weeks (oviposition period) using adults from the control and from those test item concentrations where the corrected mortality was < 50.0 %.
Endpoints:	Pre-imaginal mortality of exposed larvae and pupae. Additionally reproductive capacity of adult survivors
Test Conditions:	Temperature: 24 °C - 25 °C; relative humidity: 68 - 82 %; photoperiod: 16 h light : 8 h dark; light intensity: 1070 - 1630 lux

Results and discussion:

Table 1. Pre-imaginal mortality and reproduction of *Coccinella septempunctata*

	Rate [1]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [fertile eggs per female per day]
Control	--	7.5	--	8.3
CHR/H/CPD	138 g a.s./ha	12.5 n.s.	5.4	7.8
Perfekthion	50 mL/ha	100.0 *	100.0	-

1) Application rate in 200 L water/ha

2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces
(Fisher's Exact Test, α = 0.05: n.s. = not significant, * = significant)

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

Mortality

Table 2. Pre-imaginal mortality of *Coccinella septempunctata*

treatment group	mortality [a] [%]		corrected mortality [%]
control	7.5		-
138 g a.s./ha	12.5	n.s.	5.4
reference item	100.0	*	100.0

[Fisher's Exact Test, α = 0.05; n.s. = not significant, * = significant]

[a] the tabulated results represent rounded values calculated on the exact raw data;
40 individuals per treatment group, exposure on treated leaves, 16 days after application

Effects of CHR/H/CPD on the Reproductive Capacity of Adult Coccinella septempunctata

Reproduction of C. septempunctata was > 2 fertile eggs per viable female per day at 138 g a.s./ha, so the reproductive output is within the historical data base for control beetles and therefore this parameter is considered as not impacted by the treatment (Schmuck et al. 2000).See table 3

Table 3. Reproduction of adult *Coccinella septempunctata*

treatment group [a]	eggs per female [b] per day	fertile eggs per female per day	larval hatching rate [%]
control	8.8 ± 6.5	8.3 ± 6.5	92.9 ± 7.4
138 g a.s./ha	9.4 ± 4.7	7.8 ± 4.3	83.9 ± 18.5

note: the tabulated results represent mean and standard deviation calculated on the exact raw data;
1 replicate per treatment group with all survived beetles

[a] adults developed from larvae exposed to spray residues on leaves

[b] oviposition started 1 week after the first egglaying was observed in the control and lasted 14 days

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:
- mortality of the control group was 7.5% (criterion: a maximum of 30%),
- reference item mortality: 100.0 % (corrected mortality) (criterion: >40%corrected mortality)

- control reproduction rate: 8.3 fertile eggs per female per day (mean value (criterion ≥ 2 fertile eggs per viable female per day

A 2.3.1.7.1 Study 2

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.
LR_{50} = 512 g product/ha (138 g a.s./ha)

Reference:	KCP 10.3.1.6/02
Report	CHR/H/CPD: Effects on the Lacewing Chrysoperla carnea under Extended Laboratory Conditions ,M. Moll; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681047
Guideline(s):	GLP compliant study based on Vogt et al. 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/CPD; batch no. 18/08/2016
Test Species:	Lacewing (Chrysoperla carnea), larvae (2 days old); source: Katz Biotech AG, Baruth, Germany
Test Design:	This study encompassed 3 treatment groups (1 dose rate of the test item, control, reference item) with 40 replicates each containing 1 larva. The larvae were exposed to dried residues on treated leaf surfaces (bean leaves). Exposure time lasted until pupae were transferred to the reproduction units for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition, the reproduction performance, i.e. egg deposition and larval hatching rate, was determined (2 checks/week, 24 hours period each check)
Endpoints:	Larval and pupal mortality, additionally reproductive capacity for female survivors.
Test Conditions:	Temperature: 25 °C - 26 °C; relative humidity: 71 % - 90 %; photoperiod: 16 h light : 8 h dark; light intensity: 1030 lux - 1360 lux.

Results :

Table 1. Effects of CHR/H/CPD on pre-imaginal mortality and reproduction of *Chrysoperla carnea*, exposed to fresh dried residue in the laboratory

	Rate [1]	Mortality [%] [2]	Mortality corr. [%] [3]	Reproduction [eggs/female/day]	Larval hatching rate [%]
Control	--	0.0	--	22.0	95.5
CHR/H/CPD	138 g a.s./ha	7.5 n.s.	7.5	20.5	98.0
Reference Item (Perfekthion)	140 mL/ha	82.5 *	82.5	-	-

1) Application rate in 200 L deionised water/ha

2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Fisher's Exact Test, α = 0.05: n.s. = not significant, * = significant)

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

At the dose rate of 138 g a.s./ha CHR/H/CPD showed no adverse effect on mortality of the lace-wing Chrysoperla carnea.
Fecundity was > 15 eggs per female per day and fertility was > 70 % larval hatching rate at 138 g a.s./ha. This indicates that there was no negative effect of the test item on reproduction of Chrysoperla carnea.

Effects of CHR/H/CPD on Larvae and Pupae of Chrysoperla carnea
Pre-imaginal Mortality: See Table 2:

Table 2. Pre-imaginal mortality of *Chrysoperla carnea*

treatment group	mortality [a] [%]		corrected mortality [%]
control	0.0		-
138 g a.s./ha	7.5	n.s.	7.5
reference item	82.5	*	82.5

[test item: Fisher's Exact Test, α = 0.05; n.s. = not significant, * = significant; reference item: Fisher's Exact Test, α = 0.05; * = significant]

[a] the tabulated results represent rounded values calculated on the exact raw data; 23 days after test start; 40 individuals per treatment group

Effects of CHR/H/CPD on the Reproduction of Chrysoperla carnea

Fecundity was > 15 eggs per female per day and fertility was > 70 % larval hatching rate at 138 g a. s./ha. This indicates that there was no negative effect of the test item on reproduction of Chrysoperla carnea. See Table 3:

Table 3. Reproduction of *Chrysoperla carnea*

treatment group [a]	eggs per female per day	larval hatching rate [%]
control	22.0 ± 3.6	95.5 ± 4.0
138 g a.s./ha	20.5 ± 0.9	98.0 ± 0.8

note: the tabulated results represent mean and standard deviation calculated on the exact raw data; 2 reproduction units per treatment group with all survived lacewings
[a] adults developed from larvae exposed to spray residues on leaves

TEST VALIDITY CRITERIA
The following validity criteria were met during the study:
- mortality of the control group was 0.0% (criterion: a maximum of 20%),
- reference item mortality: 82.5 % corrected mortality (criterion: Pre-imaginal mortality should result in at least 50 % (preferably < 100 %) corrected mortality)
- fecundity in the Control Group: 22.0 eggs per female per day (mean number) (criterion: ≥ 15 eggs per female per day (mean value)
- fertility in the Control Group: 95.5 % larval hatching rate (mean value) (criterion: ≥ 70 % larval hatching rate (mean value)

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 1

zRMS comment:
The study is acceptable and is valid for risk assessment. The study followed an approved guideline OECD 222 (2016) and was conducted with GLP. All validity criteria were met.
Mortality NOEC ≥ 250 mg prod./kg soil dry weight.
The NOEC for reproduction is equal to 125 mg prod./kg soil
The EC_{50} was estimated to be >250 mg prod./kg soil. No EC_{50} was estimated.

Reference:	KCP 10.4.1/01
Report	CHR/H/CPD 300SL: Effects on Reproduction and Growth of Earthworms Eisenia andrei in Artificial Soil, D. Straube; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany; Study No: 125681022
Guideline(s):	According to the OECD Guideline No. 222 (2016)
Deviations:	No

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

Materials and methods

Test Item:	CHR/H/CPD 300SL; batch no.: 18/08/2016
Test Species:	Earthworm (Eisenia andrei), adult worms (with clitellum and weight range 304 to 586 mg), approximately 9 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 worms each.
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Test Concentration:	Control, 15.63, 31.25, 62.5, 125 and 250 mg CHR/H/CPD 300SL/kg soil
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.6 to 5.7, pH at experimental end 5.7 to 6.0; water content 34.7% to 35.9% (50.4% to 52.1% of maximum water holding capacity, WHC) at experimental start and 36.9% to 38.9% (53.5% to 56.4% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux

Results and discussion:

No mortality was observed in any treatment group.
The body weight changes of the earthworms after 4 weeks exposure to CHR/H/CPD 300SL were not statistically significantly different compared to the control up to and including the highest test concentration of 250 mg test item/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).
The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 125 mg test item/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 250 mg test item/kg soil the reproduction was statistically significantly reduced compared to the control. No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table 1. Effect of CHR/H/CPD 300SL on earthworms (*Eisenia andrei*) in a 56-day reproduction study

CHR/H/CPD 300SL [mg/kg soil]	**Control**	**15.63**	**31.25**	**62.5**	**125**	**250**
Mortality (day 28) [%]	0	0	0	0	0	0
Statistical Significance	-	-	-	-	-	-
Body weight change (day 28) [%]	42.4	44.8	36.5	41.2	35.1	33.6
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	217	226	223	201	203	187
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	*
Reproduction in [%] of control (day 56)	-	104.2	102.6	92.7	93.5	86.2
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg soil]					
NOEC (day 28 mortality and weight)	**≥250**					
LOEC (day 28 mortality and weight)	**>250**					
LC_{50} [2)]	**>250**					
NOEC (day 56 reproduction)	**125**					
LOEC (day 56 reproduction)	**250**					
EC_{50} [2)]	**>250**					

The results represent rounded values calculated on the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Williams t-test, α = 0.05, one-sided smaller
[2)] estimated value

Observations of the earthworms

No mortality was observed in any treatment group. Therefore, the NOEC for mortality was determined to be ≥250 mg test item/kg soil. The LOEC for mortality was estimated to be >250 mg test item/kg soil. The LC50 was estimated to be >250 mg test item/kg soil.

Table 3. Number of live adult earthworms and mortality after 4 weeks

Treatment group	container #	number of live adults start	number of live adults 4 weeks	mortality of the adults (4 weeks) % per container	% mean ± SD[1]	signifi-cance
control	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
15.63	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
31.25	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
62.5	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
125	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
250	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		

test item dosages are given as mg test item/kg artificial soil dry weight

- = not relevant

[1] = mean ± standard deviation of 4 replicates (8 in the control)

Body Weights of the Adult Worms

The body weight changes of the adult worms after 4 weeks exposure to CHR/H/CPD 300SL are shown in Table 4. The body weight changes in the test item treated groups were not statistically significantly different compared to the control up to and including the highest test concentration of 250 mg test item/kg soil (Williams t-test, $\alpha = 0.05$, one sided-smaller). Therefore, the NOEC for body weight changes was determined to be ≥250 mg test item/kg soil. The LOEC was estimated to be >250 mg test item/kg soil.

Table 4. Body weight changes of the adult earthworms

Treatment group	container	mean body weights per earthworm		body weight change per earthworm				
		at start	after 4 weeks	mean per container		mean ± SD [1]		signifi-
	#	mg	mg	mg	%	mg	%	cance
control	I	406.2	607.5	201.3	49.6			
	II	425.1	618.1	193.0	45.4	183	42.4	-
	III	425.3	572.8	147.5	34.7	± 16	± 4.3	
	IV	432.6	622.5	189.9	43.9			
	V	434.3	618.0	183.7	42.3			
	VI	438.5	620.5	182.0	41.5			
	VII	440.3	624.6	184.3	41.9			
	VIII	457.0	641.5	184.5	40.4			
15.63	I	419.9	663.0	243.1	57.9			
	II	429.9	630.6	200.7	46.7	194	44.8	n.s.
	III	434.4	550.0	115.6	26.6	± 55	± 13.1	
	IV	449.3	665.3	216.0	48.1			
31.25	I	421.8	601.6	179.8	42.6			
	II	428.5	599.0	170.5	39.8	157	36.5	n.s.
	III	434.5	576.7	142.2	32.7	± 21	± 5.7	
	IV	447.8	585.1	137.3	30.7			
62.5	I	422.4	574.8	152.4	36.1			
	II	427.5	623.0	195.5	45.7	178	41.2	n.s.
	III	434.6	662.2	227.6	52.4	± 42	± 9.7	
	IV	442.3	578.3	136.0	30.7			
125	I	422.8	594.2	171.4	40.5			
	II	426.4	553.9	127.5	29.9	151	35.1	n.s.
	III	436.9	639.1	202.2	46.3	± 44	± 10.3	
	IV	441.7	545.6	103.9	23.5			
250	I	423.0	540.0	117.0	27.7			
	II	425.9	574.0	148.1	34.8	146	33.6	n.s.
	III	437.8	577.1	139.3	31.8	± 25	± 5.3	
	IV	441.2	619.2	178.0	40.3			

test item dosages are given as mg test item/kg artificial soil dry weight
the results represent rounded values calculated on the exact raw data
[1] = mean ± standard deviation of 4 replicates (8 in the control)
- = not relevant
n.s. = not significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller

Reproductive Assessment

The numbers of juvenile worms found 8 weeks after application are shown in Table 5. The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 125 mg test item/kg soil (Williams t-test, α = 0.05, one-sided smaller). At the test concentration of 250 mg test item/kg soil reproduction was statistically significantly reduced compared to the control. Therefore, the NOEC for reproduction was determined to be 125 mg test item/kg soil. The LOEC for reproduction was determined to be 250 mg test item/kg soil. The EC50 was estimated to be >250 mg test item/kg soil.

Table 5. Reproduction of the earthworms after 8 weeks

Treatment group	container #	number of juvenile earthworms per container	mean ± SD [1]	% of control	significance
control	I	215			
	II	202	217	-	-
	III	223	± 23		
	IV	199			
	V	266			
	VI	220			
	VII	217			
	VIII	193			
15.63	I	171			
	II	214	226	104.2	n.s.
	III	274	± 44		
	IV	245			
31.25	I	203			
	II	225	223	102.6	n.s.
	III	200	± 29		
	IV	262			
62.5	I	206			
	II	195	201	92.7	n.s.
	III	206	± 6		
	IV	197			
125	I	215			
	II	186	203	93.5	n.s.
	III	221	± 18		
	IV	189			
250	I	177			
	II	173	187	86.2	*
	III	220	± 22		
	IV	178			

test item dosages are given as mg test item/kg artificial soil dry weight
the results represent rounded values calculated on the exact raw data
[1] = mean ± standard deviation of 4 replicates (8 in the control)
- = not relevant
n.s. = not significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller
* = significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller

Feeding Activity and Behavioural Abnormalities

Feeding Activity: In all treatment groups, food was consumed. The results show that the turnover of biomass of those earthworms exposed to the different rates of the test item was comparable to the control (see Table 6).

Behavioural Abnormalities: No behavioural abnormalities were observed and all worms burrowed into the soil within 15 minutes after introduction

Table 6. Amount of food added to the test containers

Treatment group	container	time after application					total of added food	
		day 1	1 week	2 weeks	3 weeks	4 weeks		mean ± SD[1]
	#	g	g	g	g	g	g	g
control	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	
	V	5.0	5.0	5.0	5.0	5.0	25.0	
	VI	5.0	5.0	5.0	5.0	5.0	25.0	
	VII	5.0	5.0	5.0	5.0	5.0	25.0	
	VIII	5.0	5.0	5.0	5.0	5.0	25.0	
15.63	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	
31.25	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	
62.5	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	
125	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	
250	I	5.0	5.0	5.0	5.0	5.0	25.0	25.0 ± 0.0
	II	5.0	5.0	5.0	5.0	5.0	25.0	
	III	5.0	5.0	5.0	5.0	5.0	25.0	
	IV	5.0	5.0	5.0	5.0	5.0	25.0	

test item dosages are given as mg test item/kg artificial soil dry weight

[1] = mean ± standard deviation of 4 replicates (8 in the control)

Concentration (NOEC) for mortality and growth of the earthworm Eisenia andrei was determined to be ≥250 mg test item/kg soil, i.e. the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >250 mg test item/kg soil. The LC50 was estimated to be >250 mg test item/kg soil.
The NOEC for reproduction was determined to be the concentration of 125 mg test item/kg soil. The LOEC for reproduction was determined to be 250 mg test item/kg soil. The EC50 was estimated to be >250 mg test item/kg soil.

VALIDITY CRITERIA

Control Mortality:
Should not exceed 10% over initial 4-week test period
Control mortality was 0% and so this validity criterion was met.
Reproduction of Control:
Should be ≥ 30 worms per replicate container
The number of juvenile worms per replicate was 193 to 266 and so this validity criterion was met.
Coefficient of Variation of Reproduction in Control:
Should not exceed 30%
Was 10.6% and so this validity criterion was met

A 2.4.1.2 Earthworms - field studies

No additional studies were performed.

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

A 2.4.2.1.1 Study 1

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guidline and all validity criteria given in OECD 232 (2016) are met.
$NOEC_{reproduction}$ > 1000 mg prod./kg dry soil.

Reference:	KCP 10.4.2/01
Report	CHR/H/CPD 300SL: Effects on Reproduction of the Collembola Folsomia candida in Artificial Soil, D. Straube; ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681016
Guideline(s):	according to the OECD Guideline No. 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/CPD 300SL; batch no.: 18/08/2016
Test Species:	Collembola Folsomia candida, 10-12 days old, from cultures held at the laboratory
Test Design:	28-d exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was placed into glass vessels before the Collembola were introduced on top of the soil; 5 concentrations and one control; 4 replicates/concentration with 10 Collembola each (8 replicates for the control). Feeding of Collembola with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 d.

Endpoints:	Mortality of adult Collembola, behavioural effects, number of juveniles.
Test Concentration:	Control, 62.5, 125, 250, 500 and 1000 mg CHR/H/CPD 300SL/kg soil
Test Conditions:	Artificial soil based on OECD 226; initial pH 5.9 to 6.0, pH at experimental end 5.8; water content at experimental start 20.0% to 20.6% (51.4% to 52.7% of the maximum water holding capacity); at experimental end 18.3% to 18.9% (47.0% to 48.4% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark, light intensity within the range of 400 to 800 lux.

Results and discussion:

A mortality of up to 10% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction of the Collembolan exposed to CHR/H/CPD 300SL was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups. The results are shown in Table 1.

Table 1. Effect of CHR/H/CPD 300SL on Collembola (*Folsomia candida)* in a 28-day reproduction study

CHR/H/CPD 300SL [mg/kg soil]	Control	62.5	125	250	500	1000
Mortality (day 28) [%]	8	10	5	3	3	8
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	511	594	585	497	519	432
Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 28)	-	116	114	97	102	85
	Endpoints [mg test item/kg soil]					
NOEC (mortality)	≥1000					
LOEC (mortality)	>1000					
LC_{50} [3)]	>1000					
NOEC (reproduction)	≥1000					
LOEC (reproduction)	>1000					
EC_{50} [3)]	>1000					

n.s. = not significantly different compared to the control
- not applicable
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value

CHR/H/CPD 300SL caused no statistically significant effects on mortality and reproduction of Folsomia candida up to and including the concentration of 1000 mg test item/kg soil. The LC50 was estimated to be >1000 mg test item/kg soil.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil. The EC50 was estimated to be >1000 mg test item/kg soil.

VALIDITY CRITERIA

Control Mortality:
Should be ≤ 20%
Control Reproduction:
Should reach ≥ 100 juveniles per container
Mean number of juvenile Collembola per replicate was 392 to 593, validity criterion was met
Mean mortality was 8%, validity criterion was met
Coefficient of Variation of the Control Reproduction:
Should be less than 30%
Was 12.5%, validity criterion was met.

A 2.4.2.1.2 Study 2

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guidline and all validity criteria given in OECD 232 (2016) are met.
$NOEC_{reproduction}$ > 1000 mg prod./kg dry soil.

Reference:	KCP 10.4.2/02
Report	CHR/H/CPD 300SL: Effects on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil, D.Straube; ibacon GmbH Study Arheilger Weg 17 64380 Rossdorf Germany ; STUDY CODE: 125681089
Guideline(s):	according to the OECD Guideline No. 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/CPD 300SL; batch no.: 18/08/2016;
Test Species:	Predatory mite Hypoaspis aculeifer, adult females, approximately 10 days after reaching the adult stage (31 days after placing adult females in clean rearing vessels), cultured by ibacon
Test Design:	14 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; 5 concentrations and one control were tested; 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites (Tyrophagus putrescentiae) ad libitum at test start and on day 2, 4, 7, 9 and 11. Assessment of adult mortality and reproduction performed after 14 days
Endpoints:	Adult mortality, number of juveniles
Test Concentration:	Control, 62.5, 125, 250, 500 and 1000 mg CHR/H/CPD 300SL/kg soil
Test Conditions:	Artificial soil based on OECD 226; initial pH 5.9 to 6.0, pH at experimental end 5.8 to 5.9; water content at experimental start 20.0% to 20.6% (51.4% to 52.7% of the maximum water holding capacity); at experimental end 19.0% to 19.6% (48.8% to 50.3% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark

(within the range of 400 to 800 lux)

Results and discussion:

Mortality of adult females

The mortality data are shown in Table 3. Mortality of Hypoaspis aculeifer in the test item treated group ranged from 0% to 5%. The values were not statistically significantly different compared to the control, where 1% of the adult mites died (Fisher's Exact Test, α = 0.05, one-sided greater). Therefore, the NOEC for mortality was determined to be ≥1000 mg test item/kg soil. The LOEC for mortality was estimated to be >1000 mg test item/kg soil. The LC50 was estimated to be >1000 mg test item/kg soil.

Table 3. Mortality of adult *Hypoaspis aculeifer* after 14 days

Treatment Group	number of surviving females per replicate								mean mortality	standard deviation	signifi-cance[1]
	1	2	3	4	5	6	7	8	[%]	[%]	
control	10	10	10	10	10	10	10	9	1	± 4	-
62.5	9	10	10	10	-	-	-	-	3	± 5	n.s.
125	10	9	10	10	-	-	-	-	3	± 5	n.s.
250	10	10	10	10	-	-	-	-	0	± 0	n.s.
500	10	10	9	10	-	-	-	-	3	± 5	n.s.
1000	9	10	10	9	-	-	-	-	5	± 6	n.s.

the results represent rounded values calculated from the exact raw data

test item dosages are given as mg/kg artificial soil dry weight

[1] Fisher's Exact Test, one-sided greater, α = 0.05

- not applicable

n.s. not statistically significantly different compared to the control

Observations

No differences in morphology of the mites between the test item treated groups and the control were observed.

Impact on reproduction

The reproduction data are shown in Table 4. There were no statistically significant effects on reproduction of Hypoaspis aculeifer up to and including the concentration of 1000 mg test item/kg soil (Dunnett's t-test, α = 0.05, one-sided smaller). Therefore, the NOEC for reproduction was determined to be ≥1000 mg test item/kg soil and the LOEC for reproduction was estimated to be >1000 mg test item/kg soil.

The EC values could not be determined by statistical analysis since there was no adequate concentration response. Therefore, the EC50 was estimated to be >1000 mg test item/kg soil.

Table 4. Reproduction of *Hypoaspis aculeifer* after 14 days

Treatment Group	number of juveniles per replicate[1]								mean	standard deviation	% of control	signifi-cance[2]
	1	2	3	4	5	6	7	8				
control	178	173	181	177	180	199	163	182	179	± 10	-	-
62.5	185	175	186	181	-	-	-	-	182	± 5	102	n.s.
125	191	180	198	179	-	-	-	-	187	± 9	104	n.s.
250	199	186	193	198	-	-	-	-	194	± 6	108	n.s.
500	172	208	167	190	-	-	-	-	184	± 19	103	n.s.
1000	168	168	172	181	-	-	-	-	172	± 6	96	n.s.

the results represent rounded values calculated from the exact raw data

test item dosages are given as mg test item/kg artificial soil dry weight

[1] mean of two counts; numbers underlined are median of three counts

[2] Dunnett's t-test, α = 0.05, one-sided smaller

- not applicable

n.s. not significantly different compared to the control

Conclusion

The No Observed Effect Concentration (NOEC) of CHR/H/CPD 300SL for mortality and reproduction of Hypoaspis aculeifer was determined to be ≥1000 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for mortality and reproduction was estimated to be >1000 mg test item/kg soil. The LC50 and EC50 values were estimated to be >1000 mg test item/kg soil.

Table 1. Effect of CHR/H/CPD 300SL on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study

CHR/H/CPD 300SL [mg/kg soil]	Control	62.5	125	250	500	1000
Mortality (day 14) [%]	1	3	3	0	3	5
Statistical significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 14)	179	182	187	194	184	172
Reproduction in [%] of control (day 14)	-	102	104	108	103	96
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.

	Endpoints [mg/kg soil]
NOEC (mortality)	≥1000
LOEC (mortality)	>1000
LC_{50} [3)]	>1000
NOEC (reproduction)	≥1000
LOEC (reproduction)	>1000
EC_{50} [3)]	>1000

n.s. = not statistically significantly different compared to the control

[1)] Fisher's Exact Test, α = 0.05, one-sided greater

[2)] Dunnett's t-test, α = 0.05, one-sided smaller

[3)] estimated value

- not applicable

Results of the reference test

Summary of results of most recent test (ibacon Project 105653089, performed in Nov 2016)

[mg Perfekthion/kg soil dry weight]	Control	3.95	5.71	8.29	12.00	17.34
[mg dimethoate/kg soil dry weight]		1.55	2.24	3.25	4.70	6.80
Mortality (day 14) [%]	5	5	5	33	98	93
Statistical significance[1)]	-	n.s.	n.s.	*	*	*
No. of juveniles (day 14)	198	196	215	181	24	0
Reproduction in [%] of control (day 14)	-	99	109	92	12	0
Statistical significance[2)]	-	n.s.	n.s.	*	*	*

	Endpoints [mg dimethoate/kg soil dry weight]
NOEC (mortality)	2.24
LC_{50} (mortality)[3)]	3.71
NOEC (reproduction)	2.24

	EC_{10}	EC_{20}	EC_{50}
EC Values (reproduction)	3.30	3.52	3.97
and 95% confidence limits[3)]	3.27 to 3.33	3.49 to 3.55	3.95 to 4.00

n.s. = not statistically significantly different compared to the control
* = statistically significantly different compared to the control
[1)] Fisher's Exact Test, α = 0.05, one-sided greater
[2)] Williams t-test, α = 0.05, one-sided smaller
[3)] Probit analysis
- not applicable

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the control:
• Mean mortality was 1% (criterion: ≤ 20%).
• Control Reproduction: 163 to 199 (criterion: ≥ 50 juveniles at the end of the test.
• the coefficient of variation for the number of juveniles: 5.6% (criterion: ≤ 30%).

A 2.4.2.2 KCP 10.4.2.1 Species level testing

No additional studies were performed.

A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

No additional studies were performed.

A 2.5 KCP 10.5 Effects on soil transformation

zRMS comment:
The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria given in OECD 216 (2000) are met.
The NOEC for Nitrate-Nitrogen concentrations at Day 28 is therefore 1.0605 mg a.i./kg dry soil.

Acute toxicity data for formulation CHR/H/CPD were evaluated during first registration.

Report:	KIIIA1 10.7.1/02, E.Grzebisz, 2014
Title:	CHR/H/CPD - Soil Microorganisms: Nitrogen transformation test
Document No:	Institute of Organic Industry, Pszczyna, Poland, Study Code G/07/12

Guidelines:	OECD Guideline 216, EU method C.21
GLP	Yes

Material and methods:

- CHR/H/CPD, Batch No: 22.01.2014 production date 22.01.2014, CoA issued by Pestinowa, Jaworzno, Poland,
- content of a.s.= 304.6 g a.s. according to Certificate of Analysis No 12/14 issued by Pestinowa, Jaworzno, Poland,
- Soil was amended with a nitrogen source, powdered Lucerne, and treated by incorporation of the test substance at 0.2121 and 1.0605 mg a.i./kg dry soil. An untreated control was also included in the study. The soil was incubated for a period of 28 days and samples were taken after treatment (Day 0) and on Day 7, 14 and 28. The soil samples were extracted by mixing the soil with a 0.1M KCl solution, centrifuging and the supernatant removed. The liquid extracts were kept frozen until analysis and analysed for Nitrate-Nitrogen.

- This study was designed to investigate the potential chronic effects of test substance on the nitrogen transformation activity of soil micro-organisms.

Findings:
The nitrate concentrations were within 25% of the control, with a difference of 12.770 and 8.868% at the 0.2121 and 1.0605 mg a.i./kg dose levels at Day 28, respectively.

The Validity criteria:

The following validity criterion was met for the study, therefore these data are considered to be valid.

- Variation in Nitrate-Nitrogen concentrations between replicate control samples should be less than ± 15%.

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

No additional studies were performed.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1.1 Study 1

zRMS comment:
The study is considered valid and accepted for risk assessment. It is GLP, performed according to OECD 208 (2006) and all validity criteria are met.
Results, according plant dry weight:
ER_{50} = 305 g product/ha (78.9 g a.s./ha) for sunflower,
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for cabbage,
ER_{50} = 67.2 g product/ha (17.4 g a.s./ha) for pea,
ER_{50} = 10.6 g product/ha (2.7 g a.s./ha) for carrot, (carrot was the most sensitive dicotyledon species).
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for perennial ryegrass,
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for perennial oats.

Some phytotoxic symptoms were observed after 14 days of the exposure:
- deformations (sunflower, pea, carrot)
- stunted growth (sunflower, pea, carrot)
- wilting (sunflower, pea)
The following order of the test plant sensitivity was noticed:
pea, carrot > sunflower > perennial ryegrass, oats, cabbage

Reference:	KCP 10.6/01
Report	Clopyralid 300 SL (CHR/H/CPD) Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test;P. Pieczka, Łukasiewicz Research Network – Institute Of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicological Studies Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/205/18
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Clopyralid 300 SL (CHR/H/CPD) active substances: clopyralid –25.88 ± 0.16 % (Appendix No. 1)
Test Design:	number of rates: 8 application rates for sunflower, cabbage, pea, carrot, perennial ryegrass and oats + control; number of replicates: 4 replicates/rate for carrot, perennial ryegrass, oats; 7 replicates/rate for cabbage, sunflower and pea. The total number of plants per application rate – 20 for carrot, oats, perennial ryegrass and 21 for cabbage, sunflower and pea test termination: 14 days after the emergence of 50% of the control seedlings
Test species:	sunflower (Helianthus annuus), cabbage (Brassica oleracea var. capitata), pea (Pisum sativum), carrot (Daucus carota), perennial ryegrass (Lolium perenne), oats (Avena sativa)
Endpoints:	ER25, ER50, NOER
Test Concentration:	0.4, 0.8, 2.7, 8.5, 25.9, 77.3, 231.8 and 695.5 g test item/ha (i.e. 0.1, 0.2, 0.7, 2.2, 6.7, 20.0, 60.0 and 180.0 g clopyralid/ha)– sunflower, cabbage, pea, carrot, perennial ryegrass and oats.
Test Conditions:	volume of deionised water used to prepare the highest rate: 300 L water/ha temperature: 17.8 – 24.9 °C, humidity: 47.1 – 91.5 %, lighting: 16 h light : 8 h dark; light intensity: 83.2 – 117.8 µE/m2/s; carbon dioxide concentration: 316 – 357 ppm

Results and discussion:

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of the test item/ ha for all test species are given below.

Endpoint	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	157.6	>695.5	166.0	54.1 (0.3 – 346.7)	>695.5	>695.5
NOER	77.3	≥ 695.5	77.3	25.9	≥ 695.5	≥ 695.5
Shoot length (plants without roots)						
ER_{50}	217.5	>695.5	85.3	171.1 (141.6 – 229.6)	>695.5	>695.5
NOER	77.3	≥ 695.5	25.9	25.9	≥ 695.5	≥ 695.5
Plant dry weight (plants without roots)						
ER_{50}	305.0	>695.5	67.2	10.6 (3.1 – 41.9)	>695.5	>695.5
NOER	231.8	≥ 695.5	25.9	2.7	≥ 695.5	≥ 695.5

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of clopyralid / ha for all test species are given below.

Endpoint	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	40.8	>180	43.0	14.0 (0.1 – 89.7)	>180	>180
NOER	20.0	≥ 180	20	6.7	≥ 180	≥ 180
Shoot length (plants without roots)						
ER_{50}	56.3	>180	22.1	44.3 (36.6 – 59.4)	>180	>180
NOER	20.0	≥ 180	6.7	6.7	≥ 180	≥ 180
Plant dry weight (plants without roots)						
ER_{50}	78.9	>180	17.4	2.7 (0.8 – 10.8)	>180	>180
NOER	60.0	≥ 180	6.7	0.7	≥ 180	≥ 180

Sunflower (Helianthus annuus)

After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, seedling emergence of sunflower was not delayed when compared with the control. The death of sunflower plants was observed at the rates equal to 231.1 and 695.5 g of the test item/ha.

After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the sunflower shoot length was between 0 and 110.7% of the control shoot length.

After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the sunflower shoot weight was between 0 and 115.9% of the control shoot weight.

After the application of the test item at the rates ranging from 8.5 to 695.5 g of the test item/ha, the plant damage as wilting, deformations and stunted growth was observed.

Cabbage (Brassica oleracea ver. capitata)

After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, seedling emergence of cabbage was not delayed when compared with the control. The death of cabbage plants was not observed.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the cabbage shoot length was between 82.7 and 115.8% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the cabbage shoot weight was between 88.1 and 117.4% of the control shoot weight.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the plant damage was not observed.

Pea (*Pisum sativum*)
After the application of the test item at the rates equal to 0.4 and 231.8 g of the test item/ha, seedling emergence of pea was not delayed when compared with the control. At the rate equal to 695.5 g of the test item/ha the seedling emergence of pea was not observed. The death of plant was observed at the rates equal to 77.3 and 231.8 g of the test item/ha.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the pea shoot length was between 0.0 and 123.7% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the pea shoot weight was between 0.0 – 110.4% of the control shoot weight.
After the application of the test item at the rates equal to 77.3 to 231.8 g of the test item/ha, the plant damage as wilting, deformations and stunted growth was observed.

Carrot (Daucus carota)
After the application of the test item at the rate equal to 231.8 g of the test item/ha, seedling emergence of carrot was delayed by one day when compared with the control. At the rate equal to 695.5 g of the test item/ha the seedling emergence of carrot was not observed. At the rates ranging from 25.9 to 231.8 g of the test item/ha the death of plants was observed.
After the application of the test item at the rates between 0.4 to 77.3 g of the test item/ha, the carrot shoot length was between 0.0 and 113.0% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 77.3 g of the test item/ha, the carrot shoot weight was between 0.0 and 102.7% of the control shoot weight.
After the application of the test item at the rates ranging from 8.5 to 695.5 g of the test item/ha, the plant damage as deformations and stunted growth was observed.

Perennial ryegrass (Lolium perenne)
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, seedling emergence of perennial ryegrass was not delayed when compared with the control. The death of one perennial ryegrass plant was observed only at the control group.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the perennial ryegrass shoot length was between 101.7 and 124.1% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the perennial ryegrass shoot weight was between 96.2 to 116.7% of the control shoot weight.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the plant damage was not observed.

Oats (Avena sativa)
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, seedling emergence of oats was not delayed when compared with the control. The death of plants were only observed at control group and rate equal to 25.9 g of the test item/ha.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the oats shoot length was between 94.4 and 112.6% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the oats shoot weight was between 92.5 and 122.2% of the control shoot weight.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the plant damage was not observed.

VALIDITY CRITERIA
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Clopyralid 300 SL (CHR/H/CPD) on seedling emergence and seedling growth of terrestrial plants were met:
- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
95.2% - sunflower,
85.7% - cabbage,
90.5% - pea,
90.0% – carrot,
90.0% – perennial ryegrass,
85.0% – oats,
- the mean survival of the emerged control seedlings (validity criterion: at least 90%) was as follows:
100.0% - sunflower,
100.0% - cabbage,
118.8 % - pea,
100.0% – carrot,
100.0% – perennial ryegrass,
100.0% – oats,
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

A 2.6.2.1.2 Study 2

zRMS comment:
The study is considered valid and accepted for risk assessment. It is GLP, performed according to relevant guideline OECD 227 (2006) and all validity criteria are met.
Results, according plant dry weight:
ER_{50} = 120.7 g product/ha (31.2 g a.s./ha) for sunflower,
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for cabbage,
ER_{50} = 25 g product/ha (6.5 g a.s./ha) for pea, (pea was the most sensitive dicotyledon species).
ER_{50} = 369.7 g product/ha (95.7 g a.s./ha) for carrot,
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for perennial ryegrass,
ER_{50} > 695.5 g product/ha (>180 g a.s./ha) for perennial oats.

Some phytotoxic symptoms were observed on pea, sunflower, cabbage and carrot after 21 days of the exposure. These were:
- stunted growth
- deformations
- wilting
- chlorosis
- necrosis
In cultivation of perennial ryegrass and oats there were no changes in appearance.
The following order of the test plant sensitivity was noticed: sunflower, pea> carrot > cabbage, perennial ryegrass, oats.

Reference:	KCP 10.6/02
Report	Clopyralid 300 SL (CHR/H/CPD)Terrestrial Plant Test: Vegetative Vigour Test; P. Pieczka; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G/204/18

Guideline(s):	according to the OECD Guideline No. 227 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Clopyralid 300 SL (CHR/H/CPD) batch no: 1 active substance: clopyralid – 25.88 ± 0.16 %
Test species:	sunflower (Helianthus annuus), cabbage (Brassica oleracea var. capitata), pea (Pisum sativum), carrot (Daucus carota), perennial ryegrass (Lolium perenne), oats (Avena sativa).
Test Design:	number of rates: 8 application rates + control; number of replicates: 4 replicates/rate for carrot, oats, perennial ryegrass and 7 replicates/rate for cabbage, pea, sunflower . The total number of plants per application rate – 20 for carrot, oats, perennial ryegrass and 21 for sunflower, pea, cabbage. Test termination: 21 days after the spraying.
Endpoints:	ER25, ER50, NOER
Test Concentration:	0.4, 0.8, 2.7, 8.5, 25.9, 77.3, 231.8 and 695.5 g test item/ha (i.e. 0.1, 0.2, 0.7, 2.2, 6.7, 20.0, 60.0 and 180.0 g clopyralid/ha) – sunflower, cabbage, pea, carrot, perennial ryegrass and oats.
Test Conditions:	temperature: 17.8 – 24.9°C, humidity: 47.1 – 91.5%, controlled light – dark cycles (16h:8h), light intensity: 89.7 – 126.3 µE/m2/s, carbon dioxide concentration: 312 – 337 ppm

Results and discussion:

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of the test item/ ha for all test species are given below:

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	177.8 (144.4 – 216.3)	> 695.5	396.9	> 695.5	> 695.5	> 695.5
NOER	77.3	≥ 695.5	231.8	> 695.5	> 695.5	> 695.5
Shoot length (plants without roots)						
ER_{50}	122.2 (77.7 – 225.4)	> 695.5	30.6 (17.9 – 56.9)	> 695.5*	> 695.5	> 695.5
NOER	0.8	≥ 695.5	2.7	77.3	≥ 695.5	≥ 695.5
Plant dry weight (plants without roots)						
ER_{50}	120.7 (70.4 – 272.0)	> 695.5	25.0 (13.8 – 47.5)	369.7 (318.2 – 434.3)	> 695.5	> 695.5
NOER	2.7	≥ 695.5	8.5	77.3	≥ 695.5	≥ 695.5

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of clopyralid/ha for all test species are given below:

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	46.0 (37.4 – 56.0)	> 180	102.7	> 180	> 180	> 180
NOER	20.0	≥ 180	60.0	> 180	> 180	> 180
Shoot length (plants without roots)						
ER_{50}	31.6 (20.1 – 58.3)	> 180	7.9 (4.6 – 14.7)	> 180*	> 180	> 180
NOER	0.2	≥ 180	0.7	20	≥ 180	≥ 180
Plant dry weight (plants without roots)						
ER_{50}	31.2 (18.2 – 70.4)	> 180	6.5 (3.6 – 12.3)	95.7 (82.4 – 112.4)	> 180	> 180
NOER	0.7	≥ 180	2.2	20.0	≥ 180	≥ 180

Sunflower (Helianthus annuus)

After the application of the test item at the rates ranging from 0.4 to 25.9 g of the test item/ha, plant mortality was not observed. Mortality of plant was observed at concentrations between 77.3 to 695.3 g of the test item/ha and it was between 4.8 – 100% in comparison to the control group.

After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the sunflower shoot length was between 0 – 96.5% of the control shoot length.

After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the sunflower shoot dry weight was between 0 – 111.5% of the control shoot weight.

Cabbage (Brassica oleracea var. capitata)
After the application of the test item the plant mortality was observed only at concentrations equal to 0.4 and 0.8 g of the test item/ha and it was equal to 4.8% in comparison to the control group. At the rates between 2.7 to 695.5 g of the test item/ha no plant mortality was noticed.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the cabbage shoot length was between 91.4 – 106.9% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the cabbage shoot dry weight was between 97.3 – 108.8% of the control shoot weight.
After the application of the test item at the rates ranging from 77.3 to 695.5 g of the test item/ha, the plant damage as deformations were observed.

Pea (Pisum sativum)
After the application of the test item at the rates ranging from 0.4 to 77.3 g of the test item/ha, plant mortality was not observed. Mortality of plants was observed at the rates equal to 231.8 and 695.5 g of the test item/ha and it was between 4.8 – 100% in comparison to the control group.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the pea shoot length was between 0 – 111.0% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the pea shoot dry weight was between 0 – 105.3% of the control shoot weight.
After the application of the test item at the rates ranging from 2.7 to 695.5 g of the test item/ha, the plant damage as deformations, chlorosis, necrosis wilting and stunted growth were observed.

Carrot (Daucus carota)
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, plant mortality was not observed.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the carrot shoot length was between 65.9 – 118.1% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the carrot shoot dry weight was between 31.9 – 113.6% of the control shoot weight.
After the application of the test item at the rates ranging from 2.7 to 695.5 g of the test item/ha, the plant damage as deformations, chlorosis and stunted growth were observed.

Perennial ryegrass (Lolium perenne)
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, plant mortality was not observed.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the perennial ryegrass shoot length was between 95.5 – 112.9% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the perennial ryegrass shoot dry weight was between 92.3 – 107.8% of the control shoot weight.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the plant damage was not observed.

Oats (Avena sativa)
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, plant mortality was not observed.
After the application of the test item at the rates between 0.4 to 695.5 g of the test item/ha, the oats shoot length was between 94.5 – 105.7% of the control shoot length.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the oats shoot dry weight was between 91.6 – 111.1% of the control shoot weight.
After the application of the test item at the rates ranging from 0.4 to 695.5 g of the test item/ha, the plant damage was not observed.

CONCLUSIONS

1. The test item, i.e. Clopyralid 300 SL (CHR/H/CPD) applied at rates ranging from 0.4 to 695.5 g of the test item/ha had no impact on vegetative vigour of perennial ryegrass and oats. The test item, had an im-

pact on vegetative vigour of sunflower, pea, carrot and slightly on cabbage.
2. The test item did not cause mortality of carrot, perennial ryegrass and oats at rates ranging from 0.4 to 695.5 g of the test item/ha. The test item caused mortality of pea at rates equal to 231 and 695.5 g of the test item/ha and sunflower at rates: 77.3; 231.8 and 695.5 g of the test item/ha. The mortality of cabbage at rates equal to 0.4 and 0.8 g of the test item/ha was accidental and it was not related with the test item.
3. On the basis of NOER, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibit the process of growth of sunflower, pea and carrot.
4. On the basis of NOER, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of sunflower, pea and carrot.
5. Some phytotoxic symptoms were observed on pea, sunflower, cabbage and carrot after 21 days of the exposure. These were:
- stunted growth
- deformations
- wilting
- chlorosis
- necrosis
In cultivation of perennial ryegrass and oats there were no changes in appearance
6. The following order of the test plant sensitivity was noticed:
Sunflower, pea> carrot > cabbage, perennial ryegrass, oats.

VALIDITY CRITERIA
The following validity criteria were met:
- the seedling emergence (validity criterion: at least 70%) was as follows:
88.3 – 90.5% – sunflower,
81.0 – 85.7%– cabbage,
73.8 – 85.7%– pea,
87.5 – 97.5% – carrot,
87.5 – 92.5% – perennial ryegrass,
87.5 – 97.5% – oats,
- the mean survival of the emerged control seedlings was 100% in sunflower, cabbage, pea, carrot, perennial ryegrass and oats,
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

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

No additional studies were performed.

A 2.8 KCP 10.8 Monitoring data

No additional studies were performed.