FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: CHR/H/CFF 250 EC

Product name(s): Hapi 250 EC/ Turango 250 EC

Chemical active substance(s):

Florasulam, 10 g/L
Fluroxypyr-acid, 120 g/L (fluroxypyr-meptyl, 172.9 g/L)
Cloprylid, 120 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: March 2023

MS Finalisation date: 07.2024; 08.2024; 09.2024; 11.2024; 04.2025; 07.2025

Version history

When	What
07.2024	Applicant update and zRMS PL Evaluation
08.2024	Applicant update and zRMS PL Evaluation
09.2024	Assessment by ZRMS
11.2024	The final Registration Report
04.2025	zRMS updated
07.2025	zRMS updated

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9 Ecotoxicology (KCP 10)

In the following document, data for active substances - Fluroxypyr - was described during its inclusion on Annex 1 process in respectively 2009. Were reference to active substance data in the current risk assessment has been made, it was based on the data which protection for expired 10 years from date of inclusion of active substances on Annex I.
Data matching studies for florasulam have been evaluated by Poland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the authorization of CHR/H/CFF 250 EC INNVIGO is allowed to refer to EU approved reports
Data matching studies for clopyralid have been evaluated by RMS - Finland. As a result of the assessment all re-ports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CFF 250 EC INNVIGO is allowed to refer to EU approved reports

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

GAP rev. , date: 2021-01-13

PPP product name:		Formulation type:	EC [a, b]
product code:	CHR/H/CFF		
Active substance 1:	clopyralid	Conc. of as 1:	120 g/l [c]
Active substance 2:	fluroxypyr	Conc. of as 2:	120 g/l [c]
Active substance 3:	florasulam	Conc. of as 3:	10 g/l (c)
Safener:	-	Conc. of safener:	- [c]
Synergist:	-	Conc. of synergist:	- [c]
Applicant:	Innvigo Sp. z o.o.	Professional use:	☒
Zone(s):	Central [d]	Non professional use:	☐
Verified by MS:	no		

Field of use: herbicide

1	2	3	4	5	6	7	8	9	15	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)	ZRMs Conclusion
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			
Zonal uses (field or outdoor uses, certain types of protected crops)														
1	PL	Winter wheat (TRZAW), Winter triticale (TTLWI)	F	dicotyledonous weeds	Spray, medium sprayer	spring BBCH 21-33	a)1 b)1	n/a	a) 0.4 - 0.5 l/ha b) 0.4 - 0.5 l/ha	a) 0.1 - 0.125 kg a.s./ha (0.048 CLO + 0.048 FLUROX + 0.004 FLO) –(0.06 CLO + 0.06 FLUROX + 0.005 FLO) b) 0.1 - 0.125 kg a.s./ha (0.048 CLO + 0.048 FLUROX + 0.004 FLO) –(0.06 CLO + 0.06 FLUROX + 0.005 FLO)	200-400	n/a		**A**
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)														
2														
3														

Minor uses according to Article 51 (zonal uses)														
4	PL	Spelt *Triticum spelta* (3SPWC) Emmer wheat *Triticum dicoccum* (TRZDI) Einkorn wheat *Triticum monococcum (TRZMO)* Durum wheat *Triticum durum* (TRZDW) Spring Rye *Secale cereale* (SECCS)	F	Monaocots and dicots weeds	Spray, medium sprayer	BBCH 21-33	a)1 b)1	n/a	a) 0.4 - 0.5 l/ha b) 0.4 - 0.5 l/ha	a) 0.1 - 0.125 kg a.s./ha (0.048 CLO + 0.048 FLUROX + 0.004 FLO) –(0.06 CLO + 0.06 FLUROX + 0.005 FLO) b) 0.1 - 0.125 kg a.s./ha (0.048 CLO + 0.048 FLUROX + 0.004 FLO) –(0.06 CLO + 0.06 FLUROX + 0.005 FLO)	200-400		.	**A**
5														
Minor uses according to Article 51 (interzonal uses)														
6														
7														

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at 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
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

zRMS comment: All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

9.1.1 Overall conclusions

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

CHR/H/CFF 250 EC 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/CFF 250 EC pose no unacceptable risk to aquatic organisms according to the label.

9.1.1.3 Effects on bees (KCP 10.3.1)

CHR/H/CFF 250 EC 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/CFF 250 EC pose no unacceptable risk to NTA according to the label

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/CFF 250 EC 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/CFF 250 EC pose no unacceptable risk to non-target terrestrial plants according to the label with appropriate buffer zone and drift reducing techniques.:

- 5 m buffer zone
- 1 m and use of 75 % drift reducing nozzles

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 9.1-2: Critical use pattern of CHR/H/CFF 250 EC grouped according to criterion

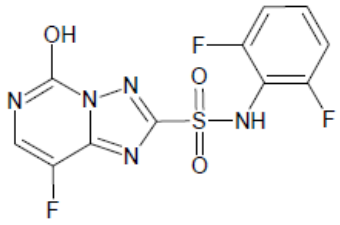
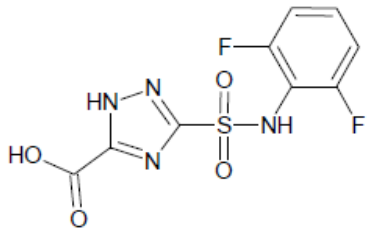
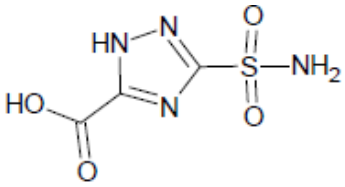
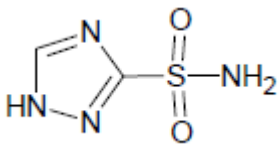
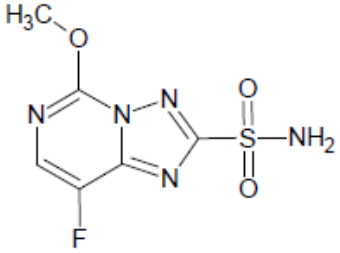
Grouping according to crop, application rate, number of application, timing criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**

Grouping according to crop, application rate, number of application, timing criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	Winter Cereals BBCH 21-33 543.1 g [product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of CHR/H/CFF 250 EC is indicated in the table.

Table 9.1-3: Metabolites of florasulam potentially relevant for exposure assessment

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
5-OH florasulam	345.26		Soil (lab): max 71.6 % at 3-7 d (n= 5) Maximum detected in aquatic environment: 99.0%	Yes
DFP-ASTCA	304.20		Soil (Lab): max 17.8 % at 14-59 d (n= 5) Maximum detected in aquatic environment: 8.9%	Yes
ASTCA	192.13		Soil (Lab): max 40.0 % (n= 4) at 59-100 d Maximum detected in aquatic environment: 53.8%	Yes
TSA	148.14		Soil (Lab): max 15.9 % (n= 4) at 14 - 100 d Maximum detected in aquatic environment: 0.0001	Yes
ASTP	247.20		Maximum detected in aquatic environment: 21.9%	Yes

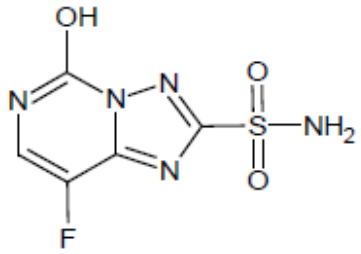
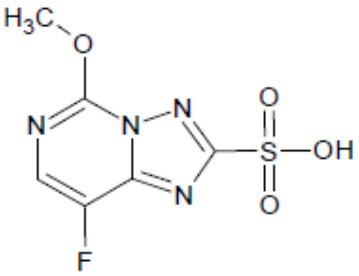
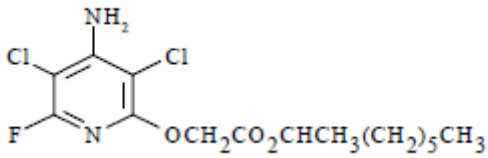
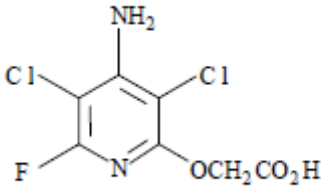
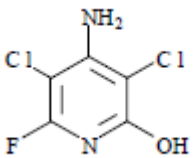
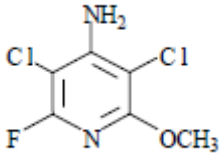
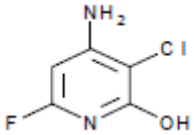
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
5-OH ASTP	233.18		Maximum detected in aquatic environment: 28.9%	Yes
TPSA	248.17		Maximum detected in aquatic environment: 58.3%	Yes

Table 9.1-4: Metabolites of fluroxypyr potentially relevant for exposure assessment

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
Fluroxypyr-MHE	367.3 g/mol		100%	Yes
Fluroxypyr acid	255 g/mol		Maximum occurrence observed: 100% in aquatic system (calculations performed as for parent)	Yes
Pyridinol	197 g/mol		Maximum occurrence observed (% molar basis with respect to the parent) Soil: 23.9 % Water: 44 Sediment: 11.5	Yes

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
Methoxypyridine	211 g/mol		Maximum occurrence observed (% molar basis with respect to the parent) Soil: 38.2 Water: not water/sediment metabolite Sediment: not water/sediment metabolite	Yes
3-CP	162 g/mol		Maximum occurrence observed (% molar basis with respect to the parent) Water: 17.9 Sediment: 6.5	Yes

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Species	Substance	Exposure System	Results	Reference
Japanese quails Coturnix coturnix japonica	Florasulam	Oral 1 d Acute	LD50= 1046 mg a.s./kg bw per day	EFSA Journal 2015; 13(1):3984
Mallard duck (Anas platyrhynchos)	Florasulam	Dietary Reproductive toxicity	Endpoint use in long-term risk assessment is LD50 for florasulam of 1046 mg/kg bw divided by 10. The resulting value is lower than the NOEC from reproductive study for florasulam of 1500 mg/kg diet multiplied by a factor 0.1.	EFSA Journal 2015; 13(1):3984
Mallard duck	Clopyralid	Acute	LD_{50} =1465 mg/kg bw per day	EFSA Journal 2018;16(7):5389
Mallard duck	Clopyralid	Long term	NOEC=118 mg/kg bw per day	EFSA Journal 2018;16(7):5389
Bobwhite quail	Fluroxypyr- MHE	Acute	> 2000 mg/kg bw/day	EFSA Journal 2011;9(3):2091
Bobwhite quail	Fluroxypyr-acid	Acute	> 2000 mg/kg bw/day	EFSA Journal 2011;9(3):2091

Species	Substance	Exposure System	Results	Reference
Mallard Duck	Fluroxypyr-MHE	Long-term	57.8 mg/kg bw/day	EFSA Journal 2011;9(3):2091
Mallard Duck	Fluroxypyr-acid	Long termy	40.1 mg/kg bw/day	EFSA Journal 2011;9(3):2091

9.2.2 Risk assessment for spray applications

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

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

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/CFF 250 EC in cereals winter for the florasulam

Intended use					
Active substance/product	Florasulam				
Application rate (g/ha)	1 x 4.8				
Acute toxicity (mg/kg bw)	1046				
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	0.79	1317.4
Reprod. toxicity (mg/kg bw/d)	104.6				
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	0.17	609.1

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/CFF 250 EC in cereals winter for the clopyralid

Intended use					
Active substance/product	Clopyralid				
Application rate (g/ha)	1 x 60				
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	9.53	153.8
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omnivorous bird	64.8	0.53	2.06	57.3

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

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CFF 250 EC in cereals winter for the fluroxypyr-MHE

Intended use					
Active substance/product	Fluroxypyr-MHE				
Application rate (g/ha)	1 x 86.4				
Acute toxicity (mg/kg bw)	2000				
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	13.72	145.8
Reprod. toxicity (mg/kg bw/d)	57.8				
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	2.97	19.5

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

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/CFF 250 EC in cereals winter for the fluroxypyr acid

Intended use					
Active substance/product	Fluroxypyr acid				
Application rate (g/ha)	1 x 60				
Acute toxicity (mg/kg bw)	2000				
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	9.53	209.9
Reprod. toxicity (mg/kg bw/d)	40.1				
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	2.06	19.5

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

Combined risk assessment for CHR/H/CFF 250 EC mixture

At the screening assessment following formula was used:

All TER values > Trigger x n (n = number active substances in the mixture - 3)

TER_A Florasulam	**TER_A Clopyralid**	**TER_A Fluroxypyr-MHE**	**Trigger value**
1317.4	153.8	145.8	30
TER_{LT} Florasulam	**TER_{LT} Clopyralid**	**TER_{Lt} Fluroxypyr-MHE**	**Trigger value**
609.1	57.3	19.5	15

TER_A Florasulam	**TER_A Clopyralid**	**TER_A Fluroxypyr-acid**	**Trigger value**
1317.4	153.8	209.9	30
TER_{LT} Florasulam	**TER_{LT} Clopyralid**	**TER_{Lt} Fluroxypyr-acid**	**Trigger value**
609.1	57.3	19.5	15

Conclusion

The calculated TERmix and TER for individual active substance value is higher than the trigger value of 10 for acute risk assessment and higher than the trigger value of 5 for chronic risk assessment , indicating CHR/H/CFF 250 EC does not posses unacceptable acute and long-term risk for mammals.
No further risk refinement is needed.

zRMS comment:
Clopyralid

The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. The toxicity endpoints for clopyralid was line in the LoEP (EFSA Journal 2018;16(7):5389). Safe use of clopyralid for birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for Turango 250 EC no unacceptable risk for birds is expected from acute or long-term exposure.

Fluroxypyr

The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. Toxicity endpoints were used from EFSA Journal 2011;9(3):2091. Safe use of fluroxypyr 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 Turango 250 EC no unacceptable risk for birds is expected from acute or long-term exposure.

Forasulam
The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. Toxicity endpoints were used from **EFSA Journal 2015; 13(1):3984)**. Safe use of florasulam 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 Turango 250 EC no unacceptable risk for birds is expected from acute or long-term exposure.

Combined risk assessment for Turango 250 EC
The combined risk assessment for Turango 250 EC for birds was accepted by RMS.
No further risk refinement is needed.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since CHR/H/CFF 250 EC is not a product for spray applications / 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 10.35, Florasulam belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	5		
Acute toxicity (mg/kg bw)	=	1046	quotient =	0.0048
Reprod. toxicity (mg/kg bw/d)	=	104.6	quotient =	0.048

With a K(f)oc of 1.41, Clopyralid belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	1465	quotient =	0.0410
Reprod. toxicity (mg/kg bw/d)	=	118	quotient =	0.508

With a K(f)oc of 19550, Fluroxypyr-MHE belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	86.4		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.0432
Reprod. toxicity (mg/kg bw/d)	=	57.8	quotient =	1.50

With a K(f)oc of 68, Flurxypyr-acid belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	40.1	quotient =	1.50

zRMS comment: **Agreed.**

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Florasulam is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Fluroxypyr (Fluroxypyr-MHE rapidly degradate to Fluroxypyr acid) is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Clopyralid is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment: Agreed**. zRMS comment:** Agreed. However, the log Pow of methoxypyridine amounts to 3.09 and thus does exceed the trigger value of 3, indicating a potential risk of secondary poisoning. Therefore, a risk assessment for effects due to secondary poisoning is required. The risk assessment was performed by zRMS.
Assessment of the risk for earthworm-eating mammals due to exposure to methoxypyridine via bioaccumulation in earthworms (secondary poisoning).

Parameter	Methoxypyridine	Comments
$PEC_{soil\ accumulation}$ (mg/kg soil)	0.0974	
log P_{ow} / P_{ow}	3.09	
Koc	321.4	Arithmetic mean (n = 4)
foc	0.02	Default
BCF_{worm}	0.137	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.0133	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.014	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	4.01	
TERlt	286	

TER values shown in bold fall below the relevant trigger.

Methoxypyridine is not water/sediment metabolite. Due to risk assessment for fish-eating mammals via secondary poisoning is not it is not necessary.

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 florasulam, clopyralid, fluroxypyr was found acceptable. CHR/H/CFF 250 EC 750 WG pose no unacceptable risk to birds with according to the label

zRMS comment: Agreed. Based on the intended use on for Turango 250 EC no unacceptable risk for birds is expected from acute or long-term exposure.

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

However, the provision of further data on the formulation CHR/H/CFF 250 EC is not considered essential, because 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 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Mouse	Florasulam	Acute	LD50>5000 mg a.s./kg bw	EFSA Journal 2015; 13(1):3984
Rat	Florasulam	Long term	NOEL>100 mg a.s./kg bw/d	EFSA Journal 2015; 13(1):3984
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
Rat	Fluroxypyr MHE	Acute	>2000 mg/kg bw	EFSA Journal 2011;9(3):2091
Rat	Fluroxypyr acid	Acute	➢ 1388.89 mg/kg bw/day (Endpoint obtained by recalculation of the acute fluroxypyr-meptyl endpoin)	EFSA Journal 2011;9(3):2091

Species	Substance	Exposure System	Results	Reference
Rabbit	Fluroxypyr MHE	Long-term	144 mg/kg bw/day (Endpoint obtained by recalculation of the long term fluroxypyr-acid endpoin – worst case)	EFSA Journal 2011;9(3):2091
Rabbit	Fluroxypyr acid	Long-term	100 mg/kg bw/day	EFSA Journal 2011;9(3):2091

9.3.2 Risk assessment for spray applications

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

9.3.2.1 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 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CFF 250 EC in winter cereals for florasulam

Intended use					
Active substance/product	Florasulam				
Application rate (g/ha)	1 × 5				
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	0.59	8445.9
Reprod. toxicity (mg/kg bw/d)	100				
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	0.13	781.28

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 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CFF 250 EC in winter cereals for clopyralid

Intended use	
Active substance/product	Clopyralid
Application rate (g/ha)	1 × 60
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	7.10	703.8
Reprod. toxicity (mg/kg bw/d)	50				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small herbivorous mammal	48.3	0.53	1.54	32.55

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

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CFF 250 EC in winter cereals for fluroxypyr-MHE

Intended use	
Active substance/product	Fluroxypyr-MHE
Application rate (g/ha)	1 × 86.4
Acute toxicity (mg/kg bw)	2000
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	10.23	195.5
Reprod. toxicity (mg/kg bw/d)	~~200~~ 144 mg/kg bw/day (Endpoint obtained by recalculation of the long term fluroxypyr-acid endpoin – worst case)				
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	2.21	65.11

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 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/CFF 250 EC in winter cereals for fluroxypyr-acid

Intended use	
Active substance/product	Fluroxypyr-acid
Application rate (g/ha)	1 × 60
Acute toxicity (mg/kg bw)	1388.89
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	7.1	195.5
Reprod. toxicity (mg/kg bw/d)	100				
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	1.54	65.11

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.

Combined risk assessment for CHR/H/CFF 250 EC mixture

At the screening assessment following formula was used:

All TER values > Trigger x n (n = number active substances in the mixture - 3)

TER_A Florasulam	**TER_A Clopyralid**	**TER_A Fluroxypyr-MHE**	**Trigger value**
8445.9	703.8	195.5	30
TER_{LT} Florasulam	**TER_{LT} Clopyralid**	**TER_{Lt} Fluroxypyr-MHE**	**Trigger value**
781.28	32.55	65.11	15

Combined risk assessment for CHR/H/CFF 250 EC mixture

At the screening assessment following formula was used:

All TER values > Trigger x n (n = number active substances in the mixture - 3)

TER_A Florasulam	**TER_A Clopyralid**	**TER_A Fluroxypyr-acid**	**Trigger value**
8445.9	703.8	195.5	30
TER_{LT} Florasulam	**TER_{LT} Clopyralid**	**TER_{Lt} Fluroxypyr-acid**	**Trigger value**
781.28	32.55	65.11	15

Conclusion

The calculated TERmix and TER for individual active substance value is higher than the trigger value of 10 for acute risk assessment and higher than the trigger value of 5 for chronic risk assessment , indicating CHR/H/CFF 250 EC does not posses unacceptable acute and long-term risk for mammals. No further risk refinement is needed.

zRMS comment:

Clopyralid

The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. The toxicity endpoints for clopyralid was line in the LoEP (EFSA Journal 2018;16(7):5389). Safe use of clopyralid for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for Turango 250 EC no unacceptable risk for mammals is expected from acute or long-term exposure.

Fluroxypyr

The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. Toxicity endpoints were used from EFSA Journal 2011;9(3):2091. Safe use of fluroxypyr for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for Turango 250 EC no unacceptable risk for mammals is expected from acute or long-term exposure.

Forasulam

The risk assessment at first-tier assessment step is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. Toxicity endpoints were used from **EFSA Journal 2015; 13(1):3984).** Safe use of florasulam for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. Based on the intended use on for Turango 250 EC no unacceptable risk for mammals is expected from acute or long-term exposure.

Combined risk assessment for Turango 250 EC
The combined risk assessment for Turango 250 EC for mammals was accepted by RMS.
No further risk refinement is needed.

9.3.2.2 Higher-tier risk assessment

Not required.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc of 10.35, Florasulam belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	5		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.0048
Reprod. toxicity (mg/kg bw/d)	=	100	quotient =	0.048

With a K(f)oc of 1.41, Clopyralid belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.0410
Reprod. toxicity (mg/kg bw/d)	=	50	quotient =	0.508

With a K(f)oc of 19550, Fluroxypyr-MHE belongs to the group of less/more sorptive substances. To achieve a con-

cise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	86.4		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.0432
Reprod. toxicity (mg/kg bw/d)	=	144	quotient =	1.50

With a K(f)oc of 68, Flurxypyr-acid belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter/spring cereals:

Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	1388.89	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	100	quotient =	1.50

zRMS comment: Agreed.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Florasulam is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Fluroxypyr (Fluroxypyr-MHE rapidly degradate to Fluroxypyr acid) is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Clopyralid is below 3 and thus no exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment: Agreed. However, the log Pow of methoxypyridine amounts to 3.09 and thus does exceed the trigger value of 3, indicating a potential risk of secondary poisoning. Therefore, a risk assessment for effects due to secondary poisoning is required. The risk assessment was performed by zRMS.

Assessment of the risk for earthworm-eating mammals due to exposure to methoxypyridine via bioaccumulation in earthworms (secondary poisoning).

Parameter	Methoxypyridine	Comments
$PEC_{soil\ accumulation}$ (mg/kg soil)	0.0974	
log P_{ow} / P_{ow}	3.09	
Koc	321.4	Arithmetic mean (n = 4)
foc	0.02	Default
BCF_{worm}	0.137	$BCF_{worm/soil}$ = $(PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.0133	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.017	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TERlt	588	

TER values shown in bold fall below the relevant trigger.

Methoxypyridine is not water/sediment metabolite. Due to risk assessment for fish-eating mammals via secondary poisoning is not it is not necessary.

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 florasulam, clopyralid, fluroxypyr was found acceptable. CHR/H/CFF 250 EC 750 WG pose no unacceptable risk to mammals with according to the label.

zRMS comment: Agreed. Based on the intended use on for Turango 250 EC no unacceptable risk for mammals is expected from acute or long-term exposure.

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 florasulam, clopyralid, fluroxypyr and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of florasulam, clopyralid, fluroxypyr. 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 – Florasulam and relevant metabolites

Species	Substance	Exposure System	Results mg/L	Reference
Oncorhynchus mykiss, Lepomis macrochirus	Florasulam	Acute static	96-h LC50 >100 nom	EFSA Journal 2015; 13(1):3984
Pimephales promelas	Florasulam	Chronic flow through (juveniles)	33-d NOEC ELS 2.9 mm	EFSA Journal 2015; 13(1):3984
Daphnia magna	Florasulam	Acute static	48-h EC50 >292 m	EFSA Journal 2015; 13(1):3984
Daphnia magna	Florasulam	Chronic semi-static	21-d NOEC= 23.4 nom	EFSA Journal 2015; 13(1):3984
Chironomus riparius	Florasulam	Chronic semi-static	28 day NOEC=	EFSA Journal 2015;

Species	Substance	Exposure System	Results mg/L	Reference
			10 nom	13(1):3984
Pseudokirchneriella subcapitata	Florasulam	Static	72-h ErC50= 0.00894 mm	EFSA Journal 2015; 13(1):3984
Lemna gibba	Florasulam	Semi-static	14-day EC50= 0.00118 im	EFSA Journal 2015; 13(1):3984
Oncorhynchus mykiss	5-OH-florasulam	Acute static	96-h LC50 >91 nom	EFSA Journal 2015; 13(1):3984
Daphnia magna	5-OH-florasulam	Acute static	48-h EC50 >96.7 mm	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	5-OH-florasulam	Static	72-h EbC50=21.32 mm 72-h ErC50= 21.57 mm	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-florasulam	Semi-static	7-d EC50=0.0378 mm	EFSA Journal 2015; 13(1):3984
Daphnia magna	DFP-ASTCA	Acute static	48-h EC50 >0.030 nom	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	DFP-ASTCA	Static	72-h EyC50=96 nom	EFSA Journal 2015; 13(1):3984
Lemna gibba	DFP-ASTCA	Semi-static	7-d EyC50 & ErC50 >100 nom	EFSA Journal 2015; 13(1):3984
Daphnia magna	ASTCA	Acute static	48-h EC50 >0.030 nom	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	ASTCA	Static	72-h & 96-h EC50, EbC50 & ErC50>9.2 mm	EFSA Journal 2015; 13(1):3984
Lemna gibba	ASTCA	Semi-static	7-d & 14-d EC50 >10.2 nom	EFSA Journal 2015; 13(1):3984
Daphnia magna	TSA	Acute static	48-h EC50 >0.030 nom	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	TSA	Static	72 h EC50, EyC50& ErC50>94 mm	EFSA Journal 2015; 13(1):3984
Lemna gibba	TSA	Semi-static	7-d EyC50 & ErC50 >100 nom	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	5-OH-ASTP	Static	72-h & 96-h EC50, EyC50 & ErC50>100 nom	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-ASTP	Semi-static	7-d EyC50 & ErC50 >100 nom	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	ASTP	Static	72-h & 96-h EC50, EyC50 & ErC50>100 nom	EFSA Journal 2015; 13(1):3984
Lemna gibba	ASTP	Semi-static	7-d EyC50 (frond no.)=88 mm	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results mg/L	Reference
Higher-tier studies (micro- or mesocosm studies)				
No further tests submitted				

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

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

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 capricornu-tum	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 9.5-3: **Endpoints and effect values relevant for the risk assessment for aquatic organisms – Fluroxypyr**

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Fluroxypyr-MHE	96h	LC50 > 0.225 mg/L	EFSA Journal 2011;9(3):2091
Oncorhynchus mykiss	Fluroxypyr-MHE	21 d	NOEC= 0.2 mg/L	EFSA Journal 2011;9(3):2091
Lepomis macrochirus	Fluroxypyr-acid	96 h	LC_{50} = 14.3 mg/L	EFSA Journal 2011;9(3):2091
Oncorhynchus mykiss	fluroxypyr- acid	21-d	NOEC= 100 mg/L	EFSA Journal 2011;9(3):2091
Oncorhynchus mykiss	Pyridinol	96 h	LC_{50} = 39 mg/L	EFSA Journal 2011;9(3):2091
Oncorhynchus mykiss	3-CP	96 h	LC_{50} = 95.1 mg/L	EFSA Journal 2011;9(3):2091
Daphnia magna	Fluroxypyr-MHE	48 h	EC_{50} > 0.183 mg/L	EFSA Journal

Species	Substance	Exposure System	Results	Reference
				2011;9(3):2091
Daphnia magna	Fluroxypyr-MHE	21 d	NOEC= 0.0605 mg/L mg/L	EFSA Journal 2011;9(3):2091
Daphnia magna	Fluroxypyr-acid	48 h	EC_{50}> 100 mg/L	EFSA Journal 2011;9(3):2091
Daphnia magna	fluroxypyr- acid	21 d	NOEC=56 mg/L	EFSA Journal 2011;9(3):2091
Daphnia magna	Pyridinol	48 h	EC_{50}> 49 mg/L	EFSA Journal 2011;9(3):2091
Daphnia magna	3-CP	48 h	EC_{50}> 7.56 mg/L	EFSA Journal 2011;9(3):2091
Chironomus riparius	Fluroxypyr-MHE	48 h	EC_{50}> 9.6 mg/L	EFSA Journal 2011;9(3):2091
Skeletonema costatum	Fluroxypyr-MHE	72 h	EC50 = 0.208 mg/l	EFSA Journal 2011;9(3):2091
Navicula pelliculosa	fluroxypyr- acid	96h	72h EbC50 = 26.0 mg/L 72h ErC50= 35.3 mg/L 96 h EC50 = 36.2 mg/L	EFSA Journal 2011;9(3):2091
Navicula pelliculosa	Pyridinol	120 h	120 d EC50 (cell density) > 3 mg/L 72h ErC50 = 2.7 mg/L 72 h EC50 = 0.640 mg/L	EFSA Journal 2011;9(3):2091
Selenastrum capricornutum	3-CP	96 h	72h EC50 = 35.0 mg/L 72h ErC50 = 46.3 96h EC50 (cell density) = 35.8 mg/L 96h EbC50 = 42.6 mg/L	EFSA Journal 2011;9(3):2091
Anabaena flos-aquae	Methoxypyridine	120 h	72h EC 50 < 1.12 mg/L 72 ErC50 = 3.16 mg/L 120 days EC50 (cell density) = 1.80 mg/L 120 ErC50 = 2.23 mg/L	EFSA Journal 2011;9(3):2091
Lemna gibba	Fluroxypyr-MHE	14 d	EC50 > 2.31 mg/L 72	EFSA Journal 2011;9(3):2091
Lemna gibba	Fluroxypyr-acid	14 d	LC50 = 12.3 mg/L	EFSA Journal 2011;9(3):2091
Lemna gibba	Pyridinol	14 d	EC50 > 10.6 mg/L	EFSA Journal 2011;9(3):2091
Lemna gibba	Methoxypyridine	14 d	EC50 > 100 mg/L	EFSA Journal

Species	Substance	Exposure System	Results	Reference
				2011;9(3):2091
Higher-tier studies (micro- or mesocosm studies) SANCO 7469/VI/98-Final 3 July 2003				
Not required				

Table 9.5-4: **Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/H/CFF 250 EC**

Species	Substance	Exposure System	Results	Reference
Daphnia magna	CHR/H/CFF 250 EC	48 h, s	EC_{50} = 4.95 mg/L $_{nom}$	Z. Kaceprek-Karetta, Study code:W-03-20
Raphidocelis subcapitata	CHR/H/CFF 250 EC	72 h	ErC50 = 1.84 mg/L EyC50= 0.40 mg/L	G. Hodorek, Study code: W-01-20
Anabaena flos-aquae	CHR/H/CFF 250 EC	72 h, static	ErC50= 7.53 mg test item/L EyC50 = 4.40 mg test item/L	K. Brzozowska-Wojoczek, Study code: W-04-20
Lemna gibba	CHR/H/CFF 250 EC	7d, ss	EC50 (7-day) Yield (frond number)= 0.106 [mg test item/L] EC50 (7-day) Growth rate (frond number)= 0.310 [mg test item/L] EC50 (7-day) Yield (dry weight)= 0.197 [mg test item/L] EC50 (7-day) Growth rate (dry weight) = 4.674 [mg test item/L]	E. Nierzędska, Study code: W-02-20
Myriophyllum spicatum	CHR/H/CFF 250 EC	14d,	Fresh weight: E_rC_{50} = 0.02 mg/L E_yC_{50}= 0.015 mg/L Dry weight E_rC_{50} = 0.035 mg/L E_yC_{50}= 0.035 mg/L Shot length: E_rC_{50} = 0.029 mg/L E_yC_{50}= 0.037 mg/L	L. Kolek, Study code: ETOX-2024-1
Higher-tier studies (micro- or mesocosm studies)				

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

concentrations

9.5.1.1 Justification for new endpoints

No new data for active substances is presented with this application.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the 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 9.5-5: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Florasulam for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/CFF 250 EC in cereals**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***P.Subcapitata***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EbC50	NOEC	EC50
(µg/L)		100000	2900	292000	23400	8.94	10000	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118
Exposure	**PEC $_{gl-max}$(µg/L)**							
Step 1								
	1.69	0.00169	0.00583	0.00058	0.00072	1.89038	0.00169	14.3220
Step 2								
	0.09	0.00009	0.00031	0.00003	0.00004	0.10067	0.00009	0.7627

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 5-OH Florasulam of Florasulam for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/CFF 250 EC in cereals

5-OH Florasulam									
Group		Fish acute	Fish prolonged	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint		LC_{50}		EC_{50}	-	-	EbC50	-	EC50

5-OH Florasulam									
Group		Fish acute	Fish prolonged	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
(µg/L)		91000	-	96700	-		21320		37.8
AF		100	-	100	-	-	10	-	10
RAC (µg/L)		910	-	967	-	-	2132	-	3.78
Exposure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	2.72	0.00299	-	0.00281	-	-	0.00128	-	0.7196

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite DFP-ASTCA of Florasulam for each organism group based on FOCUS Steps 1-2 calculations for the use of CHR/H/CFF 250 EC in cereals

DFP-ASTCA									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint		LC_{50}		EC_{50}	-	-	EbC50	-	EC50
(µg/L)		-	-	30	-		96000		100000
AF		-	-	100	-	-	10	-	10
RAC (µg/L)		-	-	0.3	-	-	9600	-	10000
Exposure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	0.34	-	-	1.133	-	-	0.00004	-	<0.0001

DFP-ASTCA									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Step 2									
PEC/RAC	0.05	-	-	0.1667	-	-	0.00001	-	<0.0001

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite ASTCA of Florasulam for each organism group based on FOCUS Steps 1-2 calculations for the use of CHR/H/CFF 250 EC in cereals

ASTCA									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint (µg/L)		- -		EC_{50} 30	- -	-	EbC50 9200	-	EC50 10200
AF		-	-	100	-	-	10	-	10
RAC (µg/L)		-	-	0.3	-	-	920	-	1020
Exposure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	0.75	-	-	2.50000	-	-	0.00082	-	0.0007
Step 2									
PEC/RAC	0.09	-	-	0.3000	-	-	0.00010	-	0.0001

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite TSA of Florasulam for each organism group based on FOCUS Steps 1,2 calculations for the use of CHR/H/CFF 250 EC in cereals

TSA

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint		-		EC_{50}	-	-	EbC50	-	EC50
(µg/L)			-	30	-		94000		100000
AF		-	-	100	-	-	10	-	10
RAC (µg/L)		-	-	0.3	-	-	9400	-	10000
Exposure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	0.11	-	-	0.3667	-	-	0.00001	-	<0.0001

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 5-OH-ASTP of Florasulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals

5-OH-ASTP									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint		-	-	-	-	-	EC50	-	EC50
(µg/L)			-	-	-		100000		100000
AF		-	-	-	-	-	10	-	10
RAC (µg/L)									
Exposure	**PEC $_{gl-max}$ (µg/L)**								

5-OH-ASTP									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Step 1									
PEC/RAC	0.29	-	-	-	-	-	0.00003	-	<0.0001

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite ASTP of Florasulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals

ASTP									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint (µg/L)		-	-	-	-	-	EC50	-	EC50
			-	-	-		100000		88000
AF		-	-	-	-	-	10	-	10
RAC (µg/L)		-	-	-	-	-	10000	-	8800
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**								
Step 1									
PEC/RAC	0.23	-	-	-	-	-	0.00002	-	<0.0001

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite TPSA of Florasulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals

TPSA									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**

TPSA									
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Americamysis bahia***	***Daphnia magna***	***Pseudokirchneriella***	***Chironomus riparius***	***Lemna Gibba***
Endpoint (µg/L)		-	- -	-	-	-	EC50 >100000	-	EC50 >100000
AF		-	-	-	-	-	10	-	10
RAC (µg/L)		-	-	-	-	-	10000	-	10000
Exposure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	0.65	-	-	-	-	-	0.00007	-	0.0001

Table 9.5-13: **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/CFF 250 EC**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Selenastrum capricornutum***	***Chironomus riparius***	***Myriophyllum spicatum***
Endpoint (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
Exposure	**PEC $_{gl-max}$(µg/L)**							

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Step 1	21.51	0.02053	0.01899	0.02072	0.01206	0.00684	0.00410	0.0684
Step 2								
	3.24	0.00324	0.00300	0.00327	0.00191	0.00108	0.00065	0.0108

Table 9.5-14: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Fluroxypyr-MHE for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/CFF 250 EC 500 SC in cereals**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	Sed. dwell. prolonged	**Aquatic plants**
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Skeletonema costatum***	***Chironomus riparius***	***Lemna Gibba***
Endpoint (µg/L)		LC_{50} 225	NOEC 200	EC_{50} 183	NOEC 60.5	EC50 208	NOEC 130	EC50 2310
AF		100	10	100	10	10	10	10
RAC (µg/L)		2.25	20	1.83	6.05	20.8	13	231
Exposure	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	1.86	0.82667	0.09300	1.01639	0.30744	0.08942	0.14308/16.14692*	0.0081
Step 2								
	0.35111	0.03950	0.43169	0.13058	0.03798	0.06077	0.0034/ 0.46308*	0.35111

- Calculation PEC sw/ PECsed, where PECsed = 209.91 µg/L for Step 1, and 6.02 µg/L for Step 2

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

Table 9.5-15: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Fluroxypyr acid for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/CFF 250 EC 500 SC in cereals**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna Gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EbC50	EC50
(µg/L)		14300	100 000	100 000	56 000	26 000	12 300
AF		100	10	100	10	10	10
RAC (µg/L)		143	10 000	1 000	5 600	2 600	1 230
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	18.89	0.13210	0.00189	0.01889	0.00337	0.00727	0.01536
Step 2							
	3.49	0.02441	0.00035	0.00349	0.00062	0.00134	0.00284

Table 9.5-16: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Pyridinol for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals in acid/neutral soils**

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna Gibba***
Endpoint		LC_{50}	EC_{50}	EbC50	EC50
(µg/L)		39 000	49 000	640	3 200
AF		100	100	10	10
RAC (µg/L)		390	490	64	320

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
PEC/RAC	6.55	0.01679	0.01337	0.10234	0.02047

Table 9.5-17: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Pyridinol for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals in alkaline soils**

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna Gibba***
Endpoint (µg/L)		LC_{50} 39 000	EC_{50} 49 000	EbC50 640	EC50 3 200
AF		100	100	10	10
RAC (µg/L)		390	490	64	320
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
PEC/RAC	11.48	0.02944	0.02343	0.17938	0.03588

Table 9.5-18: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 3-CP of Fluroxypyr for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals**

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenestrum capricornutum***

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Endpoint		LC_{50}	EC_{50}	EC50
(µg/L)		95 100	7 560	35 000
AF		100	100	10
RAC (µg/L)		951	75.6	3 500
Exposure	**PEC gl-max (µg/L)**			
Step 1				
PEC/RAC	3.19	0.00335	0.04220	0.00091

Table 9.5-19: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Metoxypyridine of fluroxypyr for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/CFF 250 EC in cereals**

Group		**Algae**	**Higher Plant**
Test species		***Anabaena flos-aquae***	***Lemna gibba***
Endpoint		EC50	EC50
(µg/L)		1120	10 600
AF		10	10
RAC (µg/L)		112	1 060
Exposure	**PEC gl-max (µg/L)**		
Step 1			
PEC/RAC	4.43	0.03955	0.00418

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

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of CHR/H/CFF 250 EC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/CFF 250 EC in winter cereals

Intended use	Winter cereals
Formulation	CHR/H/CFF 250 EC
Application rate (g[prod]/ha)	1 X 541.3
Entry into surface water via spraydrift (Drift alculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	3.4777
5	0.9426
Entry into surface water via spraydrift (Drift alculator from SWASH)	
Buffer zone (m)	**For Fish risk assessment → please refer to the active substance risk assessment**
1	
Buffer zone (m)	**PEC/RAC ratio** **Daphnia magna =EC_{50} 4 950 µg/L** **RAC=49.5 (AF=100)**
1	0.0703
Buffer zone (m)	**PEC/RAC ratio** **Raphidocelis subcapitata =EC50 400 µg/L** **RAC=40 (AF=10)**
1	0.0869
Buffer zone (m)	**PEC/RAC ratio** **Anabaena flos-aquae =EC50 4400 µg/L** **RAC=440 (AF=10)**
1	0.008
Buffer zone (m)	**PEC/RAC ratio** **Lemna Gibba =EC50 106 µg/L** **RAC=10.6 (AF=10)**
1	0.328

Buffer zone (m)	PEC/RAC ratio *Myriophyllum spicatum* EC_{50} = 20 µg/L RAC=2 (AF=10)
1	1.74
5	0.4713

Based on the calculated concentrations of the formulation CHR/H/CFF 250 EC (spray drift) respectively its active ingredients Florasulam, Clopyralid and Fluroxypyr (run-off and drainage) in surface water (PECSW according to FOCUS STEP 1-2, STEP 3), the calculated RAC/PEC (mix) values for the risk resulting from an exposure of aquatic organisms to CHR/H/CFF 250 EC according to the GAP of the formulation achieve the acceptability criterium <1 for run-off exposure, therefore no risk mitigations are required.

The following formula was used to derive the surrogate EC_{50} for the mixture of active substances with known toxicity assuming dose additivity:

Decision scheme for mixture toxcitiy risk assessment for CHR/H/CFF 250 EC:

Step 1. Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. ($EC_{x a.s.}$): Go to 7
For both formulation ($EC_{x PPP}$) and a.s. ($EC_{x a.s.}$): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for daphnia, algae and macrophytes.. → Go to 2

Measured toxicity data for fish is only available for a.s. → Go to 7

STEP 2. Check the plausibility of the measured formulation toxicity (EC_xPPP) against the calculated mixture toxicity EC_{xmix}-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (EC_xPPP) by means of the model deviation ratio (MDR = EC_{xmix}-CA/EC_xPPP).

If MDR = 0.2–5 (CA approximately holds for the mixture)
If MDR > 5 (mixture more toxic than CA)
If MDR < 0.2 (mixture less toxic than CA)

Equation 13:

$$EC_{X_{mix-CA}} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{X_i}} \right)^{-1}$$

Equation 15:

$$MDR = \frac{EC_{X_{mix-CA}}\,(calculated\ mixture\ toxicity)}{EC_{X_{PPP}}\,(measured\ mixture\ toxicity)}$$

Calculation of the acute mixture toxicity of the formulation

Table 1. Composition of CHR/H/CFF 250 EC

Name/code of the product	CHR/H/CFF 250 EC		
Name of the active substance A	clopyralid		
Name of the active substance B	fluroxypyr		
Name of the active substance C	florasulam		
Density [g product/cm^3]	1.0826		
	Nominal [g a.s./kg or L product]	Fraction considering density [%]	$p_{i\ mix}$ = Fraction of active substance i in the mixture with $\sum p_{i\ mix} = 100$ [%]
Concentrations of the active substance clopyralid in the product	120	11.1%	48.0%
Concentrations of the active substance fluroxypyr in the product	120	11.1%	48.0%
Concentrations of the active substance florasulam in the product	10	0.9%	4.0%

Table 2. Toxicity of CHR/H/CFF 250 EC and active substance

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	Toxicity of the a.s. clopyralid (ECx A) [mg a.s./L]	Toxicity of the a.s. fluroxypyr (ECx B) [mg a.s./L]	Toxicity of the a.s. florasulam (ECx C) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
LC50 fish		0.000	99	14.3	100	0.01
EC50 daphnids	4.95	1.143	99	100	292	0.01
ErC50 algae	0.4	0.092	30	26	0.00894	0.1
ErC50 higher plant	0.106	0.024	89	12.3	0.00118	0.1

Table 3. Calculation of toxicity exposure in CHR/H/CFF 250 EC

Toxicity per fraction of the a.s. clopyralid (1/TUA) [mg a.s./L]	Toxicity per fraction of the a.s. fluroxypyr (1/TUB) [mg a.s./L]	Toxicity per fraction of the a.s. florasulam (1/TUC) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) (ECx mix-CA = 1/∑ (TUi)) [mg a.s./L]	Model deviation ratio (MDR = ECx mix-CA/ECx PPP)	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) (at lower exposure tier)
206.25	29.79166667	2500	25.763	#DZIEL/0!	1.049
206.25	208.3333333	7300	102.192	89.401	1.018
62.5	54.16666667	0.2235	0.222	2.401	0.335
185.4166667	25.625	0.0295	0.029	1.204	0.331

Answer: MDRs for algaes and lemnas are between 0.2-5. Therefore , go to Step 3
MDRS for daphnias are above 5 . Therefore, goto Step 10

Step 10. Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of heterogeneous input data (a.s.) and of co-formulants ignored in the CA calculation. Does the apparent synergism remain?

Answer: No. Therefore , go to Step 3

Step 3. Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECx PPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECx PPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECx mix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).

Table 4. Results of compare ECmix-CA(a.s. in PPP) to ECmix-CA (a.s. in PECmix)

		Triggers	
Endpoint/Test species	**ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix)**	**0.8-1.2**	**<0.8 or >1.2**
EC50 daphnids	1.018	Yes	-
E_rC_{50} algae	0.499	-	Yes
EC_{50} higher plant	0.545	-	Yes

Answer: Calculated factors gives results outside 0.8-1.2 for algae and plants, Therefore, go to step 5. Calculated factor for daphnias is between 0.8-1.2, so go to step 4.

STEP 4. Conduct a mixture RA based on calculated mixture toxicity

Table 7. Results of exposure of mixture toxicity's calculation to aquatic species

Exposure		**(lower exposure tier)**	**(higher exposure tier)**	
Exposure tier (FOCUS step)	Clopyralid	Step 2	Not relevant	
PECsw [mg a.s./L]		0.003240		
Exposure tier (FOCUS step)	Fluroxypyr	Step 2		
PECsw [mg a.s./L]		0.003490		
Exposure tier (FOCUS step)	Florasulam	Step 2		
PECsw [mg a.s./L]		0.000090		
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.006820		
Endpoint/Test species	Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	ETRmix = PECmix/ECx PPP		Triggers
EC50 daphnids	1.143	0.006	Not relevant	0.10

AnswerETRmix exposure tier are below the triggers. Therefore, CHR/H/CFF 250 EC no poses unacceptable mixture toxicity to aquatic species:

STEP 5. Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECx PPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?

Table 6. Results of toxicity driver's calculation

		Clopyralid		Fluroxypyr		Florasulam		**Triggers**	
Endpoint/Test species	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix-CA}$) [mg a.s./L]	Toxicity per fraction (1/TU_i) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix-CA}$ x (1/$EC_{x\ mix-CA}$-TU_i) [%]	Toxicity per fraction (1/TU_i) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix-CA}$ x (1/$EC_{x\ mix-CA}$-TU_i) [%]	Toxicity per fraction (1/TU_i) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix-CA}$ x (1/$EC_{x\ mix-CA}$-TU_i) [%]	>=90% for one a.s.	>=90% for no a.s.
ErC50 algae	0.222	62.500	0.4%	54.167	0.4%	0.224	99.2%	Yes	
ErC50 higher plant	0.029	185.417	0.0%	25.625	0.1%	0.030	99.9%	Yes	

Equation 14:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{EC_{X_i}}$$

Answer: Toxicity drivers for algaes and lemnas is Florasulam.

STEP 7. Is there evidence that synergistic interactions between mixture components might occur (e.g. based on toxicological knowledge from literature or from counter-checking measured and calculated mixture toxicity in other species) which cannot be ruled out for the given species with sufficient certainty?

Answer: No. Therefore, got to Step 8.

STEP 8. Conduct a mixture RA based on calculated mixture toxicity

Table 7. Results of exposure of mixture toxicity's calculation to aquatic species

Exposure		**(lower exposure tier)**	**(higher exposure tier)**	
Exposure tier (FOCUS step)	Clopyralid	Step 2	Not relevant	
PECsw [mg a.s./L]		0.003240		
Exposure tier (FOCUS step)	Fluroxypyr	Step 2		

PECsw [mg a.s./L]		0.003490										
Exposure tier (FOCUS step)	Florasulam	Step 2										
PECsw [mg a.s./L]		0.000090										
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.006820										
Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]										
LC50 fish		24.560	Not relevant									
Endpoint/Test species		$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$										Triggers
LC50 fish		<0.001	Not relevant									0.10

Answer: ETRmix exposure tier are below the triggers. Therefore, CHR/H/CFF 250 EC no poses unacceptable mixture toxicity to aquatic species:

9.5.1 Overall conclusions

The risk for the entry routes run-off and drainage is acceptable without buffer zones for the intended use of CHR/H/CFF 250 EC .

The use CHR/H/CFF 250 EC according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1) with apply 5 meters 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, florasulam, fluroxypyr and for product Turango 250 EC based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organism without applying buffer zone.

However, as aquatic plants are the most sensitive group of aquatic organisms, further studies should be provided at Member State level. The study with *Myriophyllum* and product Turango 250 EC should be conducted in accordance with OECD 239 and the root weight and the shoot weight should be measured separately. A final conclusion on the risk to the aquatic environment from the formulation Turango 250 EC can only be drawn after the studies with the formulation and aquatic plants are made available. This should be addressed during product authorisation at Member State level.

DATA GAP: In case formulation *Myriophyllum*: 1. Risk assessment for aquatic plants (*M. spicatum*) has been not performed (insufficient data set - data gap). 2. The new study the product Turango 250 EC and *M.spicatum* should be performed. January 2024 updated.

Updated July 2024
To address the current data gap for *Myriophyllum spicatum* conducted by Applicant according to the OECD Guidelines. The new study for *Myriophyllum spicatum* with formulated product Turango 250 EC has been accepted by zRMS. Toxicity data and risk assessment for *Myriophyllum spicatum* was available for the PPP Turango 250 EC and a low risk was demonstrated for this species. The use Turango 250 EC

according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1) with apply 5 meters buffer zone.

The use Turango 250 EC according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1) with apply 5 meters 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 Florasulam, Clopyralid and Fluroxypyr. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of Florasulam, Tribenuron-methy and Fluroxypyr. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** 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	Florasulam	Oral	LD_{50} > 100 µg a.s/bee	EFSA Journal 2015; 13(1):3984
Apis mellifera	Florasulam	Contact	LD_{50} >100 µg a.s/bee	EFSA Journal 2015; 13(1):3984
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	Fluroxypyr-MHE	Oral	LD_{50}> 100 µg a.s./bee	EFSA Journal 2011;9(3):2091
Apis mellifera	Fluroxypyr-MHE	Contact	LD_{50} > 100 µg /bee	EFSA Journal 2011;9(3):2091
Apis mellifera	Fluroxypyr-acid	Oral	LD_{50} = 37.1 µg a.s./bee	EFSA Journal 2011;9(3):2091
Apis mellifera	Fluroxypyr-acid	Contact	LD_{50} > 180 µg /bee	EFSA Journal 2011;9(3):2091
Apis mellifera	CHR/H/CFF 250 EC	Acute Oral	LD_{50} > 200 µg/bee	E. Kulec-Płoszczyca, Study code: B-15-20
Apis mellifera	CHR/H/CFF 250 EC	Acute Contact	LD_{50} > 200 µg/bee	E. Kulec-Płoszczyca, Study code: B-134-22
Apis mellifera	CHR/H/CFF 250 EC	Chronic Oral	LC50 > 666.7 mg/kg	E. Kulec-Płoszczyca,

Species	Substance	Exposure System	Results	Reference
			LDD50 > 17.2 µg/bee/day NOEC ≥ 666.7 mg/kg NOEDD ≥ 17.2 µg/bee/day	Study code: B-18-20
Apis mellifera	CHR/H/CFF 250 EC	Larval Repeated Exposure	LD50 = 112.594 µg/larva NOED= 50 µg/larva	A. Wozniak, Study code: 0038/0066/E
Bumblebee	CHR/H/CFF 250 EC	Acute Oral	LD_{50} > 20 µg/bumblebee	Extrapolated from bee study and divide by factor 10 as a worst case
Bumblebee	CHR/H/CFF 250 EC	Acute Contact	LD_{50} > 20 µg/bumblebee	Extrapolated from bee study and divide by factor 10 as a worst case
Higher-tier studies (tunnel test, field studies)				

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).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the acute risk for bees due to the use of CHR/H/CFF 250 EC in winter cereals

Intended use **Active substance** **Application rate (g/ha)**	Cereals winter Florasulam 1 × 5		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	5	0.05
Contact toxicity	100		0.05
Intended use **Active substance** **Application rate (g/ha)**	Cereals winter/spring Clopyralid 1 × 60		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	60	0.60
Contact toxicity	98.1		0.61

Intended use	Cereals winter/spring		
Active substance	Fluroxypyr-MHE		
Application rate (g/ha)	1 × 86.4		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	86.4	0.864
Contact toxicity	100		0.864
Product	Fluroxypyr acid		
Application rate (g/ha)	1 × 60		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	37.1	60	1.62
Contact toxicity	180		0.33
Product	CHR/H/CFF 250 EC		
Application rate (g/ha)	1 × 541.3		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	541.3	2.71
Contact toxicity	200		2.71

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

a) Screening step

Table 9.6-2: Screening step assessment of the chronic risk for bees and assessment for honey bees larvae due to the use of CHR/H/CFF 250 EC in cereals.

Intended uses	Winter cereals			
Product	CHR/H/CFF 250 EC			
Application rate (g product/ha)	1 × 541.3			
Test design	**Endpoints (µg/bee/d or µg/larva)**	**Ef x SV**	**ETR**	**Trigger**
Chronic oral toxicity	LDD50 > 17.2 µg/bee/day	7.6	**0.239**	0.03
Larvae toxicity	NOED= 50 µg ai/larva	4.4	0.05	0.2

Table 9.6-3: First-tier assessment of the chronic risk for bees due to the use of CHR/H/CFF 250 EC in winter cereals

~~Type of assessment~~	~~Step~~	~~Formula~~	~~Endpoint~~	~~Application rate AR (kg~~	~~Shortcut value SV~~ ~~Downward~~	~~Shortcut value SV~~ ~~Sideward~~	~~ETR calculations~~	~~Trigger value~~

				prod/ha)					
Chronic oral exposure adult bees Honeybees	Screenieng	ETRchronic adult oral = AR * SV/10 d LDD50	LD50>17.2.5 µg /bee/Day	0.5413	7.6	10.6	0.239	0.334	<0.03
Chronic oral exposure adult bees Honeybees	1st tier	ETRchronic adult oral = AR * Ef * SV * twa/10 d LDD50	LD50>17.2 µg /bee/Day	0.5413	7.6	10.6	0.02	0.02	<0.03
Repeated larval Exposure	Screening	ETRlarvae = AR * SV/NOEDlarvae	NOED= 50 µg of test item/larva	0.5413	4.4	6.1	0.05	0.07	<0.2

*Ef- exposure factor. According to the EFSA GD 2013, for field crops Ef= 0.0092 and twa =0.72. The twa value of 0.72 is based on a default DT50 of 10 days and a 10-day time window.
The protection goal is met if the calculated value is smaller or equal to the trigger. If the calculated value is greater than the trigger value then proceed with the 1st tier risk assessement.

Intended use	Cereals					
Product	CHR/H/CFF 250 EC					
Application rate (g product/ha)	1 × 541.3					
Test design	**Scenario/BBCH**	**Shortcut Value (downward spray)**	**TWA**	**fDep/ Ef**	**ETR**	**Trigger**
Chronic oral toxicity LDD_{50} > 15.5 NOEDD ≥ 17.2 µg/bee/day	Treated crop/ BBCH 10-29	0.92	0.72	1	0.021	< 0.03
	Treated crop/ BBCH 30-39					
	Weeds/ BBCH 10-29	2.9		1	**0.066**	
	Weeds/ BBCH 30-39			0.5	**0.033**	
	field margin/ BBCH 10-29	2.9		0.0092	0.001	
	field margin/ BBCH 30-39					
	adjacent crop/ BBCH 10-29	5.8		0.0033	<0.001	
	adjacent crop/ BBCH 30-39					
	next crop/BBCH 10-29	0.54		1	0.012	
	next crop/ BBCH 30-39					

On the basic information from SPe8 phrase in order to improve these risk assessments for cereals the following restrictions are necessary:

- Do not apply when flowering weeds are present/Erase flowering weeds before application

But CHR/H/CFF 250 EC is herbicide, therefore it can be assumed that no weeds will be in the field after application.

Updated July 2024
The chronic risk assessment for bees was provided by Applicant. This calculation was accepted by zRMS. First tier chronic evaluation of the risk to adult bees exposed to Turango 250 EC resulted with ETR value above the trigger in weeds scenario indicating potentially unacceptable risk (Weeds/ BBCH 10-29 Weeds/ BBCH 30-39). No data enabling refinement of the risk was available. How-ever, Turango 250 EC is herbicide, therefore it can be assumed that no weeds will be in the field after application.
On the basic information from SPe8 phrase in order to improve these risk assessments for cereals the following restrictions are necessary:
• Do not apply when flowering weeds are present/Erase flowering weeds before application
Nevertheless, since the EFSA Bee Guidance Document is yet to be implemented (2013), this result should be treated as indication of area that should be covered in the future, once the guidance document is officially noted and accepted. Further assessments from chronic exposure could be required at national level.

SPe8:
Dangerous to bees. To protect bees and other pollinating insects do not apply when flowering weeds are present. Remove weeds before flowering.

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

Table 9.6-4: First-tier assessment of the acute risk for bumblebee due to the use of CHR/H/CFF 250 EC in winter cereals

Product	CHR/H/CFF 250 EC		
Application rate (g/ha)	1 × 541.3		
Test design	**LD_{50} (lab.) (µg/bumblebee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	20	541.3	27.1
Contact toxicity	20		27.1

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

zRMS comment: Agreed.

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/CFF 250 EC applied at the maximum use rate in cereals winter/spring 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 **Turango 250 EC**. 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 florasulam, clopyralid, fluroxypyr and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of Florasulam, Clopyralid and Fluroxypyr. 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 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	CHR/H/CFF 250 EC	Laboratory test glass plates (2D)	LR_{50} and ER_{50}> 0.5 L//ha which is equivalent to 541.3 g/ha	E. Kulec-Płoszczyca, Study code: B-131-22
Aphidius rhopalosiphi (adults)	CHR/H/CFF 250 EC	Laboratory test glass plates (3D)	LR_{50} and ER_{50}> 0.5 L//ha which is equivalent to 541.3 g/ha	E. Kulec-Płoszczyca, Study code: B-132-22
Chrysoperla Carnea	CHR/H/CFF 250 EC	Laboratory test glass plates (2D)	LR_{50} and ER_{50} >0.5 L//ha which is equivalent to 541.3 g/ha	E. Kulec-Płoszczyca, Study code: B-11-21
Coccinella septempunctata	CHR/H/CFF 250 EC	Laboratory test glass plates (2D)	LR_{50} > 0.5 L//ha which is equivalent to 541.3 g/ha ER_{50} > 25 L/ha which is equivalent to 270.65 g/ha For this dose the reproduction effect is	E. Kulec-Płoszczyca, Study code: B-133-22

Species	Substance	Exposure System	Results	Reference
			below 50%	
Field or semi-field tests				
Typhlodromus pyri (protonymphs)	CHR/H/CFF 250 EC	Aged residue study	The effects of freshly-dried and field-aged foliar residues of CHR/H/CFF 250 EC on the predatory mite Typhlodromus pyri were evaluated in a series of extended laboratory tests. When applied to sweetcorn plants at a rate equivalent to 0.5 L test item/ha, fresh-dried residues and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).	L. Fallowfield, Study code: CHR-23-03

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CHR/H/CFF 250 EC in cereals winter

Intended use	Cereals winter/spring		
Active substance/product	CHR/H/CFF 250 EC		
Application rate (g/ha)	1 × 541.3		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**

Typhlodromus pyri	> 541.3	541.3	1
Aphidius rhopalosiphi	> 541.3		1
Chrysoperla Carneo	> 541.3		1
Coccinella septempunctata	> 541.3*		1

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.

* 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.

* LR_{50} > 541.3 g/ha - for this dose the reproduction effect is a little above 50% (54.3%). However, RMS has accepted this endpoint for risk assessment. Justification: It is only slightly above the limit value and for the remaining arthropod species the estimated risk is acceptable.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CHR/H/CFF 250 EC in cereals winter

Intended use	Cereals winter
Active substance/product	CHR/H/CFF 250 EC
Application rate (g/ha)	1 x 541.3
MAF	~~1~~ 5(2D)** n.a. (3D)*** 10 (2D)# n.a. (3D)***
vdf	1

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	> 543.1	2.77	2.99** 1.49#	5	0.03** 0.014#
Aphidius rhopalosiphi	> 543.1		14.99		0.14
Chrysoperla carnea	> 543.1		2.99** 1.49#		0.03** 0.014#
Coccinella septempunctata	> 543.1		2.99** 1.49#		0.03** 0.014#

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.

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

***not applicable

Some Member states require the use of VDF 10 in off-field risk assessment for non-target arthropods other than bees. The risk assessment for non-target arthropods other than bees with VDF=10 was also added by zRMS.

9.7.2.3 Additional higher-tier risk assessment

~~Not relevant.~~

Regards to $NOER_{fecundity}$ for *T. pyri* is lower than 0.125 L/ha below is provided aged residue study for CHR/H/CFF 250 EC on *T.pyri:*

Results

The results for bioassays initiated at 0 and 14 DAT are summarised below.

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean % mortality at 7 DAI [a)]	Corrected % mortality at 7 DAI [b)]	Mean number eggs/female (7-14 DAI) [c)]	Reduction in reproduction [%] [d)]
0 DAT	Control	-	11.3	-	7.5	-
	CHR/H/CFF 250 EC	0.5	13.8	2.8	7.9	-5.8
	Toxic reference	-	100 *	100	~	-
14 DAT	Control	-	3.0	-	11.6	-
	CHR/H/CFF 250 EC	0.5	10.0 *	7.2	10.4	10.9

a) For each bioassay, treatment mortalities were compared to the respective control using either the chi^2 2x2 table test or Fisher's exact binomial test ($\alpha = 0.05$, one-sided, > respective control), a statistically significant effect is denoted by an asterisk (*).

b) Mortality corrected for respective control treatment deaths using Abbott's formula. A positive value indicates an increase.

c) Treatments were compared to the respective control using Student's t-test for homogenous variances ($\alpha = 0.05$, one-sided, < respective control), there were no significant differences.

d) Percentage reduction in numbers of eggs per female, relative to the respective control. A positive value indicates a decrease and a negative value indicates an increase in egg production, relative to the respective control.

~ indicates no assessments were made for this treatment.

Conclusions:
The effects of freshly-dried and field-aged foliar residues of CHR/H/CFF 250 EC on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to sweetcorn plants at a rate equivalent to 0.5 L test item/ha, fresh-dried residues and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

All hazard quotients (HQ) are considerably less than 2, indicating that CHR/H/CFF 250 EC applied at the maximum use rate in cereals winter/spring poses no risk to non-target arthropods. No risk mitigation needed.

zRMS comment:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2. The calculations of the risk assessment for $PER_{in\text{-}field}$ for 4 indicator species - *T. pyri*, *A. rhopalosiphi*, *Ch. carnea* and *C. septempunctata* based on extended laboratory studies were accepted by zRMS. The HQ values were below 1 for these species. In addition, based on the results from extended laboratory tests for 4 indicator species - *T. pyri*, *A. rhopalosiphi*, *Ch. carnea* and *C. septempunctata* the $PER_{off\text{-}field}$ of **Turango 250 EC** with the risk off -field for these species were corrected by zRMS (corrected VDF for 2-D studies). PEROff-field was below rate with ≤50 % effect. For LR_{50} > 541.3 g/ha for *Coccinella septempunctata* - this dose the reproduction effect is a little above 50% (54.3%). However, RMS has accepted this endpoint for risk assessment. Justification: It is only slightly above the limit value and for the remaining arthropod species the estimated risk is acceptable. It should be considered by MSs level. Finally, the risk off-field for NTA is also considered acceptable.

Updated April 2024
The aged residue study for **Turango 250 EC** on *T.pyri* was provided by Applicant. The study was accepted by zRMS. The effects of freshly-dried and field-aged foliar residues of CHR/H/CFF 250 EC on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to sweetcorn plants at a rate equivalent to 0.5 L test item/ha, fresh-dried residues and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

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 florasulam, clopyralid, fluroxypyr and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of florasulam, clopyralid and fluroxypyr. 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	Florasulam	Acute (14d) Incorporated into soil / 10% OM	LC50 > 1320 mg a.s./kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	Florasulam	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.203 mg a.s./kg d.w.soil	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results	Reference
Eisenia foetida	5-OH-florasulam	Acute (14d) Incorporated into soil / 10% OM	LC50 > 1120 mg a.s./kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	5-OH-florasulam	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.14 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	DFP-ASTCA	Acute (14d) Incorporated into soil / 10% OM	LC50 > 0.1 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	DFP-ASTCA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.0304 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	ASTCA	Acute (14d) Incorporated into soil / 10% OM	LC50 > 100 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	ASTCA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1.0 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	TSA	Acute (14d) Incorporated into soil / 10% OM	LC50 > 0.1 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	TSA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10.0 mg /kg d.w.soil	EFSA Journal 2015; 13(1):3984
Falsomia candida	5-OH-florasulam	Mixed into sub-strate 28 d, chronic 5 % peat content	NOEC = 2.5 mg/kg d.w. soil	EFSA Journal 2015; 13(1):3984
Falsomia candida	DFP-ASTCA	Mixed into sub-strate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Falsomia candida	ASTCA	Mixed into sub-strate 28 d, chronic 5 % peat content	NOEC = 12.5 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Eisenia foetida	Clopyralid	Overspray / 10% OM; chronic	NOEC = 1.97 mg a.s/kg dry soil	EFSA Journal 2018;16(7):5389
Eisenia fetida	Fluroxypyr-MHE	Mixed into sub-strate 56 d, chronic 10 % peat content	NOEC = 3.92 mg /kg soil NOEC= 1.96 mg /kg soil corrected*	EFSA Journal 2011;9(3):2091
Eisenia fetida	Fluroxypyr- acid	Mixed into sub-strate 14 d, acute	NOEC = 3.05 mg/kg	EFSA Journal 2011;9(3):2091

Species	Substance	Exposure System	Results	Reference
		10 % peat content		
Eisenia andrei	CHR/H/CFF 250 EC	Mixed into sub-strate 56 d, chronic 10 % peat content	NOEC= 180 mg/kg NOECgeomean = 92.3 mg/kg	P. Pieczka, Study code: G-01-20 Amendment 1., study code: G-01-20
Folsomia candida	CHR/H/CFF 250 EC	Mixed into sub-strate 28 d, chronic 5 % peat content	NOEC = 32 mg/kg dw NOECgeomean = 18.6 mg/kg	A. Gierbuszewska, Study code: G-02-20 Amendment 1., study code: G-01-20
Hypoaspis aculeifer	CHR/H/CFF 250 EC	Mixed into sub-strate 14 d, chronic 5 % peat content	NOEC= 320 mg/kg dw NOECgeomean = 207.0 mg/kg	P. Pieczka, Study code: G-03-20 Amendment 1., study code: G-01-20
Field studies				
Litter bag test				

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

9.8.2.1 First-tier risk assessment

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

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CHR/H/CFF 250 EC in cereals winter/spring

Intended use			
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Not required.			
Chronic effects on earthworms			

Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Florasulam	0.203	0.0053	38.3
5-OH-florasulam	0.14	0.0027	51.9
DFP-ASTCA	0.0304	0.0006	51
ASTCA	1.0	0.0011	909
TSA	10.0	0.0003	33 333
Clopyralid	1.97	0.0640	30.8
Fluroxypyr-MHE	1.96	0.0922	21.3
Fluroxypyr-acid	3.05	0.0641	47.6
CHR/H/CFF 250 EC	~~180~~ 92.3	0.577	~~312.0~~ 156.5
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)**
Florasulam	-	0.0053	-
5-OH-florasulam	2.5	0.0027	925.9
DFP-ASTCA	10	0.0006	16 666.7
ASTCA	12.5	0.0011	11 363.6
TSA	50	0.0003	166 666,7
Clopyralid	-	0.0640	-
Fluroxypyr-MHE	-	0.0922	-
Fluroxypyr-acid	-	0.0641	
CHR/H/CFF 250 EC	~~32~~ 18.6	0.577	~~55.5~~ 32.2
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)**
Florasulam	-	0.0053	-
5-OH-florasulam	1.25	0.0027	463
DFP-ASTCA	10	0.0006	16 666.7
ASTCA	100	0.0011	90 909
TSA	50	0.0003	166 666.7
Clopyralid	-	0.0640	-
Fluroxypyr-MHE	-	0.0922	-
Fluroxypyr-acid	-	0.0641	
CHR/H/CFF 250 EC	~~320~~ 207	0.577	~~554.6~~ 358.7

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/CFF 250 EC 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 **Turango 250 EC**. However, the studies for formulation of Turango 250 EC for earthworms, *Folsomia candida* and *Hypoaspis aculeifer* was accepted by zRMS ***only provisionally***. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of fluroxypyr-methyl was below 80%. The geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Aplicant should complete the calculations of toxicity endpoints for earthworms and *Folsomia candida* and *Hypoaspis aculeifer* based on geometric mean measured concentration with a risk assessment for eartworms, *Folsomia candida* and *Hypoaspis aculeifer*. The reliability of these test with the risk assessment for *Folsomia candida* and *Hypoaspis aculeifer* should be considered by MSs level.

Updated April 2024

The Aplicant provided the calculations of toxicity endpoints for earthworms and *Folsomia candida* and *Hypoaspis aculeifer* based on geometric mean measured concentration with a risk assessment for eartworms, *Folsomia candida* and *Hypoaspis aculeifer*. The calculations were accepted by RMS. 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 **Turango 250 EC**.

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 florasulam, clopyralid, iodosulfuron-methy and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of florasulam, clopyralid and fluroxypyr. 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 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Florasulam	28 d, aerobic soil type	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	EFSA Journal 2016;14(3):4419

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	5-OH-florasulam	28 d, aerobic soil type	Treatment causing <25% deviation from control: 0.036 mg/kg dry soil	EFSA Journal 2016;14(3):4419
N-mineralisation	DFP-ASTCA	28 d, aerobic soil type	Treatment causing <25% deviation from control: 0.00760 mg/kg dry soil	EFSA Journal 2015; 13(1):3984
N-mineralisation	ASTCA	28 d, aerobic soil type	Treatment causing <25% deviation from control: 1.0 mg/kg dry soil	EFSA Journal 2015; 13(1):3984
N-mineralisation	TSA	28 d, aerobic soil type	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	EFSA Journal 2015; 13(1):3984
N-mineralisation	Clopyralid	56d	< 25 % deviation in nitrate formation to the control	EFSA Journal 2018;16(7):5389
N-mineralisation	Methoxypyridine	28 d/14d, aerobic soil type	<25% effect at 0.132 mg/kg dry soil <25% effect at 0.66 mg/kg dry soil	7469/VI/98-Final 3 July 2003
N-mineralisation	CHR/H/CFF 250 EC	28 d, aerobic soil type	On the basis of the results, it was concluded that CHR/H/CFF 250 EC at the concentrations corresponding to the PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil) and 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	A. Gierbuszewska, Study code: G-17-22

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/CFF 250 EC in winter/spring cereals

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Florasulam	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	0.0053	YES
5-OH-florasulam	Treatment causing <25% deviation from control: 0.036 mg/kg dry soil	0.0027	YES
DFP-ASTCA	Treatment causing <25% deviation from control: 0.00760 mg/kg dry soil	0.0006	YES
ASTCA	Treatment causing <25% deviation from control: 1.0 mg/kg dry soil	0.0011	YES
TSA	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	0.0003	YES
Clopyralid	< 25 % deviation in nitrate formation to the control	0.0640	YES
Methoxypyridine	<25% effect at 0.132 mg/kg dry soil <25% effect at 0.66 mg/kg dry soil	0.1680	YES
CHR/H/CFF 250 EC	On the basis of the results, it was concluded that CHR/H/CFF 250 EC at the concentrations corresponding to the PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil) and 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	0.579	YES
C-mineralisation: Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/H/CFF 250 EC and its active substances Florasulam, Clopyralid, Fluroxypyr in soil are below the concentrations at which no una acceptable effects (< 25%) regarding the soil microbial activity were observed after 28 days or more of exposure, indicating that the proposed use of CHR/H/CFF 250 EC poses an acceptable risk to soil microorganisms.

zRMS comments:

The risk assessment for soil micro-organism after exposure of **Turango 250 EC** 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 **Turango 250 EC**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **Turango 250 EC.**

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 florasulam, clopyralid and fluroxypyr. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of CHR/H/CFF 250 EC were not evaluated as part of the EU assessment of florasulam, clopyralid and iodosulfurn-methyl. 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 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
Pisum sativum	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 81.7 mL test item/ha which is eqivalent to 88.7 g prod/ha	A. Wróbel, Study code: G-06-20
Linum usitatissimum	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 69.7 mL test item/ha which is eqivalent to 75.7 g prod/ha	
Daucus carota	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 47.8 mL test item/ha which is eqivalent to 51.9 g prod/ha	
Allium cepa	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 154.2 mL test item/ha which is eqivalent to 167.5 g prod/ha	
Lolium perenne	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 500 mL test item/ha which is eqivalent 543.1 g prod/ha	

Species	Substance	Exposure System	Results	Reference
Avena Sativa	CHR/H/CFF 250 EC	21 d Seedling emergence	ER50 = 500 L test item/ha which is eqivalent to 543.1g prod/ha	
Pisum sativum	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 = 49.65 mL test item/ha which is eqivalent to 53.9 g prod/ha	A. Gierbuszewska, Study code: G-05-20
Linum usitatissimum	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 = 33.90 mL test item/ha, which is eqivalent to 36.8 g prod/ha	
Daucus carota	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 = 19.30 mL test item/ha, which is eqivalent to 21 g prod/ha	
Allium cepa	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 = 149.21 mL test item/ha which is eqivalent to 162.1 g prod/ha	
Lolium perenne	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 > 500 mL test item/ha, which is eqivalent to 543.1 g prod/ha	
Avena Sativa	CHR/H/CFF 250 EC	21 d Vegetative vigour	ER50 > 500 L test item/ha which is eqivalent to 543.1 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.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CHR/H/CFF 250 EC in winter cereals

Intended use	Winter cereals

Active substance/product	CHR/H/CFF 250 EC
Application rate (g/ha)	1 x 541.3
MAF	1

Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**	
Pisum sativum	88.7 g prod/ha	0.0277	14.99	5.92	21 d Seedling emergence
Linum usitatissimum	75.7 g prod/ha	0.0277	14.99	5.05	21 d Seedling emergence
Daucus carota	51.9 g prod/ha	0.0277	14.99	15.04	21 d Seedling emergence
Allium cepa	167.5 g prod/ha	0.0277	14.99	11.17	21 d Seedling emergence
Lolium perenne	543.1 g prod/ha	0.0277	14.99	36.23	21 d Seedling emergence
Avena Sativa	543.1 g prod/ha	0.0277	14.99	36.23	21 d Seedling emergence
Pisum sativum	53.9 g prod/ha	0.0277	14.99	3.60	21 d Vegetative vigour
Linum usitatissimum	36.8 g prod/ha	0.0277	14.99	2.46	21 d Vegetative vigour
Daucus carota	21 g prod/ha	0.0277	14.99	1.40	21 d Vegetative vigour
Allium cepa	162.1 g prod/ha	0.0277	14.99	10.81	21 d Vegetative vigour
Lolium perenne	543.1 g prod/ha	0.0277	14.99	36.23	21 d Vegetative vigour
Avena Sativa	543.1 g prod/ha	0.0277	14.99	36.23	21 d Vegetative vigour

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 required

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to un-sprayed 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/CFF 250 EC in cereals winter/spring considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use	Cereals winter
Active substance/product	CHR/H/CFF 250 EC

Application rate (g/ha) **MAF**		1 × 541.3 1			
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	2.77	14.99	7.50	3.74	1.50
5	0.57	3.09	-	-	-
Toxicity value ER_{50} = 21 g/ha		**TER** **criterion: TER ≥ 5**			
1		1.40	2.8	5.61	14
5		6.80			

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

9.10.3 Overall conclusions

Based on the predicted rates of CHR/H/CFF 250 EC in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/CFF 250 EC according to the GAP of the formulation CHR/H/CFF 250 EC achieve the acceptability criteria TER ≥ 5 with applying:

- 5 m
- 1 m and use of 75 % 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} = 21 g/ha value from vegetative vigour test test and $PER_{off-field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: **Turango 250 EC** achieve the acceptability criteria TER ≥ 5 with applying:

- 5 m
- 1 m and use of 75 % drift reducing nozzles.

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

Not available.

9.12 Monitoring data (KCP 10.8)

Please refer to the point 9.5 (KCP 10.2)

9.13 Classification and Labelling

Having considered risk to the environment posed by the preparation, the following classification of product CHR/H/CFF 250 EC is proposed.

Classification	**Hazard Statement**	**Pictogram**	**Signal Word**

~~Aquatic Chronic 2, H411~~ **Aquatic acute 1, H400** **Aquatic Chronic 1, H410**	~~Toxic to aquatic life with long lasting effects.~~ **Very toxic to aquatic life** **Very toxic to aquatic life with long lasting effects.**		~~Warning~~

zRMS comment: Corrected. Aquatic acute 1, H400.Aquatic Chronic 1. H410.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2/01	Z. Kacperek-Karetta	2023	CHR/H/CFF 250 EC Daphnia magna, Acute Immobilisation Test W-03-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.2/02	G. Hodorek	2023	CHR/H/CFF 250 EC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test W-01-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP	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
			Unpublished		
KCP 10.2/03	K. Brzozowska-Wojoczek	2023	CHR/H/CFF 250 EC Anabaena flos-aquae UTEX B 1444 Growth inhibition test W-04-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.2/04	E. Nierzędska	2021	CHR/H/CFF 250 EC Lemna gibba CPCC 310, Growth inhibition test W-02-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.2/05	D. Kolek	2024	CHR/H/CFF 250 EC Water-sediment Myriophyllum spicatum toxicity test ETOX-2024-1 EcoTox Alliance Sp. z o. o.	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
			Kalinowa 2 43-520 Zaborze, Poland GLP Unpublished		
KCP 10.3/01	E. Kulec-Płoszczyca	2023	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test B-15-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.3/02	E. Kulec-Płoszczyca	2023	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test B-134-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, 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 10.3/03	E. Kulec-Płoszczyca	2023	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test B-18-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.3/04	A. Wozniak	2022	Honey bee larval toxicity test following repeated exposure of the test item CHR/H/CFF 250 EC according to OECD GD 239 ENV/JM/MONO(2016)34 0038/0066/E SORBOLAB Research Laboratory LLC, Zaniemyska Street 11, 61-029 Poznań, Poland GLP Unpublished	N	Chemirol
KCP 10.3/05	E. Kulec-Ploszczyca	2023	An extended laboratory test for evaluating the effects of CHR/H/CFF 250 EC on the predatory mite, Typhlodromus pyri (Sch.) B-131-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland	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
			GLP Unpublished		
KCP 10.3/06	E. Kulec-Ploszczyca	2023	An extended laboratory test for evaluating the effects of CHR/H/CFF 250 EC on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez) B-132-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP10.3/07	E. Kulec-Ploszczyca	2023	An extended laboratory test for evaluating effects of CHR/H/CFF 250 EC on the green lacewing, Chrysoperla carnea (Steph.) B-11-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.3/08	E. Kulec-Ploszczyca	2023	An extended laboratory test for evaluating effects of CHR/H/CFF 250 EC on the ladybird beetle,	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
			Coccinella septempunctata (L.) B-133-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP 10.3/09	L. Fallowfield	2023	CHR/H/CFF 250 EC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae) Study code: CHR-23-03 Mambo-Tox 2 Venture Road University Science Park Southampton SO16 7NP UK GLP Unpublished	N	Chemirol
KCP 10.4/01	P. Pieczka	2023	CHR/H/CFF 250 EC Earthworm reproduction test (Eisenia andrei) G-01-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP	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
			Unpublished		
KCP 10.4/02	A. Gierbuszewska	2023	CHR/H/CFF 250 EC Collembolan (Folsomia candida) Reproduction Test G-02-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.4/03	P. Pieczka	2021	CHR/H/CFF 250 EC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil G-02-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.4/04	P. Pieczka	2024	Amendment No. 1: CHR/H/CFF 250 EC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil G-02-20	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
			Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP 10.4/05	P. Pieczka	2024	Amendment No. 1: CHR/H/CFF 250 EC Earthworm reproduction test (Eisenia andrei) G-01-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.4/06	A. Gierbuszewska	2024	Amendment no. 1: CHR/H/CFF 250 EC Collembolan (Folsomia candida) Reproduction Test G-02-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, 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 10.5/01	A. Gierbuszewska	2023	CHR/H/CFF 250 EC Soil Microorganisms: Nitrogen Transformation Test G-17-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.6/01	A. Wróbel	2023	CHR/H/CFF 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test G-06-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol
KCP 10.6/02	A. Gierbuszewska	2023	CHR/H/CFF 250 EC Terrestial Plant Test: Vegetative Vigour Test G-05-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland	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**
			GLP Unpublished		

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**	**Verte-brate study Y/N**	**Owner**
KCP 10.1	-	1995	XDE-570 Herbicide: A Pilot Reproduction Study with the Mallard [redacted] GLP No Unublished	Y	DAS
KCP 10.1		1995	XDE-570 Herbicide: A Pilot Reproduction Study with the Northern Bobwhite [redacted] GLP Yes Unublished	N	DAS
KCP 10.3	Beech, P	1996	A Determination of the Oral LD50s for XDE-570 against the Honey Bee, Apis mellifera Agrochemical Evaluation Unit, Department of Biology, The University, Southampton, UK GHE-P-6705 GLP Yes Unpublished	N	DAS
KCP 10.4	Boeri, RL, Magazu, JP,	1994	XDE-570 Herbicide: Acute Toxicity to the Earthworm, Eisenia foetida TR Wilbury Laboratories Inc,	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
	Ward, TJ		DECO-ES-2798 GLP Yes Unpublished		
KCP 10.1		1994	XDE-570: An Acute Oral Toxicity Study with the Japanese Quail DECO-ES-2799 GLP Yes Unpublished	N	DAS
KCP 10.5	Ehr, RJ, Alexander, AL	1997	The Activity of DE-570 in Herbicide, Insecticide and Fungicide Screening Tests and the Herbicidal Activity of DE-570 Soil Metabolites DERBI# 60600 DowElanco GLP Yes Unpublished	N	DAS
KCP 10.2	Ehr, RJ, Schmitzer, PR, Gray, JA	1997	The Activity of DE-570 and Soil Metabolites on Acetolactate Synthase, Lemna minor, and Agrostis palustris DERBI # 60598 DowElanco GLP No Unpublished	N	DAS
KCP 10.5	Feil, N.	2010	Effects of 5-hydroxy-florasulam on the activity of the soil microflora in the laboratory DAS Report No.: 101342 (Accession Number) 2007411 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished	N	DAS
KCP 10.5	Feil, N.	2011	Effects of DFP-ASTCA metabolite of florasulam on the activity of the soil microflora	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			in the laboratory. DAS Report No.: 101343 (Accession Number) 2009901 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished		
KCP 10.5	Feil, N.	2008	Effects of ASTCA metabolite of florasulam on the activity of the soil microflora in the laboratory DAS Report No.: 080039 (Accession Number) 2000205 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished	N	DAS
KCP 10.5	Feil, N.	2011	Effects of TSA metabolite of florasulam on the activity of the soil microflora in the laboratory DAS Report No.: 110143 (Accession Number) 2010747 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished	N	DAS
KCP 10.5	Forster, J	1997	A Laboratory Assessment of the Effects of XDE-570 on Soil Microflora Respiration and Nitrogen Turnover According to BBA Guidelmes VI 1-1 (1990) Euro Laboratories Limited GHE-T-713 GLP Yes Unpublished	N	DAS
KCP 10.1	-	1995	XDE-570: [redacted] GLP Yes Unpublished	Y	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.1	-	1995	XDE-570: [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.1	-	2011	Florasulam technical: an early life-stage toxicity test with the fathead minnow (Pimephales promelas) [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.2	Hancock, G.A. Arnold, B.H., Carr, M.S., Najar, J.R.	2007	5-Hydroxy-florasulam: growth inhibition test with the aquatic plant duckweed, Lemna gibba DAS Report No.: 071032 (Accession Number) 245034 The Dow Chemical Company GLP Yes Unpublished	N	DAS
KCP 10.2	Hastings, M	1997	Preparation of Soil Extracts for Determination of the Algal Toxicity of XDE-570 Metabolites GHE-P-6616 DowElanco Europe GLP Yes Unpublished	N	DAS
KCP 10.1	-	1994	XDE-570 Herbicide: 8-day Acute Dietary LC50 Study in Japanese Quail [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.1	-	1994	XDE-570 Herbicide: 8-day Acute Dietary LC50 Study in Mallard Ducklings [redacted] GLP Yes Unpublished	Y	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2	Hughes, JS, Williams, TL, Conder, LA	1995	The Toxicity of XDE-570 to Skeletonema costatum Carolina Ecotox Inc DECO-ES-3021 GLP Yes Unpublished	N	DAS
KCP 10.2	Jenkins, CA	1997	Two Aqueous Soil Extracts Containing XDE-570 Metabolites: Growth Inhibition of Selenas-trum capricornutum (Preliminary Toxicity Screen) Huntingdon Life GHE-T-837 GLP Yes Unpublished	N	DAS
KCP 10.2	Kelly, CR	1997	To Assess the Toxicity to the Sediment Dwelling Phase of the Midge, Chironomus riparius Huntingdon Life Sciences Ltd, GHE-T-838 GLP Yes Unpublished	N	DAS
KCP 10.2	-	1996	Evaluation of the Acute Toxicity of 5-hydroxy XDE-570 to the Rainbow Trout, [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.2	Kirk, HD, Landre, AM, Hugo, JM	1996	Evaluation of the Acute Toxicity of 5-Hydroxy XDE-570 to the Daphnid, Daphnia magna Straus The Dow Chemical Company DECO-ES-3117 GLP Yes Unpublished	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2	Kirk, HD, Landre, AM, Hugo, JM, Stahl, DC	1996	Evaluation of the Chronic Toxicity of XDE-570 Herbicide to the Daphnid, Daphnia magna Straus The Dow Chemical Company DECO-ES-2944 GLP Yes Unpublished	N	DAS
KCP 10.2	Kirk, HD, Landre, AM, Massaro, LM, Hugo, JM, Stahl, DC	1995	Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Daphnid, Daphnia magna Straus. The Dow Chemical Company DECO-ES-2938 GLP Yes Unpublished	N	DAS
KCP 10.2	-	1995	Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Rainbow Trout, Oncorhynchus mykiss [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.2	-	1995	Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Bluegill, Lepomis macrochirus [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.2	Kirk, H.D. Gilles, M.M., Rick, D.L., McFadden, L.G.	2000	5-(Aminosulfonyl)-1H-1,2,4-triazole-3-carboxylic acid (florasulam M4 metabolite): growth inhibition test with the freshwater green alga, Selenastrum capricornutum DAS Report No.: 001019 Accession Number) 76271 (PRINTZ Toxicology & Environmental Research and Consulting The Dow Chemical Company GLP Yes Unpublished	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2	Kirk, H.D. Gilles, M.M., Rick, D.L., McFadden, L.G.	2000	5-(Aminosulfonyl)-1H-1,2,4-triazole-3-carboxylic acid (florasulam M4 metabolite): growth inhibition test with the freshwater aquatic plant, duckweed, Lemna gibba L. G-3 DAS Report No.: 001021 (Accession Number) 76666 The Dow Chemical Company GLP Yes Unpublished	N	DAS
KCP 10.2	Kirk, H.D.and Marino, T.A	1998	Toxicity of metabolites of XDE-570 to DaphniaMagna DAS Report No.: 981157 (Accession Number) 66206 The Toxicology Research Laboratory Health and Environmental Research Laboratories GLP Yes Unpublished	N	DAS
KCP 10.2		1995	Evaluation of the Prolonged (28-day) Toxicity of XDE-570 Herbicide to the Rainbow trout, Oncorhynchus mykiss walbaum [redacted] GLP Yes Unpublished	N	DAS
KCP 10.4	Lührs, U.	2008	Acute toxicity (14 days) of ASTCA metabolite of florasulam to the earthworm Eisenia fetida in artificial soil DAS Report No.: 080037 (Accession Number) 259941 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP No Unpublished	N	DAS
KCP 10.4	Lührs, U.	2008	Effects of ASTCA metabolite of florasulam on reproduction and growth of earthworms Ei-	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			senia fetida in artificial soil DAS Report No.: 080038 (Accession Number) 2001599 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished		
KCP 10.4.	Lührs, U.	2011	Effects of DFP-ASTCA metabolite of florasulam on reproduction of the Collembola Folsomia candida in artificial soil with 5% peat DAS Report No.: 101345 (Accession Number) 2009902 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished	N	DAS
KCP 10.4	Lührs, U.	2011	Effects of TSA metabolite of florasulam on reproduction of the Collembola Folsomia candida in artificial soil with 5% peat DAS Report No.: 110133 (Accession Number) 2009861 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP Yes Unpublished	N	DAS
KCP 10.4	Lührs, U.	2011	Effects of DFP-ASTCA metabolite of florasulam on reproduction of the predatory mite Hypo-aspis aculeifer in artificial soil with 5% peat DAS Report No.: 101348 (Accession Number) 2009903 Institut fiir Biologische Analytik und Consulting IBACON GmbH GLP No Unpublished	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2	Porch, J.R., Kendall, T.Z., Krueger, H.O	2011	TPSA metabolite of florasulam: a 96-hour toxicity test with the freshwater alga (Pseudokirch-neriella subcapitata) DAS Report No.: 101350 (Accession Number) 2008420 Wildlife International, Ltd. GLP Yes Unpublished	N	DAS
KCP 10.2	Porch, J.R., Kendall, T.Z., Krueger, H.O.	2011	Florasulam (TPSA metabolite): a 7-day staticrenewal toxicity test with duckweed (Lemna gibba G3) DAS Report No.: 101351 (Accession Number) 2008814 Wildlife International, Ltd. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M	2011	DFP-ASTCA metabolite of florasulam (X12239339): growth inhibition test with the unicellu-lar green alga, Pseudokirchneriella subcapitata DAS Report No.: 110046 (Accession Number) 2010085 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M.	2011	TSA metabolite of florasulam (X634074): growth inhibition test with the unicellular green alga, Pseudokirchneriella subcapitata DAS Report No.: 110043 (Accession Number) 2010859 ABC Laboratories, Inc. GLP Yes	N	DAS

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			Unpublished		
KCP 10.2	Rebstock, M.	2011	5-OH-ASTP metabolite of florasulam (X12251401): growth inhibition test with the unicellular green alga, Pseudokirchneriella subcapitata DAS Report No.: 110044 (Accession Number) 2010120 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M	2011	ASTP metabolite of florasulam (X516274): growth inhibition test with the unicellular green alga, Pseudokirchneriella subcapitata DAS Report No.: 110045 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M.	2011	DFP-ASTCA metabolite of florasulam (X12239339): growth inhibition test with the freshwater aquatic plant, duckweed, Lemna gibba DAS Report No.: 110039 (Accession Number) 2010084 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M.	2011	TSA metabolite of florasulam (X634074): growth inhibition test with the freshwater aquatic plant, duckweed, Lemna gibba DAS Report No.: 110040 (Accession Number) 2010161	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			ABC Laboratories, Inc. GLP Yes Unpublished		
KCP 10.2	Rebstock, M.	2011	5-OH-ASTP metabolite of florasulam (X12251401): growth inhibition test with the freshwater aquatic plant, duckweed, Lemna gibba DAS Report No.: 110041 (Accession Number) 2010087 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	Rebstock, M.	2011	ASTP Metabolite of Florasulam (X516274): Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, Lemna gibba DAS Report No.: 110042 (Accession Number) 2010018 ABC Laboratories, Inc. GLP Yes Unpublished	N	DAS
KCP 10.2	-	1997	The Bioconcentration of XDE-570 by the Rainbow Trout, Oncorhychus mykiss Walbaum [redacted] GLP Yes Unpublished	Y	DAS
KCP 10.1		1980	Acute Oral LD50 – Mallard Duck – DOWCO 290 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	DAS
KCP 10.1		1985	Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos) - Final Report. [redacted]	Y	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.1		1987	Lontrel T Herbicidal Chemical (Penta Process): Acute Oral Toxicity Study in Fischer 344 Rats. [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.1		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		1985	Report No A2A-052	Y	DAS
KCP 10.2		2000	Clopyralid: An Acute Toxicity Study with the Rainbow Trout, Oncorhynchus mykiss Walbaum [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.2		2000	Clopyralid: Toxicity to the Early Life Stages of the Fathead Minnow, Pimephales Promelas Raf-inesque. [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS
KCP 10.2	Marino, T. A., McClymont, E. L. & Staley, J. L.	2000	Clopyralid: An Acute Toxicity Study with the Daphnia, Daphnia magna Straus DAS report no. 001025, Ref. J52 Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS
KCP 10.2	Douglas, M. T., Bell, G. &	1992	An Assessment of the Effects of Lontrel T on the Reproduction of Daphnia magna	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
	Macdonald, I. A.		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		
KCP 10.2	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	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	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	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 Published (Y/N): No	N	DAS
KCP 10.2	Banman, C. S., Moore, S.	2015	Clopyralid: Toxicity to the Aquatic Macrophyte, Myriophyllum spicatum DAS Report No. 140735	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104 GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.2	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	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	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	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	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
KCP 10.4	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	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.5	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
All points	European Commission	1999	Review report for the active substance fluroxypyr. Fluroxypyr, 6848/VI/98, 15 December 1999 GLP: no published	N	DOW
KCP 10.2	[redacted]	2000	4-Amino-3-chloro-6-fluoro-2-pyridinol: An acute toxicity study with the rainbow, Oncorhynchus mykiss Walbaum [redacted] 2000-05-08 GLP: yes not published	Y	DOW
KCP 10.2	[redacted]	1996	The Bioconcentration and metabolism of Fluroxypyr 1- methylheptyl ester by the rainbow trout, Oncorhynchus mykiss Walbaum [redacted] GLP: yes not published	Y	DOW
KCP 10.2	Marino, T.A., McClymo	2000	4-Amino-3-chloro-6-fluoro-2-pyridinol: An acute toxicity studywith the Daphnia, Daphnia magna Straus Toxicology & Environmental	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
	nt, E.L. & Kern, J.M.		Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA 001010 (75245) 2000-05-16 GLP: yes not published		
KCP 10.2	Kirk, H.D., Miller, J.A., Hugo, J.M. & Landre, A.M.	1996	Evaluation of the chronic toxicity of Fluroxypyr 1-methylheptyl ester to the daphnid, Daphnia magna Straus The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3079 1996-06-15 GLP: yes not published	N	DOW
KCP 10.2	Milazzo, D.P., Hugo, J.M. & Martin, M.D.	1996	Fluroxypyr 1-methylheptylester: The toxicity to the blue-green algae, Anabaena flos-aquae The Toxicology Research Laboratory, Health and Environmental Science, The Dow Chemical Company, Midland, Michigan, USA ES-3073 1996-06-04 GLP: yes not published	N	DOW
KCP 10.2	Hoberg, J.R.	2002	Fluroxypyr 1-methylheptyl ester technical - Acute toxicity to the freshwater diatom (Navicula	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			pelliculosa) Springborn Smithers Laboratories, Wareham, MA, USA 12550.6203 (Project No.: 021014) 2002-11-15 GLP: yes not published		
KCP 10.2	Alexander, M.M.	1996	Fluroxypyr 1-methylheptyl ester: The toxicity to Skeletonema costatum Carolina Ecotox., Inc., Durham, NC, USA DECO-ES-3131 (10-04-1) 1996-05-31 GLP: yes not published	N	DOW
KCP 10.2	Hancock, G.A., Hales, C.A., McClymont, E.L. & Najar, J.R.	2004	Fluroxypyr: Growth inhibition test with the freshwater diatom, Navicula pelliculosa Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA 031132 GLP: yes not published	N	DOW
KCP 10.2	Boeri, R.L., Magazu, J.P. & Ward, T.J.	1999	4-Amino-3,5-dichloro-6-fluoro-2-pyridinol (a metabolite of Fluroxypyr): Toxicity to the freshwater bluegreen algae, Anabaena flos-aquae	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990035 1999-09-10 GLP: yes not published		
KCP 10.2	Ward, T.J., Magazu, J.P. & Boeri, R.L.	1999	4-Amino-3,5-dichloro-6-fluoro-2-pyridinol (a metabolite of Fluroxypyr): Toxicity to the freshwater diatom, Navicula pelliculosa T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990032 1999-09-01 GLP: yes not published	N	DOW
KCP 10.2	Ward, T.J., Magazu, J.P. & Boeri, R.L.	1999	4-Amino-3,5-dichloro-6-fluoro-2-pyridinol (a metabolite of Fluroxypyr): Toxicity to the saltwater diatom, Skeletonema costatum T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990036 1999-09-13 GLP: yes not published	N	DoW
KCP 10.2	Kirk, H.D., Gilles,	2000	4-Amino-3-chloro-6-fluoro-2-pyridinol: Growth inhibition test with the freshwater green alga,	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
	M.M., McClymont, E.L. & McFadden, L.G.		Selenastrum capricornutum Printz Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA 001008 2000-04-24 GLP: yes not published		
KCP 10.2	Boeri, R.L., Magazu, J.P. & Ward, T.J.	1999	4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine (a metabolite of Fluroxypyr): Toxicity to the freshwater blue-green algae, Anabaena flos-aquae T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990039 1999-09-15 GLP: yes not published	N	DOW
KCP 10.2	Ward, T.J., Magazu, J.P. & Boeri, R.L.	1999	4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine (a metabolite of Fluroxypyr): Toxicity to the freshwater diatom, Navicula pelliculosa T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990037 1999-09-20 GLP: yes not published	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2	Boeri, R.L., Magazu, J.P. & Ward, T.J.	1999	4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine (a metabolite of Fluroxypyr): Toxicity to the saltwater diatom, Skeletonema costatum T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990040 1999-10-19 GLP: yes not published	N	DOW
KCP 10.2	Putt, A.E.	2002	Fluroxypyr, 1-Methylheptyl ester – the full life-cycle toxicity to midge (Chironomus riparius) under static conditions Springborn Laboratories, Inc., Wareham, MA, USA 011184 2002-02-11 GLP: yes not published	N	DOW
KCP 10.2	Kirk, H.D., Milazzo, J.M., Hugo, J.M. & Martin, M.D.	1996	Fluroxypyr 1-methylhepthylester: The toxicity to the aquatic plant, duckweed Lemna gibba L. G-3 The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3074 1996-06-04	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP: yes not published		
KCP 10.2	Ward, T.J., Magazu, J.P. & Boeri, R.L.	1999	4-Amino-3,5-dichloro-6-fluoro-2-pyridinol (a metabolite of Fluroxypyr): Toxicity to the duckweed, Lemna gibba T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990033 1999-11-11 (report amendment) GLP: yes not published	N	DOW
KCP 10.2	Ward, T.J., Magazu, J.P. & Boeri, R.L.	1999	4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine (a metabolite of Fluroxypyr): Toxicity to the duckweed, Lemna gibba T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA 990038 1999-03-17 GLP: yes not published	N	DOW
KCP 10.4	Rodgers, M.H.	2000	Fluroxypyr pyridinol (MII): Acute toxicity (LC50) to the earthworm (Eisenia foetida) Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, UK GHE-T-1088 2000-06-30 GLP: yes not published	N	DOW

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.4	Rees, P.B.	1996	Fluroxypyr methoxypyridine: Acute toxicity study in the earthworm (artificial soil test) Huntingdon Life Sciences Ltd., Eye, Suffolk, UK GHE-T-645 1996-06-25 GLP: yes not published	N	DOW
KCP 10.4	Carter, J.N.	2000	Fluroxypyr pyridinol (metabolite MII): Effects on soil non-target micro-organisms Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, UK GHE-T-1089 (85899) 2000-09-20 GLP: yes not published	N	DOW
KCP 10.5	Knowles, S.J.	1994	Ready Biodegradability of Fluroxypyr-1- methylheptyl ester (Modified Sturm Test) Life Science Research Ltd., Suffolk, England, UK GHE-P-2439 1991-05-23 GLP: yes not published	N	DOW

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1.1 Daphnia magna

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 202 (2004) of the test were met. Validity criteria: 1. The percentage of immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%), 2. The dissolved oxygen concentrations in the test vessels were within the range of 7.4 – 9.3 mg/L (criterion: not less than 3 mg/L). Deviation of the study: none **Agreed toxicity endpoints:** <u>The endpoint value based on nominal test item concentration:</u> The EC_{50}/48 h value is 4.95 mg/L (95%-CL lower: 4.05 mg/L upper: 6.05 mg/L) The LOEC is 4.70 mg/L The NOEC is 2.13 mg/L <u>The endpoint values based on the geometric mean of determined concentrations of clopyralid:</u> The EC_{50}/48 h value is 496.33 µg/L (95% confidence interval: 396.27 – 611.65 µg/L). The LOEC/48 h is 475.18 µg/L. The NOEC/48 h is 191.41 µg/L. <u>The endpoint values based on the geometric mean of determined concentrations of fluroxypyr:</u> The EC_{50}/48 h value is 411.27 µg/L (95% confidence interval: 329.50 – 506.56 µg/L). The LOEC/48 h is 393.08 µg/L. The NOEC/48 h is 161.30 µg/L.

Reference:	KCP 10.2/01
Report	CHR/H/CFF 250 EC Daphnia magna, Acute Immobilisation Test.; E. Nierzędska, 2021, Study code: W-03-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item:	CHR/H/CFF 250 EC; batch no. CHE2AC2001; the content of clopyralid: 117.67 g/L; the content of florasulam: 9.82 g/L; the content of fluroxypyr: 118.65 g/L; density at 20°C: 1.0832 g/mL; manufacturing date: February 16, 2022; expiry date: February 16, 2024.
Test system:	*Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.
Test design:	Semi-static test (48 h of exposure with renewal after 24 h); 4 replicates per each test item concentration and the control; 5 *Daphnia magna* in each replicate.
Nominal test item concentration:	50, 22.7, 10.3, 4.7, 2.13 and 0.97 mg/L plus the control.
Geometric means of determined concentrations of clopyralid	4859.84, 2359.92, 1064.99, 475.18, 191.41 and 89.74 µg/L plus the control
Geometric means of determined concentrations of fluroxypyr	4716.17, 1997.35, 880.32, 393.08, 161.30 and 71.48 µg/L plus the control
Test conditions:	Temperature: 19.3 – 19.9°C; pH of the control: 7.05 – 7.70; dissolved oxygen concentration in the control: 7.4 – 9.3 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.
Chemical determinations:	The concentrations of clopyralid, florasulam and fluroxypyr-meptyl which was stoichiometrically recalculated and expressed as fluroxypyr (acid) were chemically analyzed using the high performance liquid chromatography (HPLC) with Diode Array Detection.
Endpoint value:	EC_{50}/48 h, NOEC/48 h, and LOEC/48 h.

Results and discussion:

Immobilisation of Daphnia magna exposed to the test item, CHR/H/CFF 250 EC was investigated during a 48-hour semi-static test. The definitive test was performed with a test item concentrations of 50, 22.7, 10.3, 4.7, 2.13 and 0.97 mg/L plus the control. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentrations or the control per replicate. Four replicates were used for the test item concentrations and the control, each with five Daphnia magna.

The Daphnia magna were observed for immobilisation and any abnormal behavior or appearance after 24 and 48 h of exposure. The Daphnia magna were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.
In the control and in the test item concentrations of 0.97 and 2.13 mg/L, no immobilisation of Daphnia magna was observed during exposure. At exposure termination in the test item concentrations of 50 and 22.7 mg/L, the immobilisation of Daphnia magna was 100%. In the test item concentration of 10.3 mg/L, the immobilisation of Daphnia magna was 95%, whereas in the test item concentration of 4.7 mg/L, the immobilisation of Daphnia magna was 50%. No abnormal behaviour of Daphnia magna was observed during exposure.
The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined. The concentrations of active substances were chemically analyzed using the validated liquid chromatographic method with Diode Array Detection. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.
The concentrations of active substances were chemically analyzed in samples of all fresh test item concentrations and the control collected at exposure initiation and in samples of all spent test item concentrations and the control collected at renewal. Moreover, samples of the test item concentrations of 22.7 mg/L, 0.97 mg/L and the control fresh at renewal and spent at exposure termination were chemically analyzed.
In fresh samples at exposure initiation and at renewal, the determined concentrations of clopyralid were in the range of 86.3 - 96.9%, the concentrations of florasulam were in the range of 91.8 - 98.3% and the concentrations of fluroxypyr were in the range of 80.3 – 89.7% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.
In spent samples at renewal and at exposure termination, the determined concentrations of clopyralid were in the range of 76.5 – 96.6%, the concentrations of florasulam were in the range of 93.9 – 99.6% and the determined concentrations of fluroxypyr were in the range of 56.4 – 82.7% of the nominal concentration. Therefore, the concentrations of florasulam were stable under test conditions and the concentrations of clopyralid and fluroxypyr were not stable during 24 h under test conditions.
The endpoint value was determined based on the nominal test item concentration.

Results:
The endpoint value based on nominal test item concentration:
The EC50/48 h value is 4.95 mg/L (95%-CL lower: 4.05 mg/L upper: 6.05 mg/L)
The LOEC is 4.70 mg/L
The NOEC is 2.13 mg/L

The endpoint values based on the geometric mean of determined concentrations of clopyralid:
The EC50/48 h value is 496.33 µg/L (95% confidence interval: 396.27 – 611.65 µg/L).
The LOEC/48 h is 475.18 µg/L.
The NOEC/48 h is 191.41 µg/L.

The endpoint values based on the geometric mean of determined concentrations of fluroxypyr:
The EC50/48 h value is 411.27 µg/L (95% confidence interval: 329.50 – 506.56 µg/L).
The LOEC/48 h is 393.08 µg/L.
The NOEC/48 h is 161.30 µg/L.L

TEST VALIDITY CRITERIA
In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004) and EU Method C.2.:
- the percentage of immobilisation of Daphnia magna in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 7.4 – 9.3 mg/L (criterion: not less than 3 mg/L).).

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 201 of the test were met. Validity criteria: - the biomass in the control increased by a factor of 124.0 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.7% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 25.3% (criterion: it must not exceed 35%).

Deviation of the study: no

Agreed toxicity endpoints:

The endpoint values based on nominal test item concentrations are given below:

The E_rC_{50}/72 h value is 1.84 mg/L (95% confidence interval: 1.52 – 2.25).
The LOEC/72 h value for growth rate is 0.32 mg/L.
The NOEC/72 h value for growth rate is 0.128 mg/L.
The E_yC_{50}/72 h value is 0.40 mg/L (95% confidence interval: 0.32 – 0.50).
The LOEC/72 h value for yield is lower than or equal to 0.128 mg/L.
The NOEC/72 h value for yield is lower than 0.128 mg/L.

The endpoint values based on the geometric means of determined concentrations of clopyralid are given below:

The E_rC_{50}/72 h value is 0.16 mg/L (95% confidence interval: 0.14 – 0.20).
The LOEC/72 h value for growth rate is 0.03 mg/L.
The NOEC/72 h value for growth rate is 0.014 mg/L.
The E_yC_{50}/72 h value is 0.04 mg/L (95% confidence interval: 0.03 – 0.05).
The LOEC/72 h value for yield is lower than or equal to 0.014 mg/L.
The NOEC/72 h value for yield is lower than 0.014 mg/L.

The endpoint values based on the geometric means of determined concentrations of fluroxypyr are given below:

The E_rC_{50}/72 h value is 0.13 mg/L (95% confidence interval: 0.10 – 0.17).
The LOEC/72 h value for growth rate is 0.010 mg/L.
The NOEC/72 h value for growth rate is 0.002 mg/L.
The E_yC_{50}/72 h value is 0.01 mg/L (95% confidence interval: 0.01 – 0.02).
The LOEC/72 h value for yield is lower than or equal to 0.002 mg/L.
The NOEC/72 h value for yield is lower than 0.002 mg/L.

EC_{10}/EC_{20} calculation

The endpoint values determined based on the nominal test item concentrations:
The E_rC_{20}/72 h value is 0.79 mg/L (95% confidence interval: 0.54 – 1.02) and the E_rC_{10}/72 h value is 0.51 mg/L (95% confidence interval: 0.30 – 0.70).
The E_yC_{20}/72 value is 0.13 mg/L (95% confidence interval: 0.08 – 0.18) and E_yC_{10}/72 h value is 0.07 mg/L (95% confidence interval: 0.04 – 0.11).

The endpoint values determined based on the geometric mean of determined clopyralid concentrations:
The E_rC_{20}/72 h value is 0.07 mg/L (95% confidence interval: 0.05 – 0.10) and the E_rC_{10}/72 h value is 0.05 mg/L (95% confidence interval: 0.03 – 0.07).
The E_yC_{20}/72 value is 0.01 mg/L (95% confidence interval: 0.01 – 0.02) and E_yC_{10}/72 h value is 0.01 mg/L (95% confidence interval: 0.004 – 0.011).

The endpoint values determined based on the geometric mean of determined fluroxypyr concentrations:

The E_rC_{20}/72 h value is 0.05 mg/L (95% confidence interval: 0.03 – 0.06) and the E_rC_{10}/72 h value is 0.03 mg/L (95% confidence interval: 0.01 – 0.04).

The E_yC_{20}/72 value is 0.002 mg/L (95% confidence interval: 0.001 – 0.004) and E_yC_{10}/72 h value is 0.001 mg/L (95% confidence interval: not determined – 0.002).

A 2.2.1.1.2 Pseudokirchneriella subcapitata

Reference:	KCP 10.2/03
Report	CHR/H/CFF 250 EC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test.; G. Hodorek, 2023, Study code: W-01-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Test item:	CHR/H/CFF 250 EC; batch no. CHE2AC2001, the content of clopyralid: 117.67 g/L, florasulam: 9.82 g/L, fluroxypyr-meptyl (ester): 118.65 g/L1, density at 20°C: 1.0832 g/mL; manufacturing date: February 16 2022, expiry date: February 16, 2024.
Test system:	The unicellular freshwater green algae, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata (*Korshikov*)* Hindák, *Selenastrum capricornutum* Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.
Test design:	72 hours of exposure; three replicates per each test item concentration; six replicates per control; a background for each treatment; initial algal cell density: 1 x 10_4 cells/mL.
Nominal test item concentrations:	5.0, 2.0, 0.8, 0.32 and 0.128 mg/L plus the control.
Geometric means of determined concentrations of clopyralid:	0.014, 0.030, 0.074, 0.180, 0.414 mg/L plus the control.
Geometric means of determined concentrations of fluroxypyr (acid):	0.002, 0.010, 0.043, 0.141, 0.433 mg/L plus the control.
Test conditions:	Temperature: 22.4 – 22.8°C; pH of the control: 7.37 – 8.16; mean light intensity: 6208 – 6442 lux; constant illumination and shaking; medium: AAP.
Statistics:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Wiliams Multiple Sequential t-test Procedure, Step-down Jonckheere-Terpstra Test Procedure.
Endpoint values:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

The growth of the green algae *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) exposed to the test item, CHR/H/CFF 250 EC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial densi-

ty of the algae was 1 x 104 cells/mL. The definitive test was performed with the following test item concentrations: 5.0, 2.0, 0.8, 0.32 and 0.128 mg/L plus the control.
The number of algal cells was determined with indirect method, which involves a spectrophotometric measurement of the absorbance of algal suspension at 670 nm and converting its value into the number of cells using a standard curve. The absorbance for each replicate of each test item concentration and the control were measured after 24, 48, and 72 h of exposure. Morphology observations of the algae cells were performed at exposure termination.
Calculated inhibition of growth rate for the test item concentrations ranging from 0.128 to 5.0 mg/L after 72 h of exposure was in the range of 3.8 – 92.8% when compared to the control. Inhibition of yield for the test item concentrations ranging 0.128 to 5.0 mg/L after 72 h of exposure was in the range of 16.5 – 99.6% when compared to the control.
In test item concentrations of 0.128 mg/L no differences in shape, size and colour of algal cells were reported as compared to the algae cells in the control. In the test item concentrations of 0.32 and 0.8 mg/L, swollen opalescent algae cells were observed.In the test item concentration of 2 mg/L, swollen opalescent and comma-shaped algae cells were observed. In the test item concentration of 5 mg/L, swollen opalescent and comma-shaped algae cells were observed.
The concentrations of florasulam, clopyralid and fluroxypyr-meptyl were chemically determined using validated high performance liquid chromatographic method with DAD detection. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.
At exposure initiation, the determined concentrations of florasulam were in the range of 87.0 – 101.1% of the nominal concentration. The determined concentrations of clopyralid were in the range of 80.7 – 107.2 % of the nominal concentration. The determined concentrations of fluroxypyr (acid) were in the range of 87.8 – 106.8 % of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of florasulam were in the range of 87.6 – 97.6% of the nominal concentration. The determined concentrations of clopyralid were in the range of 72.2 – 100.3% of the nominal concentration. The determined concentrations of fluroxypyr were in the range of 0.0 – 68.1% of the nominal concentration. The results confirm, that the concentrations of florasulam were stable under test conditions, whereas the concentrations of clopyralid and fluroxypyr (acid) were not stable.
The endpoint values were determined based on the nominal test item concentrations, geometric means of determined concentrations of clopyralid and and geometric means of determined concentrations of fluroxypyr (acid).

Results:
The endpoint values based on nominal test item concentrations are given below:
The E_rC_{50}/72 h value is 1.84 mg/L (95% confidence interval: 1.52 – 2.25).
The LOEC/72 h value for growth rate is 0.32 mg/L.
The NOEC/72 h value for growth rate is 0.128 mg/L.
The E_yC_{50}/72 h value is 0.40 mg/L (95% confidence interval: 0.32 – 0.50).
The LOEC/72 h value for yield is lower than or equal to 0.128 mg/L.
The NOEC/72 h value for yield is lower than 0.128 mg/L.
The endpoint values based on the geometric means of determined concentrations of clopyralid are given below:
The E_rC_{50}/72 h value is 0.16 mg/L (95% confidence interval: 0.14 – 0.20).
The LOEC/72 h value for growth rate is 0.03 mg/L.
The NOEC/72 h value for growth rate is 0.014 mg/L.
The E_yC_{50}/72 h value is 0.04 mg/L (95% confidence interval: 0.03 – 0.05).
The LOEC/72 h value for yield is lower than or equal to 0.014 mg/L.
The NOEC/72 h value for yield is lower than 0.014 mg/L.

The endpoint values based on the geometric means of determined concentrations of fluroxypyr are given below:
The E_rC_{50}/72 h value is 0.13 mg/L (95% confidence interval: 0.10 – 0.17).
The LOEC/72 h value for growth rate is 0.010 mg/L.
The NOEC/72 h value for growth rate is 0.002 mg/L.
The E_yC_{50}/72 h value is 0.01 mg/L (95% confidence interval: 0.01 – 0.02).
The LOEC/72 h value for yield is lower than or equal to 0.002 mg/L.
The NOEC/72 h value for yield is lower than 0.002 mg/L

TEST VALIDITY CRITERIA
In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:
- the biomass in the control increased by a factor of 124.0 within the 72-hour test period (criterion: at least a 16-fold growth),

- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.7% (criterion: it must not exceed 7%),

- the mean coefficient of variation for the section-by-section growth rate in the control culture was 25.3% (criterion: it must not exceed 35%).

A 2.2.1.1.3 Anabaena flos-aquae

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 201 (2006) of the test were met. Validity criteria: - the biomass in the control increased by a factor of 28.1 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.1% (criterion: it must not exceed 10%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 29.5% (criterion: it must not exceed 35%). Deviation of the study: none **Agreed toxicity endpoints:** The endpoint values based on the nominal test item concentrations: The E_rC_{50}/72 h value is 7.53 mg/L (95% confidence interval: 6.78 – 8.28). The E_yC_{50}/72 h value is 4.40 mg/L (95% confidence interval: 3.81 – 5.20). The LOEC/72 h value for growth rate and yield is 3.05 mg/L. The NOEC/72 h value for growth rate and yield is 0.95 mg/L. The endpoint values based on the geometric mean od determined fluroxypyr (acid) concentrations: The E_rC_{50}/72 h value is 0.550 mg/L (95% confidence interval: 0.504 – 0.595). The E_yC_{50}/72 h value is 0.351 mg/L (95% confidence interval: 0.311 – 0.406). The LOEC/72 h value for growth rate and yield is 0.258 mg/L. The NOEC/72 h value for growth rate and yield is 0.069 mg/L. **EC_{20}/EC_{10} based on nominal concentration of formulation Turango 250 EC:** The E_rC_{20}/72 h value is 4.32 mg/L (95% confidence interval: 3.50– 4.99) and the E_rC_{10}/72 h value is 3.23 mg/L (95% confidence interval: 2.43 – 3.90). The E_yC_{20}/72 value is 2.64 mg/L (95% confidence interval: 2.09 – 3.09) and E_yC_{10}/72 h value is 2.02 mg/L (95% confidence interval: 1.46 – 2.46). **EC_{20}/EC_{10} based on geometric mean of determine fluroxypyr (acid) concentrations:** The E_rC_{20}/72 h value is 0.343 mg/L (95% confidence interval: 0.287 – 0.387) and the E_rC_{10}/72 h value is 0.268 mg/L (95% confidence interval: 0.210 – 0.315). The E_yC_{20}/72 value is 0.232 mg/L (95% confidence interval: 0.191 – 0.264) and E_yC_{10}/72 h value is 0.187 mg/L (95% confidence interval: 0.142 – 0.220).

Reference:	KCP 10.2/03
Report	CHR/H/CFF 250 EC Anabaena flos-aquae UTEX B 1444 Growth inhibition test.; K. Brzozowska-Wojoczek, 2023, Study code: W-04-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study) No

Materials and methods:

Test item: CHR/H/CFF 250 EC; batch no. CHE2AC2001, the content of clopyrailid: 117.67 g/L, florasulam: 9.82 g/L, fluroxypyr-meptyl (ester) as fluroxypyr acid: 118.65 g/L; manufacturing date: February 16, 2022, expiry date: February 16, 2024.

Test system: The freshwater cyanobacteria, Anabaena flos-aquae (Lyng.) Bréb UTEX B 1444 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna,

Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.

Test design: 72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial cyanobacterial cell density: 1 x 10^4 cells/mL.

Nominal test item concentrations:
100, 31,3, 9.77, 3.05 and 0.95 mg/L plus the control.

Geometric mean of determined fluroxypyr (acid) concentrations:
9.499, 2.910, 0.683, 0.258 and 0.069 mg/L plus the control

Test conditions: Temperature: 22.3 – 22.8°C; pH of the control: 6.51 – 7.43; mean light intensity: 4004 - 4143 lux; constant illumination and shaking; medium: AAP.

Chemical determinations:
The concentrations of clopyralid, florasulam and fluroxypyrmeptyl were chemically analyzed using the validated liquid chromatographic method with Diode Array Detection. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.

Statistics: Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Step-down Jonckheere-Terpstra Test Procedure.

Endpoint values: E_rC_{50}/72 h, E_yC_{50}/72 h, NOEC/72 h, LOEC/72 h.

Results and discussion:

The growth of the cyanobacteria Anabaena flos-aquae exposed to the test item, CHR/H/CFF 250 EC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the cyanobacteria was 1 x 104 cells/mL. The definitive test was performed using the following test item concentrations: 100, 31.3, 9.77, 3.05 and 0.95 mg/L (with a spacing factor of 3.2) plus the control.
The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope. In case of each replicate, the number of cells was determined after 24, 48, and 72 h of exposure. Morphology observations of the cyanobacteria cells were performed at exposure termination.
In the test item concentrations in the range of 0.95 – 9.77 mg/L, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control. In the test item concentrations of 31.3 and 100 mg/L no morphological observations were performed due to lack of the cyanobacteria cells. The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically analyzed using the validated liquid chromatographic method with Diode Array Detection.
The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations. The samples of each test item concentration and the control collected at exposure initiation and at exposure termination were chemically analysed.
At exposure initiation, the determined concentrations of clopyralid were in the range of 87.3 – 112.2% of the nominal concentration, the determined concentrations of florasulam were in the range of 93.8 – 95.9% of the nominal concentration, whereas the determined concentrations of fluroxypyr (acid) were in the range of 81.3 –

86.8% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of clopyralid were in the range of 80.1 – 106.5% of the nominal concentration, the determined concentrations of florasulam were in the range of 95.2 – 101.3% of the nominal concentration, whereas the determined concentrations of fluroxypyr (acid) were in the range of 46.9 – 88.1% of the nominal concentration. Therefore, the concentrations of clopyralid and florasulam were stable under test conditions However, the concentrations of fluroxypyr (acid) were not stable under test conditions.
The endpoint values were determined based on nominal test item concentrations and geometric means of determined fluroxypyr (acid) concnetrations.

Results:
The endpoint values based on the nominal test item concentrations:
The ErC50/72 h value is 7.53 mg/L (95% confidence interval: 6.78 – 8.28).
The EyC50/72 h value is 4.40 mg/L (95% confidence interval: 3.81 – 5.20).
The LOEC/72 h value for growth rate and yield is 3.05 mg/L.
The NOEC/72 h value for growth rate and yield is 0.95 mg/L.

The endpoint values based on the geometric mean od determined fluroxypyr (acid) concentrations:

The ErC50/72 h value is 0.550 mg/L (95% confidence interval: 0.504 – 0.595).
The EyC50/72 h value is 0.351 mg/L (95% confidence interval: 0.311 – 0.406).
The LOEC/72 h value for growth rate and yield is 0.258 mg/L.
The NOEC/72 h value for growth rate and yield is 0.069 mg/L.

TEST VALIDITY CRITERIA
In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU method C.3. were met:
- the biomass in the control increased by a factor of 28.1 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.1% (criterion: it must not exceed 10%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 29.5% (criterion: it must not exceed 35%).

A 2.2.1.1.1 Lemna Gibba

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 221 (2006) of the test were met. Validity criteria: - the doubling time of frond number in the control was 1.6 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 20.9), - the average specific growth rate in the control between day 0 and day 7 was 0.434 d^{-1} (minimum requirement: higher than 0.275 d^{-1}). Deviation of the study: In the experimental part of study, no deviations occurred from the OECD Test Guideline No. 221 (2006): '*Lemna sp*., Growth Inhibition Test', EU method C.26: '*Lemna sp., Growth Inhibition Test'*. **Agreed toxicity endpoints:**

	The endpoint values based on the nominal test item concentrations: Endpoints based on the frond number: The E_rC_{50}/7 d value is 0.310 mg/L (95% confidence interval 0.157 – 0.622). The E_rC_{20}/7 d value is 0.066 mg/L (95% confidence interval 0.038 – 0.117). The E_rC_{10}/7 d value is 0.029 mg/L (95% confidence interval 0.016 – 0.054). The E_yC_{50}/7 d value is 0.106 mg/L (95% confidence interval 0.076 – 0.149). The E_yC_{20}/7 d value is 0.053 mg/L (95% confidence interval 0.041 – 0.070). The E_yC_{10}/7 d value is 0.037 mg/L (95% confidence interval 0.028 – 0.050). For growth rate and yield, the LOEC/7 d value is lower than or equal to 0.06 mg/L, whereas NOEC/7 d value is lower than 0.06 mg/L. Endpoints based on the dry weight: The E_rC_{50}/7 d value is 4.674 mg/L (95% confidence interval 1.241 – 16.269). The E_rC_{20}/7 d value is 0.124 mg/L (95% confidence interval: 0.046 – 0.338). The E_rC_{10}/7 d value is 0.019 mg/L (95% confidence interval: 0.007 – 0.051). The E_yC_{50}/7 d value is 0.197 mg/L (95% confidence interval: 0.072 – 0.554). The E_yC_{20}/7 d value is 0.027 mg/L (95% confidence interval: 0.012 – 0.064). The E_yC_{10}/7 d value is 0.010 mg/L (95% confidence interval: 0.004 – 0.024) For growth rate and yield, the LOEC/7 d value is lower than or equal to 0.06 mg/L, whereas NOEC/7 d value is lower than 0.06 mg/L.
	The endpoint values based on the geometric means of determined concentrations of clopyralid: Endpoints based on the frond number: The E_rC_{50}/7 d value is 24.420 µg/L (95% confidence interval 12.019 – 49.142). The E_rC_{20}/7 d value is 5.833 µg/L (95% confidence interval 3.259 – 10.430). The E_rC_{10}/7 d value is 2.759 µg/L (95% confidence interval 1.498 – 5.083). The E_yC_{50}/7 d value is 9.088 µg/L (95% confidence interval 6.550 – 12.593). The E_yC_{20}/7 d value is 4.336 µg/L (95% confidence interval 3.302 – 5.690). The E_yC_{10}/7 d value is 2.945 µg/L (95% confidence interval 2.213 – 3.921). For growth rate and yield, the LOEC/7 d value is lower than or equal to 4.95 µg/L, whereas NOEC/7 d value is lower than 4.95 µg/L.
	Endpoints based on the dry weight: The E_rC_{50}/7 d value is 447.845 µg/L (95% confidence interval 96.429 – 1887.035). The E_rC_{20}/7 d value is 9.133 µg/L (95% confidence interval: 2.921 – 28.752). The E_rC_{10}/7 d value is 1.194 µg/L (95% confidence interval: 0.375 – 3.802). The E_yC_{50}/7 d value is 15.895 µg/L (95% confidence interval: 5.261 – 47.622). The E_yC_{20}/7 d value is 2.335 µg/L (95% confidence interval: 0.958 – 5.819). The E_yC_{10}/7 d value is 0.857 µg/L (95% confidence interval: 0.328 – 2.240) For growth rate and yield, the LOEC/7 d value is lower than or equal to 4.95 µg/L, whereas NOEC/7 d value is lower than 4.95 µg/L.

	The endpoint values based on the geometric means of determined concentrations of fluroxypyr: Endpoints based on the frond number: The E_rC_{50}/7 d value is 18.180 µg/L (95% confidence interval 9.776 – 34.153). The E_rC_{20}/7 d value is 2.760 µg/L (95% confidence interval 1.626 – 4.659). The E_rC_{10}/7 d value is 1.030 µg/L (95% confidence interval 0.591 – 1.796). The E_yC_{50}/7 d value is 4.516 µg/L (95% confidence interval 2.841 – 7.234). The E_yC_{20}/7 d value is 1.333 µg/L (95% confidence interval 0.911 – 1.971). The E_yC_{10}/7 d value is 0.705 µg/L (95% confidence interval 0.465 – 1.067). For growth rate and yield, the LOEC/7 d value is lower than or equal to 1.63 µg/L, whereas NOEC/7 d value is lower than 1.63 µg/L. Endpoints based on the dry weight: The E_rC_{50}/7 d value is 347.120 µg/L (95% confidence interval 98.698 – 1133.603). The E_rC_{20}/7 d value is 6.416 µg/L (95% confidence interval: 2.498 – 16.535). The E_rC_{10}/7 d value is 0.797 µg/L (95% confidence interval: 0.306 – 2.077). The E_yC_{50}/7 d value is 9.915 µg/L (95% confidence interval: 3.566 – 28.052). The E_yC_{20}/7 d value is 0.862 µg/L (95% confidence interval: 0.371 – 2.045). The E_yC_{10}/7 d value is 0.240 µg/L (95% confidence interval: 0.096 – 0.602) For growth rate and yield, the LOEC/7 d value is lower than or equal to 1.63 µg/L, whereas NOEC/7 d value is lower than 1.63 µg/L.

Reference:	KCP 10.2/04
Report	CHR/H/CFF 250 EC Lemna gibba CPCC 310, Growth inhibition test.; E. Nierzędska, 2023, Study code: W-02-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 221 (2006)/ EU Method C.26.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: CHR/H/CFF 250 EC; batch no. CHE2AC2001; the content of clopyralid: 117.67 g/L; the content of florasulam: 9.82 g/L; the content of fluroxypyr (acid): 118.65 g/L; density at 20°C: 1.0832 g/mL; manufacturing date: February 16, 2022; expiry date: February 16, 2024.

Test system: Freshwater aquatic plant Lemna gibba L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.

Test design: Semi-static system (7 days of exposure with renewal every 24 h); three replicates for each test item concentration and six replicates for the control.

Nominal test item concentrations:

20, 6.25, 1.95, 0.61, 0.19, 0.06 mg/L plus control.

Geometric means of determined concentrations of clopyralid
2219.91, 638.73, 150.17, 44.15, 16.75 and 4.95 µg/L plus the control

Geometric means of determined concentrations of fluroxypyr (acid)
1629.54, 465.70, 142.00, 42.39, 12.55 and 1.63 µg/L plus the control

Test conditions: Temperature: 23.0 – 23.3ºC; pH of the control: 7.41 – 8.56;
light intensity mean: 8160 – 8235 lux; constant illumination;
test vessels: glass crystallizers with a depth of 4 cm and a diameter of 9 cm containing 150 mL of each treatment; initial
frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP.

Endpoint value: ErC50, ErC20, ErC10, EyC50, EyC20, EyC10, LOEC and NOEC, based on frond number and dry weight.

Results and discussion:
The growth of Lemna gibba exposed to the test item, CHR/H/CFF 250 EC, was investigated in a 7 day semi-static test. The test was performed in glass crystallizers with a depth of 4 cm and a diameter of 9 cm containing 150 mL of either test item concentration or the control. The initial frond number in each test item concentration and the control was nine. The following test item concentrations were used: 0.06, 0.19, 0.61, 1.95, 6.25, 20 mg/L plus the control.
The total number of fronds in each test vessel was counted twice during exposure (day 2 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time.
At exposure termination, in the test item concentrations range of 0.06 – 0.19 mg/L, no distinctive changes from the normal development of plants in the control were observed.
In the test item concentrations of 1.95 and 0.61 mg/L, overlapping of fronds was observed, whereas in the test item concentration 6.25 mg/L, shorter roots was observed. In the test item concentration of 20 mg/L, spots of chlorosis and shorter roots were observed.
The concentrations of clopyralid, florasulam and fluoxypyr-meptyl were chemically determined. The concentrations of active substances were chemically analyzed using the validated liquid chromatographic method with Diode Array Detection [SOP/C/304, SOP/C/531]. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.
The concentrations of active substances were chemically analyzed in samples of all fresh test item concentrations and the control collected at exposure initiation and in samples of all spent test item concentrations and the control collected at the first renewal. Moreover, fresh and spent samples of the test item concentration of 20 mg/L, the lowest test item concentration of 0.06 mg/L and the control at renewals and at exposure termination were chemically determined [SOP/W/83].
In fresh samples at exposure initiation and at the renewal, the determined concentrations of clopyralid were in the range of 80.1 - 119.2%, the concentrations of florasulam were in the range of 87.3 - 118.7% and the concentrations of fluroxypyr (acid) were in the range of 81.1 – 118.0% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.
In spent samples at renewal and at exposure termination, the determined concentrations of clopyralid were in the range of 49.2 – 117.4%, the concentrations of florasulam were in the range of 84.6 – 115.4% and the determined concentrations of fluroxypyr (acid) were in the range of 0 – 86.3% of the nominal concentration. Therefore, the concentrations of florasulam were stable under test conditions and the concentrations of clopyralid and fluroxypyr were not stable during 24 h under test conditions.
The endpoint value was determined based on the nominal test item concentration, geometric mean of determined concentrations of clopyralid and geometric mean of fluroxypyr (acid).

The endpoint values based on the nominal test item concentrations:
Endpoints based on the frond number:
The ErC50/7 d value is 0.310 mg/L (95% confidence interval 0.157 – 0.622).
The ErC20/7 d value is 0.066 mg/L (95% confidence interval 0.038 – 0.117).
The ErC10/7 d value is 0.029 mg/L (95% confidence interval 0.016 – 0.054).
The EyC50/7 d value is 0.106 mg/L (95% confidence interval 0.076 – 0.149).
The EyC20/7 d value is 0.053 mg/L (95% confidence interval 0.041 – 0.070).
The EyC10/7 d value is 0.037 mg/L (95% confidence interval 0.028 – 0.050).

For growth rate and yield, the LOEC/7 d value is lower than or equal to 0.06 mg/L, whereas NOEC/7 d value is lower than 0.06 mg/L.
Endpoints based on the dry weight:
The ErC50/7 d value is 4.674 mg/L (95% confidence interval 1.241 – 16.269).
The ErC20/7 d value is 0.124 mg/L (95% confidence interval: 0.046 – 0.338).
The ErC10/7 d value is 0.019 mg/L (95% confidence interval: 0.007 – 0.051).
The EyC50/7 d value is 0.197 mg/L (95% confidence interval: 0.072 – 0.554).
The EyC20/7 d value is 0.027 mg/L (95% confidence interval: 0.012 – 0.064).
The EyC10/7 d value is 0.010 mg/L (95% confidence interval: 0.004 – 0.024)
For growth rate and yield, the LOEC/7 d value is lower than or equal to 0.06 mg/L, whereas NOEC/7 d value is lower than 0.06 mg/L.
The endpoint values based on the geometric means of determined concentrations of clopyralid:
Endpoints based on the frond number:
The ErC50/7 d value is 24.420 µg/L (95% confidence interval 12.019 – 49.142).
The ErC20/7 d value is 5.833 µg/L (95% confidence interval 3.259 – 10.430).
The ErC10/7 d value is 2.759 µg/L (95% confidence interval 1.498 – 5.083).
The EyC50/7 d value is 9.088 µg/L (95% confidence interval 6.550 – 12.593).
The EyC20/7 d value is 4.336 µg/L (95% confidence interval 3.302 – 5.690).
The EyC10/7 d value is 2.945 µg/L (95% confidence interval 2.213 – 3.921).
For growth rate and yield, the LOEC/7 d value is lower than or equal to 4.95 µg/L,

Endpoints based on the dry weight:
The ErC50/7 d value is 447.845 µg/L (95% confidence interval 96.429 – 1887.035).
The ErC20/7 d value is 9.133 µg/L (95% confidence interval: 2.921 – 28.752).
The ErC10/7 d value is 1.194 µg/L (95% confidence interval: 0.375 – 3.802).
The EyC50/7 d value is 15.895 µg/L (95% confidence interval: 5.261 – 47.622).
The EyC20/7 d value is 2.335 µg/L (95% confidence interval: 0.958 – 5.819).
The EyC10/7 d value is 0.857 µg/L (95% confidence interval: 0.328 – 2.240)
For growth rate and yield, the LOEC/7 d value is lower than or equal to 4.95 µg/L, whereas NOEC/7 d value is lower than 4.95 µg/L.
The endpoint values based on the geometric means of determined concentrations of fluroxypyr:
Endpoints based on the frond number:
The ErC50/7 d value is 18.180 µg/L (95% confidence interval 9.776 – 34.153).
The ErC20/7 d value is 2.760 µg/L (95% confidence interval 1.626 – 4.659).
The ErC10/7 d value is 1.030 µg/L (95% confidence interval 0.591 – 1.796).
The EyC50/7 d value is 4.516 µg/L (95% confidence interval 2.841 – 7.234).
The EyC20/7 d value is 1.333 µg/L (95% confidence interval 0.911 – 1.971).
The EyC10/7 d value is 0.705 µg/L (95% confidence interval 0.465 – 1.067).
For growth rate and yield, the LOEC/7 d value is lower than or equal to 1.63 µg/L, whereas NOEC/7 d value is lower than 1.63 µg/L.
Endpoints based on the dry weight:
The ErC50/7 d value is 347.120 µg/L (95% confidence interval 98.698 – 1133.603).
The ErC20/7 d value is 6.416 µg/L (95% confidence interval: 2.498 – 16.535).
The ErC10/7 d value is 0.797 µg/L (95% confidence interval: 0.306 – 2.077).
The EyC50/7 d value is 9.915 µg/L (95% confidence interval: 3.566 – 28.052).
The EyC20/7 d value is 0.862 µg/L (95% confidence interval: 0.371 – 2.045).
The EyC10/7 d value is 0.240 µg/L (95% confidence interval: 0.096 – 0.602)
For growth rate and yield, the LOEC/7 d value is lower than or equal to 1.63 µg/L, whereas NOEC/7 d value is lower than 1.63 µg/L.

VALIDITY CRITERIA
In the definitive test, the following validity criteria specified in the OECD Guideline No. 221/ EU method C.26. were met:
- the doubling time of frond number in the control was 1.6 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 20.9),
- the average specific growth rate in the control between day 0 and day 7 was 0.434 d-1 (minimum requirement: higher than 0.275 d-1).

A 2.2.1.1.2 Myriophyllum spictatum

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 239 of the test were met. Validity criteria: **VALIDITY OF THE STUDY** The mean total shoot length in the control increased by a factor of 2.3 within the 14 days of exposure (criterion: at least a 2-fold growth) • The mean total shoot fresh weight in control plants increased by a factor of 2.1 within the 14 days of exposure (criterion: at least a 2-fold growth) • Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium. • The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was 32.8 % (criterion: it must not exceed 35%). **Agreed toxicity endpoints:** In the study six deviation occurred: 11.03.2024 – Deviation from SPO-H-4: Na_3PO_4 * 12 H_2O instead of Na_3PO_4 was used to prepare the sediment. Proper amount of the reagent was recalculated and used, therefore the deviation did not affect the study. 20.03.2024 – Deviation from SPO-H-4: $MgSO_4$ was used to prepare the culture medium instead of $MgSO_4$ * 7 H_2O. Proper amount of the reagent was recalculated and used, therefore the deviation did not affect the study. 12.04.2024 – Deviation from SPO-H-4: $MgSO_4$ was used to prepare the culture medium instead of $MgSO_4$ * 7 H_2O. Proper amount of the reagent was recalculated and used, therefore the deviation did not affect the study. 25.04.2024 – Deviation from SPO-H-4: $MgSO_4$ was used to prepare the culture medium instead of $MgSO_4$ * 7 H_2O. Proper amount of the reagent was recalculated and used, therefore the deviation did not affect the study. According to the Study Plan: planted shoots apices/tips should be of 6 cm (± 1 cm). For the study the shoots length was 4-7 cm. This deviation did not affect the course and results of the study. Storage of reference item: between 8:30 p.m. on April 23 and 11:00 a.m. on April 24, the temperature dropped to 15.6. The deviation does not affect the reliability of the results. According to the Study Plan: planted shoots apices/tips should be of 6 cm (± 1 cm). For the study the shoots length was 4-7 cm. This deviation did not affect the course and results of the study. **Agreed endpoints:** **The endpoint values determined on the basis of the nominal test item concentrations and active ingredient content are given below:**

	Growth rate fresh weight endpoint values after 14 days of exposure [mg/L]	Yield fresh weight endpoint values after 14 days of exposure [mg/L]
E_xC_{50}	0.02	0.015
E_xC_{20}	0.006	0.004
E_xC_{10}	0.003	0.002
LOEC	0.019	0.019
NOEC	0.006	0.006
	Growth rate dry weight endpoint values after 14 days of exposure [mg/L]	**Yield dry weight endpoint values after 14 days of exposure [mg/L]**
ExC_{50}	0.035	0.035
ExC_{20}	0.028	0.027
ExC_{10}	0.025	0.024
LOEC	0.061	0.061
NOEC	0.019	0.019
	Growth rate total shoot length endpoint values after 14 days of exposure [mg/L]	**Yield total shoot length endpoint values after 14 days of exposure [mg/L]**
ExC_{50}	0.029	0.037
ExC_{20}	0.002	0.011
ExC_{10}	0.001	0.006
LOEC	0.019	>2.000
NOEC	0.006	>=2.000

Yield fresh weight endpoint value [mg/L] for active ingredient			
Endpoint	**Clopyralid**	**Florasulam**	**Fluroxypyr**
E_rC_{50}	0.00022	0.00002	0.00021
E_rC_{20}	0.00043	0.00003	0.00042
E_rC_{10}	0.00162	0.00013	0.00158
LOEC	0.00206	0.00016	0.00200
NOEC	0.00065	0.00005	0.00063
Yield dry weight endpoint value [mg/L] for active ingredient			
Endpoint	**Clopyralid**	**Florasulam**	**Fluroxypyr**
E_rC_{50}	0.00260	0.00020	0.00253
E_rC_{20}	0.00292	0.00023	0.00284
E_rC_{10}	0.00379	0.00030	0.00368
LOEC	0.00660	0.00052	0.00642
NOEC	0.00206	0.00016	0.00200

Yield total shoot length endpoint value [mg/L] for active ingredient						
Endpoint / Time	**Clopyralid**		**Florasulam**		**Fluroxypyr**	
	7 days	**14 days**	**7 days**	**14 days**	**7 days**	**14 days**
E_rC_{50}	0.00043	0.00065	0.00003	0.00005	0.00042	0.00063
E_rC_{20}	0.00141	0.00119	0.00011	0.00009	0.00137	0.00116
E_rC_{10}	0.01201	0.00400	0.00094	0.00031	0.01168	0.00389
LOEC	0.00660	>0.21638	0.00052	>0.01701	0.00642	>0.21049
NOEC	0.00206	>=0.21638	0.00016	>=0.01701	0.00200	>=0.21049

Yield total shoot length endpoint value [mg/L] for active ingredient						
Endpoint / Time	Clopyralid		Florasulam		Fluroxypyr	
	7 days	14 days	7 days	14 days	7 days	14 days
E_rC_{50}	0.00043	0.00065	0.00003	0.00005	0.00042	0.00063
E_rC_{20}	0.00141	0.00119	0.00011	0.00009	0.00137	0.00116
E_rC_{10}	0.01201	0.00400	0.00094	0.00031	0.01168	0.00389
LOEC	0.00660	>0.21638	0.00052	>0.01701	0.00642	>0.21049
NOEC	0.00206	>=0.21638	0.00016	>=0.01701	0.00200	>=0.21049

Growth rate fresh weight endpoint value [mg/L] for active ingredient			
Endpoint	Clopyralid	Florasulam	Fluroxypyr
E_rC_{50}	0.00032	0.00003	0.00032
E_rC_{20}	0.00065	0.00005	0.00063
E_rC_{10}	0.00216	0.00017	0.00210
LOEC	0.00206	0.00016	0.00200
NOEC	0.00065	0.00005	0.00063

Growth dry weight endpoint value [mg/L] for active ingredient			
Endpoint	Clopyralid	Florasulam	Fluroxypyr
E_rC_{50}	0.00270	0.00021	0.00263
E_rC_{20}	0.00303	0.00024	0.00295
E_rC_{10}	0.00379	0.00030	0.00368
LOEC	0.00660	0.00052	0.00642
NOEC	0.00206	0.00016	0.00200

Growth rate total shoot length endpoint value [mg/L] for active ingredient						
Endpoint / Time	Clopyralid		Florasulam		Fluroxypyr	
	7 days	14 days	7 days	14 days	7 days	14 days
E_rC_{50}	0.00747	0.00011	0.00059	0.00001	0.00726	0.00011
E_rC_{20}	0.00032	0.00022	0.00003	0.00002	0.00032	0.00021
E_rC_{10}	0.00747	0.00314	0.00059	0.00025	0.00726	0.00305
LOEC	0.00206	0.00206	0.00016	0.00016	0.00200	0.00200
NOEC	0.00065	0.00065	0.00005	0.00005	0.00063	0.00063

Reference:	KCP 10.2/05
Report	CHR/H/CFF 250 EC Water-sediment Myriophyllum spicatum toxicity test.; D. Kolek, 2024, Study code: ETOX-2024-1, EcoTox Alliance Sp. z o. o. Kalinowa 2, 43-520 Zaborze, Poland
Guideline(s):	according to OECD No. 239 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Product name:	CHR/H/CFF 250 EC
Batch number:	CHE2AC2001
Active ingredients content[1]:	Clopyralid: 120.0 ± 7.20 g/L
	Florasulam: 10.0 ± 1.50 g/L
	Fluroxypr: 120.0 ± 7.20 g/L
Density at 20°C:	1.0832 g/cm^3
IUPAC name (a.i.):	3,6-Dichloro-pyridine-2-carboxylic acid

	N-(2,6-Difluorophenyl)-8-fluoro-5-methoxy-[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide
	1-Methylheptyl (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxy)acetate
CAS number (a.i.):	Clopyralid:1702-17-6
	Florasulam: 145701-23-1
	Fluroxypr: 81406-37-3
Production date:	16.02.2022
Expiry date:	16.02.2025
Storage conditions:	15.6 - 23.5°C

Biological test system
Myriophyllum spicatum shoots were used in the study.
The source of the test system: The Institute of Ichthyobiology and Aquaculture of Polish Academy of Sciences in Golysz, 43-520 Zaborze, Kalinowa 2.
Before the test, acclimation to the test conditions according to SOP-B-19 and SOP-H-13 was included. The acclimation period started 25 days before the test initiation (23.03.2024 – 17.04.2024). Stock plants were acclimatized in aquaria in order to allow space for proliferation. Sediment and water-media composition were the same as used for the test.
Stock plants were visibly free of contamination with any other organisms, including snails, filamentous algae, fungi and insects (including eggs or larvae).
The pH of the medium at the start of acclimation was 7.8, the average light intensity was between 125.85 – 128.04 µE and the average temperature was between 20.3 – 22.9°C (measured constantly during the acclimation period).

Culture media:
1) Water medium
Smart and Barko medium was used for acclimation and testing. The medium was also used for preparation of test item solutions.
The pH of the medium (water phase) at test initiation was adjusted to 7.9 for optimum plant growth. Medium composition was as follows:

Component	Lot. number	Amount of reagent added to ultra-pure water (mg/L)
CaCl2 • 2 H2O	220204092	91.7
MgSO4	210625184	33.7
NaHCO3	179A08	58.4
KHCO3	269KQV	15.4

2) Sediment
The following formulated sediment, based on the artificial sediment used in OECD Test Guideline 239, was used in this test (amount of all the ingredients listed was calculated based on their dry matter):
a) 6.8 kg peat in finely ground form (according to 2 ± 0.5% organic carbon, peat content should be 4-5%) pH 5.5 to 6.0 as possible; air dried.
b) 8.33 kg kaolin clay (kaolinite content 20%).
c) 30.06 kg fine quartz sand with more than 50% of the particles between 50 and 200 µm (quartz sand content should be 75-76 %).
d) An aqueous nutrient medium was added such that the final sediment batch contained 200 mg/Kg dry sediment of both ammonium chloride (NH4Cl) and sodium phosphate (Na3PO4) and the moisture content of the final mixture was in a range of 30-50%.
e) Calcium carbonate of chemically pure quality (CaCO3) was added to adjust the pH of the final mixture of the sediment to 7.03.
For the test, the sediment was transferred into a glass containers, which fit into the glass beakers (the sediment surface area should cover approximately 90% of the vessel surface area). The glass containers was filled with the sediment such that the sediment surface is level.

Results and discussion:

The impact of CHR/H/CFF 250 EC on the vegetative growth of Myriophyllum plants growing in standardized media (water, sediment and nutrients) was tested during a 14 days toxicity study. The test was performed using the following test item concentrations: 0.00596, 0.0191, 0.061, 0.195, 0.625, 2.000 mg/L (separating factor 3.2) in four replicates each, determined based on the preliminary non-GLP study (results are provided in Appendix No. 1). Control was prepared in six replicates. Effects on growth was determined from quantitative assessments of shoot length, fresh weight and dry weight, as well as qualitative observations of symptoms such as chlorosis, necrosis or growth deformities. To quantify substance-related effects, growth in the test solutions was compared with that of the controls and the concentration bringing about a specified x% inhibition of growth (e.g. 50%) was determined and expressed as the EC50.
The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) was statistically determined from estimates of average specific growth rates and yield, if possible.

The endpoint values determined on the basis of the nominal test item concentrations and active ingredient content are given below. Endpoint values for active ingredients were calculated based on Table 1 available in Appendix No.4.

	Growth rate fresh weight endpoint values after 14 days of exposure [mg/L]	Yield fresh weight endpoint values after 14 days of exposure [mg/L]
E_xC_{50}	0.02	0.015
E_xC_{20}	0.006	0.004
E_xC_{10}	0.003	0.002
LOEC	0.019	0.019
NOEC	0.006	0.006
	Growth rate dry weight endpoint values after 14 days of exposure [mg/L]	**Yield dry weight endpoint values after 14 days of exposure [mg/L]**
ExC_{50}	0.035	0.035
ExC_{20}	0.028	0.027
ExC_{10}	0.025	0.024
LOEC	0.061	0.061
NOEC	0.019	0.019
	Growth rate total shoot length endpoint values after 14 days of exposure [mg/L]	**Yield total shoot length endpoint values after 14 days of exposure [mg/L]**
ExC_{50}	0.029	0.037
ExC_{20}	0.002	0.011
ExC_{10}	0.001	0.006
LOEC	0.019	>2.000
NOEC	0.006	>=2.000

Yield fresh weight endpoint value [mg/L] for active ingredient			
Endpoint	**Clopyralid**	**Florasulam**	**Fluroxypyr**
E_rC_{50}	0.00161	0.00013	0.00159
E_rC_{20}	0.00043	0.00003	0.00042
E_rC_{10}	0.00026	0.00002	0.00021
LOEC	0.00204	0.00016	0.00201
NOEC	0.00064	0.00005	0.00063
Yield dry weight endpoint value [mg/L] for active ingredient			
Endpoint	**Clopyralid**	**Florasulam**	**Fluroxypyr**

E_rC_{50}	0.00376	0.00029	0.0037
E_rC_{20}	0.00290	0.00023	0.00285
E_rC_{10}	0.00258	0.00020	0.00254
LOEC	0.00655	0.00051	0.00645
NOEC	0.00204	0.00016	0.00201

Yield total shoot length endpoint value [mg/L] for active ingredient

Endpoint / Time	Clopyralid		Florasulam		Fluroxypyr	
	7 days	14 days	7 days	14 days	7 days	14 days
E_rC_{50}	0.01192	0.00397	0.00093	0.00031	0.01173	0.00391
E_rC_{20}	0.00140	0.00118	0.00011	0.00009	0.00137	0.00116
E_rC_{10}	0.00043	0.00064	0.00003	0.00005	0.00042	0.00063
LOEC	0.00655	>0.21477	0.00051	0.01678	0.00645	>0.21141
NOEC	0.00204	>=0.21477	0.00016	0.01678	0.00201	>=0.21141

Growth rate fresh weight endpoint value [mg/L] for active ingredient

Endpoint	Clopyralid	Florasulam	Fluroxypyr
E_rC_{50}	0.00215	0.00017	0.00211
E_rC_{20}	0.00064	0.00005	0.00063
E_rC_{10}	0.00032	0.00003	0.00032
LOEC	0.00204	0.00016	0.00201
NOEC	0.00064	0.00005	0.00063

Growth dry weight endpoint value [mg/L] for active ingredient

Endpoint	Clopyralid	Florasulam	Fluroxypyr
E_rC_{50}	0.00376	0.00029	0.00370
E_rC_{20}	0.00301	0.00024	0.00296
E_rC_{10}	0.00269	0.00021	0.00264
LOEC	0.00655	0.00051	0.00645
NOEC	0.00204	0.00016	0.00201

Growth rate total shoot length endpoint value [mg/L] for active ingredient

Endpoint / Time	Clopyralid		Florasulam		Fluroxypyr	
	7 days	14 days	7 days	14 days	7 days	14 days
E_rC_{50}	0.00741	0.00311	0.00058	0.00024	0.00729	0.00307
E_rC_{20}	0.00032	0.00022	0.00003	0.00002	0.00032	0.00021
E_rC_{10}	0.00011	0.00011	0.00001	0.00001	0.00011	0.00011
LOEC	0.00204	0.00204	0.00016	0.00016	0.00201	0.00201
NOEC	0.00064	0.00064	0.00005	0.00005	0.00063	0.00063

VALIDITY CRITERIA
The mean total shoot length in the control increased by a factor of 2.3 within the 14 days of exposure (criterion: at least a 2-fold growth)
• The mean total shoot fresh weight in control plants increased by a factor of 2.1 within the 14 days of exposure (criterion: at least a 2-fold growth)
• Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium.

• The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was 32.8 % (criterion: it must not exceed 35%).

A 2.2.1 **Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms**

A 2.2.2 **KCP 10.2.3 Further testing on aquatic organisms**

A 2.3 **KCP 10.3 Effects on arthropods**

A 2.3.1 **KCP 10.3.1 Effects on bees**

A 2.3.1.1.1 KCP 10.3.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 213 (1998) of the test were met. Validity criteria: − the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%). − the LD_{50}/24 h of the reference item (dimethoate) was 0.25 µg a.i./bee (criterion: 0.1 – 0.3 µg a.i./bee). Deviation: none **Agreed toxicity endpoints:**

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD_{50} [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	**0.0**	**> 200.0**
12.5	30	0	**0.0**	
25.0	30	1	**3.33**	
50.0	30	0	**0.0**	
100.0	30	1	**3.33**	
200.0	30	4	**13.33**	

Reference:	KCP 10.3.1/01
Report	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test.; E. Kulec-Płoszcyca, 2023, Study code: B-15-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	CHR/H/CFF 250 EC content: 117.67 g/L of clopyralid (CAS No. 1702-17-6) 9.82 g/L of florasulam (CAS No.145701-23-1) 118.65 g/L of fluroxypyr (CAS No. 81406-37-3) batch no.: CHE2AC2001 production date: 16.02.2022 expiry date: 16.02.2024
Biological test system: **– age:** **– source:**	the honeybee, *Apis mellifera* L., strain: carnica approximately 3 weeks an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,
Test design:	– the test item: - exposure duration: 48 hours - number of doses: 5 doses and a control - number of replicates: 3 replicates - number of bees: 10 bees/replicate – the reference item: - exposure duration: 24 hours - number of doses: 3 doses - number of replicates: 3 replicates - number of bees: 10 bees/replicate
Test item doses:	12.5, 25.0; 50.0; 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses:	0.1, 0.2 and 0.4 µg a.i./bee and a control (0.0 µg/bee)
Test conditions:	
– temperature:	25°C
– relative air humidity:	63.5 − 64%
Photoperiod:	24h darkness, except during application and assessments
Statistical analysis:	regression analysis using the probit method
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, – the oral LD_{50}/24 h of the reference item (dimethoate).

Results and discussion

The acute oral toxicity study of CHR/H/CFF 250 EC was conducted to determine the LD50 value or to demonstrate that it is higher than the highest tested dose. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results.
Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of 50% sucrose solution, containing the test item at the doses mentioned above, using a micropipette. During the entire experiment, the insects were caged in groups of 10.
The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure.

After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute oral toxicity test finished after the 48-hour observation.

The acute oral toxicity study of the test item, CHR/H/CFF 250 EC on honeybees (Apis mellifera L.) in the laboratory test are summarized below..

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD_{50} [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	**0.0**	**> 200.0**
12.5	30	0	**0.0**	
25.0	30	1	**3.33**	
50.0	30	0	**0.0**	
100.0	30	1	**3.33**	
200.0	30	4	**13.33**	

Conclusions:
The median lethal doses LD50/24 h and LD50/48 h are higher than the highest dose used in the test i.e. 200.0 µg/honeybee..

TEST VALIDITY CRITERIA
The following validity criteria were met during the test:
− the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
− the LD50/24 h of the reference item (dimethoate) was 0.25 µg a.i./bee (criterion: 0.13 – 0.3 µg a.i./bee).

A 2.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 214 (1998) of the test were met. Validity criteria: − the mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10.0%), − the LD_{50}/24 h of the reference item (dimethoate) was 0.22 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee). Deviation: none **Agreed toxicity endpoints:**

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD_{50} [µg/bee]
		Total		
		[no.]	[%]	
0.0 (water control)	30	0	0.0	> 200.0
0.0 (1% Triton control)	30	0	0.0	
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	0	0.0	
200.0	30	0	0.0	

Reference:	KCP 10.3.1/02
Report	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test.; E. Kulec-Płoszczyca, 2023, Study code: B-134-22, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	CHR/H/CFF 250 EC content: 117.67 g/L of clopyralid (CAS No. 1702-17-6) 9.82 g/L of florasulam (CAS No. 145701-23-1) 118.65 g/L of fluroxypyr (CAS No. 81406-37-3) batch no.: CHE2AC2001 production date: 16.02.2022 expiry date: 16.02.2024
Biological test system: **– age:** **– source:**	the honeybee, *Apis mellifera* L., strain: carnica approximately 3 weeks an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,
Test design:	the test item: − exposure duration: 48 hours − number of doses: 5 doses with surfactant and two controls: water control and control with surfactant (1% Triton) − number of replicates: 3 replicates − number of bees: 10 bees/replicate the reference item: − exposure duration: 24 hours − number of doses: 3 doses with surfactant (1% Triton) − number of replicates: 3 replicates − number of bees: 10 bees/replicate
Test item doses:	12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and water control (0.0 µg/bee), 1% Triton control (0.0 µg/bee)
Reference item doses:	0.1, 0.2 and 0.4 µg a.i./bee
Test conditions: – temperature: – relative air humidity: 16 hours light : 8 hours dark	 25°C 64%
Place:	Dark room
Statistical analysis:	regression analysis using the log-probit method
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, , – the contact LD50/24 h of the reference item (dimethoate).

Results and discussion

Mortality of honeybees, Apis mellifera, exposed to CHR/H/CFF 250 EC was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results.

A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10.
The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure.
After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation..

The acute contact toxicity study of the test item, CHR/H/CFF 250 EC on honeybees (Apis mellifera L.) in the laboratory test are summarized below:

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD_{50} [µg/bee]
		Total		
		[no.]	[%]	
0.0 (water control)	30	0	**0.0**	**> 200.0**
0.0 (1% Triton control)	30	0	**0.0**	
12.5	30	0	**0.0**	
25.0	30	0	**0.0**	
50.0	30	0	**0.0**	
100.0	30	0	**0.0**	
200.0	30	0	**0.0**	

Conclusions:
The median lethal doses LD50/24 h and LD50/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee

TEST VALIDITY CRITERIA
The following validity criteria were met during the test:
- the mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10.0%),
- the LD50/24 h of the reference item (dimethoate) was 0.22 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Chronic oral toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 245 (217) of the test were met. The following validity criteria were met during the test: − At the end of the experiment average mortality of the control groups was 4.0% (criterion: it must not exceed 15%).

− After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (dietary dose: 0.011 µg/bee/day) was 100.0% (criterion: it must be ≥ 50% on day 10 of exposure).

Deviation: none

Agreed toxicity endpoints:

The effects of CHR/H/CFF 250 EC on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested[a] dose	Number of tested bees	Total mortality			LC_{50}	LDD_{50}
[µg/30 mg/day] [µg/bee/day]	[mg/kg]	[µg/bee/day]	[no]	No.	[%]	[%]*	[mg/kg]	[µg/bee/day]
CHR/H/CFF 250 EC								
0.0 (Control)			50	2	**4.0**	–	**> 666.7**	**> 17.2**
20.0	**666.7**	**17.2**	50	2	**4.0**	**0.0**		
NOEC			**≥ 666.7 mg/kg**					
NOEDD			**≥ 17.2 µg/bee/day**					
Dimethoate (reference item)								
0.024	**0.8**	**0.011**	30	30	**100.0**	**100.0**	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and actual sucrose solution consumption in each study group
*: corrected according Abbott's formula [7]

Reference:	KCP 10.3.1/03
Report	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test.; E. Kulec-Płoszczyca, 2023, Study code: B-18-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

CHR/H/CFF 250 EC
content: clopyralid (CAS No. 1702-17-6)
specification: 120.0 ±7.20 g/L
result of the analysis: 117.67 g/L
florasulam (CAS No. 145701-23-1)
specification: 10.0 ±1.50 g/L
result of the analysis: 9.82 g/L
fluroxypyr-meptyl (ester) (CAS No. 81406-37-3)
specification: 173 g/L1
fluroxypyr (acid) specification: 120.0 ±7.20 g/L
result of the analysis: 118.65 g/L2
batch no.: CHE2AC2001
production date: 16.02.2022

	expiry date: 16.02.2024
Biological test system:	species: the honeybee, *Apis mellifera* L.; strain: carnica, source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: freshly emerged worker honeybees (max. 2 days old) from the same queen-right colony
Experimental design:	□ the test item: number of concentrations: 1 and the control number of replicates: 5 number of insects: 10 bees/replicate □ the reference item: number of concentrations: 1 number of replicates: 3 number of insects: 10 bees/replicate exposure duration: 10 days
Nominal concentration of the test item: **Nominal dose of the test item:**	666.7 mg/kg 20.0 µg/bee/day
Test item dietary dose:	17.2 µg/bee/day
Nominal concentration of the reference item (dimethoate):	0.8 mg/kg
Nominal dose of the reference item (dimethoate):	0.024 µg/bee/day
Reference item dietary dose:	0.011 µg/bee/day
Test conditions:	temperature: 32.7 – 34.5ºC; relative humidity: 50.0 – 64.4%;
Statistical analysis:	not needed due to zero mortality (after Abbott's correction)
Endpoints:	honeybee mortality after 10 days of exposure

Results and discussion

The mortality of honeybees exposed to CHR/H/CFF 250 EC was investigated during 10-days chronic oral toxicity test.
The design of the definitive test was selected on the basis of the preliminary range-finding non-GLP test results. One dose of the test item was used (limit test). The nominal concentration was 666.7 mg/kg of diet (corresponding to the nominal dose of 20.0 µg/30 mg/day).
Daily dose, consumed by the bees in the group treated with the test item at the nominal concentration of 666.7 mg/kg (20 µg/30 mg/day) was 17.2 µg/bee/day (dietary dose). Daily dose was calculated on the basis of actual consumption of a treated 50% sucrose solution in each study group and the nominal dose used for the treatment.

Each group of bees (5 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentration of 666.7 mg/kg or 50% sucrose solution alone (control group) for 10 days.
Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per 10 bees) was fed the with 2 mL of a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.024 µg/30 mg).
Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10.
Daily dose, consumed by the bees in the group treated with the reference item at the nominal concentration of 0.8 mg/kg (0.024 µg/30 mg/day) was 0.011 µg/bee/day (dietary dose).
The insects were observed for mortality and behavioral abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure.
Average consumption of a 50% sucrose solution in the control group was 26.48 mg/bee/day. Average consumption in the group treated with the test item at the concentration of 666.7 mg/kg was 25.80 mg/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg was 13.68 mg/bee/day.
The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined using the validated high performance liquid chromatographic method with DAD detection. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.
Fresh samples of the test item concentration and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 666.7 mg/kg, the determined concentration of clopyralid was 103.3% of nominal concentration, the determined concentration of florasulam was 100.1% of nominal concentration and the determined concentration of fluroxypyr-meptyl reacalculated and expressed as fluroxypyr was 111.5% of nominal concentration. The result confirm that the test item concentration was prepared correctly.
After 4 days of the storage period, in the sample of the test item of 666.7 mg/kg, the determined concentration of clopyralid was 104.1% of nominal concentration, the determined concentration of florasulam was 100.9% of nominal concentration and the determined concentration of fluroxypyr-meptyl reacalculated and expressed as fluroxypyr was 111.0% of nominal concentration.
Based on the result of chemical analyses, the concentrations of clopyralid, florasulam and fluroxypyr-meptyl were stable under storage conditions.

The validity criterion concerning mortality was met, because mortality in the control was 4.0% after 10 days of exposure [1].
The percentage of mortality of the honeybees exposed to the test item, at the concentration of 666.7 mg/kg (dietary dose 17.2 µg/bee/day) at exposure termination (after 10 days), was 0.0%, after Abbott's correction.
On the basis of the obtained mortality results the LC50 is higher than 666.7 mg/kg, and the LDD50 value is higher than 17.2 µg/bee/day. The NOEC value is higher than or equal 666.7 mg/kg, the NOEDD value is higher than or equal 17.2 µg/bee/day.
The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality was equal to 100.0% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

The effects of CHR/H/CFF 250 EC on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested[a] dose	Number of tested bees	Total mortality			LC_{50}	LDD_{50}
[µg/30 mg/day] [µg/bee/day]	[mg/kg]	[µg/bee/day]	[no]	No.	[%]	[%]*	[mg/kg]	[µg/bee/day]
CHR/H/CFF 250 EC								
0.0 (Control)			50	2	**4.0**	–	**> 666.7**	**> 17.2**
20.0	**666.7**	**17.2**	50	2	**4.0**	**0.0**		
NOEC			**≥ 666.7 mg/kg**					
NOEDD			**≥ 17.2 µg/bee/day**					
Dimethoate (reference item)								
0.024	**0.8**	**0.011**	30	30	**100.0**	**100.0**	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and actual sucrose solution consumption in each study group
*: corrected according Abbott's formula [7]

TEST VALIDITY CRITERIA
The following validity criteria were met during the test:
− At the end of the experiment average mortality of the control groups was 4.0% (criterion: it must not exceed 15%),
− After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (dietary dose: 0.011 µg/bee/day) was 100.0% (criterion: it must be ≥ 50% on day 10 of exposure).

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 239 of the test were met. The following validity criteria were met during the test: • in control cumulative larval mortality from day 3 to day 8 was 13.890% (required: ≤15%), • in control the adults emergence rate on day 22 was 83.333% (required: ≥70%), Table 12. Deviation: none **Agreed toxicity endpoints:**

Final results of the study

Parameter	Concentration [mg of test item/kg of food]	Parameter	Dose [µg of test item/larva]
LC_{10}	168.598 (n.d. – n.d.)*	LD_{10}	25.938 (n.d. – n.d.)*
LC_{20}	279.073 (n.d. – n.d.)*	LD_{20}	42.934 (n.d. – n.d.)*
LC_{50}	731.846 (n.d. – n.d.)*	LD_{50}	112.594 (n.d. – n.d.)*
NOEC	325.000	NOED	50.000
LOEC	650.0**	LOED	100.0**

LC_{10} test item concentration causing mortality of 10% population
LC_{20} test item concentration causing mortality of 20% population
LC_{50} test item concentration causing mortality of 50% population
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowest test item concentration causing statistically significant differences in relations to the control
LD_{10} test item dose causing mortality of 10% population
LD_{20} test item dose causing mortality of 20% population
LD_{50} test item dose causing mortality of 50% population
NOED the highest test item dose not causing statistically significant differences in relations to the control
LOED the lowest test item dose causing statistically significant differences in relations to the control
* upper and lower confidence limits (95%) given in the brackets
** **values determined based on the analysis of the results**

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

Reference:	KCP 10.3.1/04
Report	Honey bee larval toxicity test following repeated exposure of the test item CHR/H/CFF 250 EC according to OECD GD 239 ENV/JM/MONO(2016)34.; A. Wozniak, 2022, Study code: 0038/0066/E, SORBOLAB Research Laboratory LLC, Zaniemyska Street 11, 61-029 Poznań, Poland
Guideline(s):	according to the OECD GD 239 ENV/JM/MONO(2016)34
Deviations:	Deviations from the Study plan were found concerning changes in temperature and humidity during larval, pre-pupal and pupal/imago stage during the range-finding, definitive and reference test. The above deviations did not affect the test result. The study met the validity criteria. Deviations from the Study plan are described in details in point 6.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Test design	stability test: tested concentrations and control in one replicate range-finding, definitive test, reference test: tested concentrations and control in one replicate; 36 larvae per replicate, 12 larvae from 3 different breeding

Test cages	stability test: volumetric flask of 100 mL volume range-finding, definitive, reference test: 48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator; from day 15 of the test – transparent plastic boxes placed in test room
Exposition time	4 days (from day 3 to day 6)
Duration of the test	stability test: 72 hours range-finding, definitive, reference test: 22 days
Tested concentrations (doses)	stability test: control (0 g of test item/L of solution) 0.05 g of test item/L of solution, corresponding to 0.65 mg of test item/kg of food 50 g of test item/L of solution, corresponding to 650 mg of test item/kg of food range-finding test: control (0 mg of test item/kg of food), corresponding to 0 µg of test item/larva (0 g of test item/L of solution) 0.65 mg of test item/kg of food, corresponding to 0.1 µg of test item/larva (0.05 g of test item/L of solution) 6.5 mg of test item/kg of food, corresponding to 1 µg of test item /larva (0.50 g of test item/L of solution) 65 mg of test item/kg of food, corresponding to 10 µg of test item /larva (5.0 g of test item/L of solution) 650 mg of test item/kg of food, corresponding to 100 µg of test item /larva (50.0 g of test item/L of solution) definitive test: control (0 mg of test item/kg of food)), corresponding to 0 µg of test item/larva (0 g of test item/L of solution) 40.63 mg of test item/kg of food, corresponding to 6.25 µg of test item/larva (3.125 g of test item/L of solution) 81.25 mg of test item/kg of food, corresponding to 12.5 µg of test item/larva (6.25 g of test item/L of solution) 162.5 mg of test item/kg of food, corresponding to 25.0 µg of test item/larva (12.5 g of test item/L of solution) 325 mg of test item/kg of food, corresponding to 50.0 µg of test item/larva (25.0 g of test item/L of solution) 650.00 mg of test item/kg of food, corresponding to 100.00 µg of test item/larva (50.0 g of test item/L of solution) reference test: control I - 0 mg of reference item/kg of food control II (with acetone) - 0 mg of reference item/kg of food fenoxycarb 0.32 mg of reference item/kg of food, i.e. 0.35 ng of reference item/µL of diet corresponding to 49.28 ng of reference item/larva

Test condition	stability test: average temperature 5.707°C (minimum temperature 4.9°C; maximum temperature 8.0°C); darkness range-finding test: - for larval stage (day 1-8): average temperature 34.344°C (minimum temperature 33.0°C; maximum temperature 34.6°C); average relative humidity 93.937% (minimum humidity 82.1%; maximum humidity 97.1%), darkness* - for pre-pupal stage (day 8-15): average temperature 34.378°C (minimum temperature 33.4°C; maximum temperature 34.6°C); average relative humidity 76.310% (minimum humidity 58.2%; maximum humidity 95.1%), darkness* - for pupal/imago stage (day 15-22): average temperature 34.736°C (minimum temperature 30.9°C; maximum temperature 39.1°C); average relative humidity 63.156% (minimum humidity 53.2%; maximum humidity 74.4%), darkness* definitive test and reference test: - for larval stage (day 1-8): average temperature 34.112°C (minimum temperature 32.8°C; maximum temperature 34.4°C); average relative humidity 88.846% (minimum humidity 58.6%; maximum humidity 91.3%), darkness* - for pre-pupal stage (day 8-15): average temperature 34.236°C (minimum temperature 34.2°C; maximum temperature 34.3°C); average relative humidity 72.246% (minimum humidity 71.2%; maximum humidity 76.7%), darkness* - for pupal/imago stage (day 15-22): average temperature 33.732°C (minimum temperature 32.3°C; maximum temperature 36.5°C); average relative humidity 62.383% (minimum humidity 56.2%; maximum humidity 72.7%), darkness*

The assessment test of the test item CHR/H/CFF 250 EC toxicity on honey bee larvae (*Apis mellifera* L.) was conducted in accordance with the OECD GD 239 ENV/JM/MONO(2016)34 Guideline.
During the test, the impact on the successive stages of development of the honey bee, resulting from the repeated exposure of the larval stage to the test item, were determined. The aim of the study was to determine the concentration of the test item causing the mortality of 50% of the population in the test (LC50 value) and the dose of the test item causing the mortality of 50% of the population after 22 days (LD50 value). The values of NOEC and NOED, LC10/LD10 and LC20/LD20 were determined for emerged adults (survival) on the 22nd day of the study.

Final results
In course of the experiment, the test item has shown apitoxic effect in mortality of following developmental stages of bees after 22 days of the test.
At the end of the study, the concentration and the dose causing 10%, 20% and 50% mortality of the population in the test (LC10, LC20, LC50 and LD10, LD20, LD50 values) were determined, as well as NOEC and NOED values were determined at 22nd day.

Parameter	Concentration [mg of test item/kg of food]	Parameter	Dose [µg of test item/larva]
LC_{10}	168.598 (n.d. – n.d.)*	LD_{10}	25.938 (n.d. – n.d.)*
LC_{20}	279.073 (n.d. – n.d.)*	LD_{20}	42.934 (n.d. – n.d.)*
LC_{50}	731.846 (n.d. – n.d.)*	LD_{50}	112.594 (n.d. – n.d.)*
NOEC	325.000	NOED	50.000
LOEC	650.0**	LOED	100.0**

LC_{10} test item concentration causing mortality of 10% population
LC_{20} test item concentration causing mortality of 20% population
LC_{50} test item concentration causing mortality of 50% population
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowest test item concentration causing statistically significant differences in relations to the control
LD_{10} test item dose causing mortality of 10% population
LD_{20} test item dose causing mortality of 20% population
LD_{50} test item dose causing mortality of 50% population
NOED the highest test item dose not causing statistically significant differences in relations to the control
LOED the lowest test item dose causing statistically significant differences in relations to the control
* upper and lower confidence limits (95%) given in the brackets
** **values determined based on the analysis of the results**

Validity criteria
The test met the validity criteria (acc. to OECD GD 239 OECD GD 239 ENV/JM/MONO(2016)34):
- in control group cumulative larval mortality from day 3 to day 8 was 13.89% (required: ≤15%),
- in control group the adults emergence rate on day 22 was 75.00% (required: ≥70%),
-for fenoxycarb as reference item, the adults emergence rate on day 22 was 16.67% (required: ≤20%),

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.4.1 Typhlodromus pyri

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: – mortality of the control group was 6.7% on day 7 of exposure (criterion: a maximum of 20%), – mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 94.6% on day 7 of exposure (criterion: from 50 to 100%), – the cumulative mean number of eggs per female in the control group was 8.0 (required: ≥ 4 eggs per female).

Deviation: The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/36: 'An extended laboratory test for evaluating the effects of plant protection products on the predatory mite, *Typhlodromus pyri* (Sch.)', other references given in section 9 and the SOP's listed in section 10 of the report and the Study Plan. According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen was used. However, in the experiment additional food in the form of the two-spotted spider mite (T. *urticae*) eggs, was used. Another food source prevents the mites from escaping from discs.

Agreed toxicity endpoints :

Parameter (endpoint)					
Mortality			**Reproduction**		
Test item rate [L/ha]	**Total**		**Test item rate [mL/ha]**	**Mean number of eggs/ female (Rr) [no.]**	**Reproduction reduction Pr [%]**
	[%]	**Corrected[a] [%]**			
control	6.7	–	**control**	8.0	–
0.125	8.3	1.8	**0.125**+	6.1	23.8
0.25	10.0	3.6	**0.25**+	5.8	27.6
0.5	11.7	5.4	**0.5**+	4.0	49.9
LR_{50}	**> 0.5 L/ha** > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr		**ER_{50}**	**> 0.5 L/ha** > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	
$NOER_{mortality}$	**≥ 0.5 L/ha** ≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr		**$NOER_{reproduction}$**	**< 0.125 L/ha** < 14.7 g of clopyralid + 1.2 g of florasulam + 14.8 g of fluroxypyr	
Reference item: dimethoate					
Rate [g/ha]	**Total [%]**	**Corrected[a] [%]**	**Reproduction**		
4.0	95.0	94.6	not assessed		

+: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]
[a]: mortality corrected according to the formula of Abbott [1]

Reference: KCP 10.3.1/05

Report: An extended laboratory test for evaluating the effects of CHR/H/CFF 250 EC on the predatory mite, Typhlodromus pyri (Sch.).; E. Kulec-Płoszczyca, 2023, Study code: B-131-22, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the ESCORT 1 (Barrett K.L. et al., 1994)
and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents
and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000))

Deviations: No

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

Materials and methods

Test item:	Name:	CHR/H/CFF 250 EC
Active substance:		117.67 g/L of clopyralid (CAS No. 1702-17-6) 9.82 g/L of florasulam (CAS No.145701-23-1) 118.65 g/L of fluroxypyr (CAS No. 81406-37-3)
Batch number:		CHE2AC2001
Manufacture date:		16.02.2022
Expiry date:		16.02.2024
Biological test system:		the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: *Phytoseiidae)*
– age:		24-hour-old protonymphs
– source:		a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented from a commercial breeder
Experimental design:		5 study groups: − a control group (0.0 L/ha) − CHR/H/CFF 250 EC at the rate of 0.125 L/ha − CHR/H/CFF 250 EC at the rate of 0.25 L/ha − CHR/H/CFF 250 EC at the rate of 0.5 L/ha − reference item: dimethoate at the rate of 4.0 g/ha number of replicates: 3/group number of mites in each replicate: 20
Test conditions:		
– temperature:		24 – 26°C
– relative air humidity:		64 – 76%
– photoperiod:		16 h light : 8 h dark
– light intensity:		843 lux
Statistical analysis:		Probit analysis using linear max. likelihood regression Chi2 2x2 Table Test with Bonferroni Correction Shapiro Wilk's Test on Normal Distribution Levene's Test on Variance Homogenity (with Residuals) Williams Multiple Sequential t-test Procedure
Endpoints:		
		– mite mortality after 7 days of the treatment – LR_{50} and $NOER_{mortality}$ – reproduction reduction (Pr) after 14 days of the treatment – ER_{50} and $NOER_{reproduction}$

Results and discussion

The aim of the extended laboratory test was to evaluate the effects of the test item, CHR/H/CFF 250 EC on mortality and reproduction of the predatory mite, T. pyri (Sch.).
On the basis of the preliminary test results, it was decided to use three rates of the test item in the definitive test. These were 0.125, 0.25 and 0.5 L/ha.
The mites, T. pyri at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (Pinus sp.) and T. urticae eggs. Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and groups treated with the test item at rates 0.125, 0.25 and 0.5 L/ha 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, dimethoate was used as a reference item. The rate of the reference item was 4.0 g/ha. The control group was treated with distilled water.
In the definitive test, mortality of the control group after 7 days of exposure was 6.7%. After 7 days of exposure to CHR/H/CFF 250 EC at rates of 0.125, 0.25 and 0.5 L/ha, the percentages of mortality, corrected according to the formula of Abbott, were 1.8, 3.6 and 5.4%, respectively.
There were no statistically significant difference in mortality the group treated with the test item at all rates in comparison to the control group (Chi2 2x2 Table Test with Bonferroni Correction).
The LR50 value is higher than 0.5 L/ha (> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr).
NOERmortality is higher than or equal to 0.5 L/ha (≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr).
After 7 days of exposure to dimethoate at the rate of 4.0 g/ha, mortality corrected according to the Abbott formula was 94.6%. Therefore, the validity criterion specified in the method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.
Reproduction of the surviving mites from the control group and all groups treated with test item was assessed since mortality of these groups was < 50.0%.
The mean reproduction rate (Rr) in the control group was 8.0 eggs/female. The mean Rr after 14 days of exposure to test item at the rates of 0.125, 0.25 and 0.5 L/ha were 6.1, 5.8 and 4.0 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.125, 0.25 and 0.5 L/ha were 23.8, 27.6 and 49.9%, respectively.
There were statistically significant difference in reproduction between all group treated with the test item, i.e. 0.125, 0.25 and 0.5 L/ha and the control group (Williams Multiple Sequential t-test Procedure,|t|>|t*|).
The calculated ER50 value is higher than 0.5 L/ha (> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr). NOERreproduction is lower than 0.125 L/ha (< 14.7 g of clopyralid + 1.2 g of florasulam + 14.8 g of fluroxypyr).

The effects of CHR/H/CFF 250 EC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized in the table.

Parameter (endpoint)					
Mortality			**Reproduction**		
Test item rate [L/ha]	**Total**		**Test item rate [mL/ha]**	**Mean number of eggs/ female (Rr) [no.]**	**Repro-duction reduction Pr [%]**
	[%]	**Corrected[a] [%]**			
control	6.7	–	**control**	8.0	–
0.125	8.3	1.8	**0.125[+]**	6.1	23.8
0.25	10.0	3.6	**0.25[+]**	5.8	27.6
0.5	11.7	5.4	**0.5[+]**	4.0	49.9
LR_{50}	**> 0.5 L/ha** > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr		**ER_{50}**	**> 0.5 L/ha** > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	
$NOER_{mortality}$	**≥ 0.5 L/ha** ≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr		**$NOER_{reproduction}$**	**< 0.125 L/ha** < 14.7 g of clopyralid + 1.2 g of florasulam + 14.8 g of fluroxypyr	
Reference item: dimethoate					
Rate [g/ha]	**Total [%]**	**Corrected[a] [%]**	**Reproduction**		
4.0	95.0	94.6	not assessed		

[+]: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]
[a]: mortality corrected according to the formula of Abbott [1]

Conclusions:
Based on the results it can be stated that CHR/H/CFF 250 EC at all rates has no adverse effect on mortality of the mites. The test item at all rates has an adverse effect on reproduction of the mites.

TEST VALIDITY CRITERIA
The following validity criteria were met during the study:
− mortality of the control group was 6.7% on day 7 of exposure (criterion: a maximum of 20%),
− mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 94.6% on day 7 of exposure (criterion: from 50 to 100%),
− the cumulative mean number of eggs per female in the control group was 8.0 (required: ≥ 4 eggs per female).

A 2.3.1.4.2 Aphidius rhopalosiphi

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: – after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%), – after 48 hours, mortality of the group treated with the reference item at the rate of 20.0 g/ha was 73.3% (criterion: a minimum of 50%),

– all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
– the mean number of mummies per female in the control group was 44.0 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).
Deviations of the study: The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/28: 'An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez.)', other references given in section 9 and the SOP's listed in section 10 of the report and the Study Plan. There were no deviations from mentioned documents.

Agreed toxicity endpoints:

Parametr (endpoint)						
Mortality			**Fecundity**			
Test item [L/ha]	**Total [%]**	**LR_{50} [L/ha]**	**Test item [L/ha]**	**Mean no. of mummies /female**	**Fecundity reduction Pr [%]**	**ER_{50} [L/ha]**
Control	0.0	–	**Control**	44.0	–	–
Test item: CHR/H/CFF 250 EC						
0.125	0.0	**> 0.5**	**0.125**	34.7	21.1	**> 0.5**
0.25	0.0		**0.25**+	27.5	37.6	
0.5	0.0		**0.5**+	26.7	39.2	
$NOER_{mortality}$	**≥ 0.5 [L/ha]**		**$NOER_{fecundity}$**	**0.125 [L/ha]**		
	≥ 58.8 g clopyralid/ha + 4.9 g florasulam/ha+ 59.3 g fluroxypyr/ha			14.7 g clopyralid/ha + 1.2 g florasulam/ha + 14.8 g fluroxypyr/ha		
Reference item: dimethoate						
[g/ha]	**Mortality**		**Fecundity**			
	Total [%]					
20.0	73.3		not assessed			

+: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3], [SOP/B/67].

Reference: KCP 10.3.1/06

Report: An extended laboratory test for evaluating the effects of CHR/H/CFF 250 EC on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez), E. Kulec-Płoszczyca, 2023, Study code: B-132-22, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010)

Deviations: No

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

Materials and methods

Test item:	Name:	CHR/H/CFF 250 EC
Active substance:		117.67 g/L of clopyralid (CAS No. 1702-17-6) 9.82 g/L of florasulam (CAS No.145701-23-1) 118.65 g/L of fluroxypyr (CAS No. 81406-37-3)
Batch number:		CHE2AC2001
Manufacture date:		16.02.2022
Expiry date:		16.02.2024
Biological test system:		the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: *Braconidae*, *Aphiidinae*
– age:		adult females (24 – 48 hours after emerging from mummies)
– source:		the culture was obtained from a commercial breeder (Katz Biotech AG)
Experimental design:		− a control group (0.0 L/ha) − 0.125 L/ha – 0.25 L/ha – 0.5 L/ha − Reference item: dimethoate at the rate of 20.0 g/ha mortality assessment: 6 replicates/group; 5 females/replicate fecundity assessment: 15 replicates/group; 1 females/replicate
Test conditions:		
– temperature:		19 – 20°C
– relative air humidity:		65 –96%
– photoperiod:		16 hours light : 8 hours dark
– light intensity:		mortality and oviposition assessment: 1827 lx fecundity phase: 4953 lx
Statistical analyses:		Fecundity: – Shapiro-Wilk's Test on Normal Distribution, – Levene's Test on Variance Homogeneity, – Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm Repellency: – Shapiro-Wilk's Test on Normal Distribution, – Levene's Test on Variance Homogeneity, – Multiple Sequentially-rejective t-test After Bonferroni-Holm..
Endpoints:		– wasp mortality after 48 hours of exposure, – determination of the LR50 and the NOER-mortality, – determination of the ER50 and the NOERfecundity. – reduction in fecundity (Pr) of the surviving female wasps exposed to test item, 12 days

after the oviposition period

Results and discussion

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/CFF 250 EC on mortality and fecundity of the parasitic wasp, Aphidius rhopalosiphi. On the basis of the results of the preliminary range - finding test, it was decided to use three rates of the test item in the definitive test. These were 0.125, 0.25 and 0.5 L/ha.
Adult wasps were exposed to the test item applied to barley plants. Observations of settling behavior were made during the initial 3 hours of exposure. The aims were to determine repellent effects of CHR/H/CFF 250 EC and to check if the test insects had contact with barley plants sprayed with the test item. Settling behavior of wasps from each replicate was observed five times. Mortality was determined 2, 24 and 48 hours after the introduction of the wasps to the test arenas.
Females which survived the 48-hour exposure to test item and the ones from the control group were subjected to fecundity assessments. Fifteen female wasps from the three group treated with the test item and the control were individually introduced into the fecundity units containing barley plants infested with the aphid, Rhopalosiphum padi. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.
Mortality after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.
To verify the sensitivity of the biological test system and the precision of the test procedure, dimethoate, which is an insecticide, was used as a reference item. The rate of the reference item was 20.0 g/ha. The control group was treated with distilled water.

In the definitive test, after 48 hours mortality of the control wasps was 0.0%. The mortality, in the groups treated with CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha were 0.0%.
Based on the obtained results the LR50 value could not be estimated. It could be assumed that LR50 is higher than 0.5 L/ha (58.8 g clopyralid/ha + 4.9 g florasulam/ha + 59.3 g fluroxypyr/ha). The NOERmortality is higher than or equal to 0.5 L/ha (58.8 g clopyralid/ha + 4.9 g florasulam/ha + 59.3 g fluroxypyr/ha).
The mortality of the wasps exposed to dimethoate at the rate of 20.0 g/ha was 73.3% after 48 hours. Therefore, the validity criterion specified in the Method description was met [6]. The results showed that the test organisms were sensitive to dimethoate.
The fecundity assessment showed that the mean number of mummies per female in the control group was 44.0 (after 12 days after oviposition). As for the wasps treated with test item at the rates of 0.125, 0.25 and 0.5 L/ha the mean number of mummies per female were 34.7, 27.5 and 26.7, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.125, 0.25 and 0.5 L/ha were 21.1, 37.6 and 39.2%, respectively.
At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item at the tested rate of 0.125 L/ha and the control group (Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm).
At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the tested rates of 0.25 and 0.5 L/ha and the control group (Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm).
Based on the obtained fecundity results it could be assumed that the ER50 value is higher than 0.5 L/ha (58.8 g clopyralid/ha + 4.9 g florasulam/ha + 59.3 g fluroxypyr/ha) and the NOERfecundity is 0.125 L/ha of the test item (14.7 g clopyralid/ha + 1.2 g florasulam/ha + 14.8 g fluroxypyr/ha).

The effects of the test item, CHR/H/CFF 250 EC on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Parametr (endpoint)						
Mortality			Fecundity			
Test item [L/ha]	Total [%]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	0.0	–	Control	44.0	–	–
Test item: CHR/H/CFF 250 EC						
0.125	0.0	> 0.5	0.125	34.7	21.1	> 0.5
0.25	0.0		0.25+	27.5	37.6	
0.5	0.0		0.5+	26.7	39.2	
$NOER_{mortality}$	≥ 0.5 [L/ha]		$NOER_{fecundity}$	0.125 [L/ha]		
	≥ 58.8 g clopyralid/ha + 4.9 g florasulam/ha+ 59.3 g fluroxypyr/ha			14.7 g clopyralid/ha + 1.2 g florasulam/ha + 14.8 g fluroxypyr/ha		
Reference item: dimethoate						
[g/ha]	Mortality Total [%]		Fecundity			
20.0	73.3		not assessed			

+: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3], [SOP/B/67].

Conclusion:
On the basis of the obtained mortality results it can be concluded that CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha has no adverse effect on the mortality of the wasps.
On the basis of the obtained fecundity results it can be concluded that CHR/H/CFF 250 EC at the tested rate of 0.125 L/ha has no adverse effect on the fecundity of the wasps.
On the basis of the obtained fecundity results it can be concluded that CHR/H/CFF 250 EC at the tested rates of 0.25 and 0.5 L/ha has an adverse effect on the fecundity of the wasps

TEST VALIDITY CRITERIA
– after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
– after 48 hours, mortality of the group treated with the reference item at the rate of 20.0 g/ha was 73.3% (criterion: a minimum of 50%),
– all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
– the mean number of mummies per female in the control group was 44.0 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

A 2.3.1.4.3 Chrysoperla Carnea

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study:

The following validity criteria were met during the study [4]:

- pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 20.0%),
- corrected mortality of the reference item group was 100.0% (criterion: a minimum of 50%),
- the mean number of eggs per female per day in the control group (fecundity) was 16.4 (criterion: ≥ 15.0),
- the mean hatching rate in the control group (fertility) was 78.0 (criterion: ≥ 70%).

Deviation of the study: The study was conducted according to the ESCORT 1 (Barrett K.L. *et al.*, 1994) and the ESCORT 2 (Candolfi M.P. *et al.*, 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Vogt H. *et al.*, 2000), SOP/B/62 and other procedures related to the study and the Study Plan. In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. *et al.*, 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/62.

Agreed toxicity endpoints:

Study group [application rate]	**Parameter (endpoints)**					
	Mortality			**Reproduction**		
Test item: CHR/H/CFF 250 EC						
[L test item/ha]	**[%]**	**LR_{50}**		**Mean number of eggs/ female/ day [no.]**	**Mean hatching rate [%]**	**Reduction [%]**
		[L test item/ha]	**[g a.s./ha]**			
Control (0.0)	0.0	**> 0.5**	> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	16.4	78.0	–
0.125	0.0			16.9	79.4	(-1.9)*
0.25	0.0			14.4	67.1	13.9
0.5	0.0			14.3	64.5	17.3
$NOER_{mortality}$	**≥ 0.5 L/ha**					
	≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr					
Reference item: Dimethoate						
[g/ha]	**[%]**	**Reproduction**				
15.0	100.0	not assessed				

*: the negative value indicates that mean hatching rate in the group treated with the test item was higher than in the control group

Reference: KCP 10.3.1/07

Report: An extended laboratory test for evaluating effects of CHR/H/CFF 250 EC on the green lacewing, Chrysoperla carnea (Steph.), E. Kulec-Płoszczyca, 2023, Study code: B-11-21, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines devel-

	oped by the IOBC, BART, and EPPO Joint Initiative (Vogt H. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	CHR/H/CFF 250 EC 117.67 g/L of clopyralid (CAS No. 1702-17-6) 9.82 g/L of florasulam (CAS No.145701-23-1) 118.65 g/L of fluroxypyr (CAS No. 81406-37-3) CHE2AC2001 16.02.2022 16.02.2024
Biological test system: – age: – source:	the green lacewing, *Chrysoperla carnea* (Steph.), Neuroptera: *Chrysopidae* first instars' larvae (3 days old) a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented by commercial breeder
Experimental design:	5 study groups: - a control group (0.0 L/ha) – CHR/H/CFF 250 EC at the rates of: – 0.125 L/ha – 0.25 L/ha – 0.5 L/ha – dimethoate at the rate of 15.0 g/ha number of replicates: 30 replicates/group number of larvae: 1 larva of Chrysoperla carnea /replicate
Test conditions: – temperature: – relative air humidity: – photoperiod: – light intensity	 23.2 – 27.0°C 60.0 – 89.9% 16 hours light : 8 hours dark 1743 lux
Statistical analysis:	not conducted due to lack of mortality
Endpoints:	– cumulative mortality of larvae, pupae, and adults after emergence – LR_{50} value – reproduction of the lacewings: - fecundity (mean number of eggs/female/day)

- fertility (mean hatching rate)

Results and discussion

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/CFF 250 EC on mortality and reproductive capacity of the green lacewing, Chrysoperla carnea. In a definitive test, three test item application rates were used. These were 0.125, 0.25 and 0.5 L/ha.
To assess mortality, 3-day-old larvae of Chrysoperla carnea were exposed to dry residues of the test item on leaf discs. Eggs of the mill moth Ephestia kuehniella were offered as food. After emergence of adults, total mortality was calculated on the basis of the numbers of dead larvae, pupae, and adults which died during emergence. There were 30 replicates of each treated group. Each of them contained 1 larva of Chrysoperla carnea.
To determine possible adverse effects of the test item on fecundity and fertility of the lacewings, reproductive performance was conducted.
Total mortality of the lacewings, the mean number of eggs laid per female lacewing per day, and the mean hatching rate were the endpoints.
To control the sensitivity of the biological test system, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 15.0 g/ha. Control lacewings had contact with discs sprayed with distilled water..

Study group [application rate]	Parameter (endpoints)					
	Mortality			Reproduction		
Test item: CHR/H/CFF 250 EC						
[L test item/ha]	[%]	LR_{50}		Mean number of eggs/ female/ day [no.]	Mean hatching rate [%]	Reduction [%]
		[L test item/ha]	[g a.s./ha]			
Control (0.0)	0.0	> 0.5	> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	16.4	78.0	–
0.125	0.0			16.9	79.4	(-1.9)*
0.25	0.0			14.4	67.1	13.9
0.5	0.0			14.3	64.5	17.3
$NOER_{mortality}$	≥ 0.5 L/ha					
	≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr					
Reference item: Dimethoate						
[g/ha]	[%]	Reproduction				
15.0	100.0	not assessed				

*: the negative value indicates that mean hatching rate in the group treated with the test item was higher than in the control group

Conclusion:
The validity criterion concerning mortality was met, because mortality of the green lacewings, Chrysoperla carnea (Steph.) in the control group was 0.0%. The percentages of mortality of the green lacewings exposed to the test item at all the tested rates, i.e. of 0.125, 0.25 and 0.5 L test item/ha of the test item were 0.0%.
There was no need to conduct statistical analysis of the mortality results, since there was no mortality observed.

On the basis of the obtained results it can be concluded that the LR50 value is higher than 0.5 L test item/ha (i.e. > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr). The NOERmortality value is higher than or equal to 0.5 L/ha (≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr).
The percentage of corrected mortality of Ch. carnea (Steph.) exposed to dimethoate at rate of 15.0 g/ha was 100.0%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate. The mean number of eggs/female/day in the control group was equal to 16.4 (criterion: ≥ 15.0). The mean numbers of eggs/female/day in the groups treated with the test item at the rates of 0.125, 0.25 and 0.5 L test item/ha were equal to 16.9, 14.4 and 14.3, respectively. The mean hatching rate in the control group was 78.0% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of 0.125, 0.25 and 0.5 L test item/ha were 79.4, 67.1 and 64.5%, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates 0.125, 0.25 and 0.5 L/ha were (-1.9), 13.9 and 17.3%, respectively. The negative value indicates that mean hatching rate in the group treated with the test item was higher than in the control group.
Based on the results it can be stated that CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha has no an adverse effect on mortality of the tested organisms. The test item at the rates of 0.25 and 0.5 L /ha has an adverse effect on reproduction of the green lacewings (i.e. the mean number of layed eggs by green lacewings and on mean hatching rate)..

TEST VALIDITY CRITERIA
The following validity criteria were met during the study:
– pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 20.0%),
– corrected mortality of the reference item group was 100.0% (criterion: a minimum of 50%),
– the mean number of eggs per female per day in the control group (fecundity) was 16.4 (criterion: ≥ 15.0),
– the mean hatching rate in the control group (fertility) was 78.0 (criterion: ≥ 70%).

A 2.3.1.4.4 Coccinella Septempunctata

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: - pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 30.0%), - mean corrected mortality of the reference item group was 100.0% (criterion: a minimum of 40%), - fertility (the mean number of fertile eggs/female/day) in the control group was 6.0 (criterion: ≥ 2 fertile eggs/female). Deviations of the study: The experimental part of the study was conducted according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Schmuck V. et al., 2000), SOP/B/63 and other procedures related with the study and the Study Plan. In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/63. The deviation is due to the type of study ordered by the Sponsor. **Agreed toxicity endpoints:**

Study group	Parameters (endpoints)					
	Mortality			Reproduction		
Test item [L/ha]	[%]	LR_{50} [L test item/ha]	LR_{50} [g a.s./ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]
Test item: CHR/H/CFF 250 EC						
Control (0.0)	0.0	>0.5	> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	6.4	6.0	-
0.125	0.0			4.7	4.4	27.3
0.25	0.0			3.4	3.1	47.6
0.5	2.5			3.2	2.7	54.3
$NOER_{mortality}$	≥0.5 [L test item/ha]					
	≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr					
Reference item: dimethoate						
[g/ha]	[%]	Reproduction				
3.2	100.0	not assessed				

Reference: KCP 10.3.1/08

Report: An extended laboratory test for evaluating effects of CHR/H/CFF 250 EC on the ladybird beetle, Coccinella septempunctata (L.), E. Kulec-Płoszczyca, 2023, Study code: B-133-22, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Schmuck et al., 2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Materials and methods

Test item: CHR/H/CFF 250 EC
117.67 g/L of clopyralid (CAS No. 1702-17-6)
9.82 g/L of florasulam (CAS No.145701-23-1)

	118.65 g/L of fluroxypyr (CAS No. 81406-37-3) CHE2AC2001 16.02.2022 16.02.2024
Biological test system:	the ladybird beetle, *C. septempunctata* L. (Arthropoda: *Coccinellidae)*
– age:	4-day-old larvae
– source:	Beetles was obtained from commercial breeder (Katz Biotech AG, Germany)
Experimental design:	5 study groups: – a control group (0.0 mL/ha) – CHR/H/CFF 250 EC at the rates of: - the test item at the rate of 0.125 L/ha - the test item at the rate of 0.25 L/ha - the test item at the rate of 0.5 L/ha – dimethoate at the rate of 3.2 g/ha number of replicates: 40 replicates/group number of larvae: 1 larva of Coccinella septempunctata /replicate
Test conditions:	
– temperature:	23.1 – 27.0°C
– relative air humidity:	60.0 – 89.9%
– photoperiod:	16 hours light : 8 hours dark
– light intensity	2073 lx
Statistical analysis:	probit analysis using linear max. likelihood regression, Chi2 2x2 Table Test with Bonferroni Correction
Endpoints:	
	– preimaginal mortality of the ladybird beetles – LR_{50} – $NOER_{mortality}$ – reproductive performance of the moulted beetles over a period of 14 days (the mean number of fertile eggs/female/day) reproduction reduction (Pr)

Results and discussion

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/CFF 250 EC on mortality and reproductive capacity of the ladybird beetle, Coccinella septempunctata. In a definitive test, three test item application rates of 0.125, 0.25 and 0.5 L/ha were used.
To assess mortality of the ladybird beetles, Coccinella septempunctata L., 4-day-old larvae were exposed to the test item applied to leaf discs. There were 40 replicates of each treated group. Each replicate contained 1 larva of C. septempunctata L. The larvae were fed with the fresh aphids, Acyrthosiphon pisum until pupation. During the exposure phase, survival, condition and development of the ladybird beetles were regularly assessed until the end of pupation. After emergence of the adults, pre-imaginal mortality was calculated on the basis of the numbers of dead larvae and pupae.
After completion of mortality assessment, healthy hatched beetles from the control group and from group treated with the test item at the rates of 0.125, 0.25 and 0.5 L/ha were subjected to evaluate the reproductive performance. To allow egg-laying, adult ladybirds were transferred to separate reproduction units. The beetles had continuous access to food in the form of a honey-water solution (2:1), pine pollen (Pinus sp.) and the broad bean plants infested with the aphid, A. pisum. Reproductive performance observations, concerning the numbers of eggs laid and their fertility were made between 28 and 43 day after treatment.

To check the relative susceptibility of the test system and the sensitivity of the test method, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 3.2 g/ha. Control beetles had contact with leaf discs sprayed with distilled water..

Conclusion:

The validity criterion concerning mortality was met, because mortality of the ladybird beetle, Coccinella septempunctata L. in the control group was equal to 0.0% (≤ 30.0%). The mortality of the ladybird beetles exposed to the test item at the rates of 0.125, 0.25 and 0.5 L/ha, were 0.0, 0.0 and 2.5%, respectively.

At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.125, 0.25 and 0.5 L/ha of CHR/H/CFF 250 EC and the control group (Chi2 2x2 Table Test with Bonferroni Correstion (p(z) > Alpha).

The LR50 value is higher than 0.5 L/ha of CHR/H/CFF 250 EC (i.e. > 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr). The NOERmortality is higher than or equal to 0.5 L/ha of CHR/H/CFF 250 EC(≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr).

The mortality of the ladybird beetles exposed to the reference item at the rate of 3.2 g of dimethoate/ha, was equal to 100.0%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to dimethoate.

The mean number of fertile eggs/female/day in the control group was 6.0 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha were equal to 4.4, 3.1 and 2.7, it refers to 27.3, 47.6 and 54.3% of reproduction reduction.

It can be concluded that CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha had no adverse effect on mortality of the ladybird beetle.

Based on the results, it can be stated that CHR/H/CFF 250 EC at the rates of 0.125, 0.25 and 0.5 L/ha has no adverse effect on the reproduction capacity (i.e. mean number of fertile eggs per female per day > 2) of the ladybird beetle.

Study group	Parameters (endpoints)					
	Mortality			Reproduction		
Test item [L/ha]	[%]	LR_{50}		Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]
		[L test item/ha]	[g a.s./ha]			
Test item: CHR/H/CFF 250 EC						
Control (0.0)	0.0	>0.5	> 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr	6.4	6.0	–
0.125	0.0			4.7	4.4	27.3
0.25	0.0			3.4	3.1	47.6
0.5	2.5			3.2	2.7	54.3
$NOER_{mortality}$	≥0.5 [L test item/ha]					
	≥ 58.8 g of clopyralid + 4.9 g of florasulam + 59.3 g of fluroxypyr					
Reference item: dimethoate						
[g/ha]	[%]	Reproduction				
3.2	100.0	not assessed				

TEST VALIDITY CRITERIA
The following validity criteria were met during the study [6]:
− pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 30.0%),
− mean corrected mortality of the reference item group was 100.0% (criterion: a minimum of 40%),
− fertility (the mean number of fertile eggs/female/day) in the control group was 6.0 (criterion: ≥ 2 fertile eggs/female).

A 2.3.1.4.5 Aged Residue study

Comments of zRMS:	The study was accepted by zRMS. **Validity criteria** For a bioassay to be deemed valid (Blümel *et al*., 2000), it was considered that: a) mortality in the control treatment over the initial 7 days of a bioassay should not exceed 20%. b) corrected mortality in the toxic reference treatment should be 50-100%. c) the mean cumulative number of eggs produced between 7 and 14 days should be equal to or exceed 4.0 per female in the control treatment. All of these criteria, where relevant, were met in the 0 and 14 DAT bioassays. Deviations of the study: noene **Agreed oxicity endpoints:**

Results

The results for bioassays initiated at 0 and 14 DAT are summarised below.

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean % mortality at 7 DAI [a)]	Corrected % mortality at 7 DAI [b)]	Mean number eggs/female (7-14 DAI) [c)]	Reduction in reproduction [%] [d)]
0 DAT	Control	-	11.3	-	7.5	-
	CHR/H/CFF 250 EC	0.5	13.8	2.8	7.9	-5.8
	Toxic reference	-	100 *	100	~	-
14 DAT	Control	-	3.0	-	11.6	-
	CHR/H/CFF 250 EC	0.5	10.0 *	7.2	10.4	10.9

a) For each bioassay, treatment mortalities were compared to the respective control using either the chi² 2x2 table test or Fisher's exact binomial test (α = 0.05, one-sided, > respective control), a statistically significant effect is denoted by an asterisk (*).

b) Mortality corrected for respective control treatment deaths using Abbott's formula. A positive value indicates an increase.

c) Treatments were compared to the respective control using Student's t-test for homogenous variances (α = 0.05, one-sided, < respective control), there were no significant differences.

d) Percentage reduction in numbers of eggs per female, relative to the respective control. A positive value indicates a decrease and a negative value indicates an increase in egg production, relative to the respective control.

~ indicates no assessments were made for this treatment.

Conclusion:
The effects of freshly-dried and field-aged foliar residues of CHR/H/CFF 250 EC on the predatory mite Typhlodromus pyri were evaluated in a series of extended laboratory tests. When applied to sweetcorn plants at a rate equivalent to 0.5 L test item/ha, fresh-dried residues and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

Reference:	KCP 10.3.1/09
Report	CHR/H/CFF 250 EC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae), L. Fallowfield, 2023, Study code: CHR-23-03, Mambo-Tox 2 Venture Road University Science Park Southampton SO16 7NP UK
Guideline(s):	Blümel et al. (2000). Laboratory residual contact test with the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Product code = CHR/H/CFF 250 EC
Formulation type = emulsifiable concentrate (EC)
Sample identification = 222022300907
Batch number = CHE2AC2001

Active substances = a) clopyralid b) florasulam c) fluroxypyr
Nominal content of a.s. = a) 120.0 g/L b) 10.0 g/L c) 120.0 g/L
Analysed content of a.s. = a) 117.67 g/L b) 9.82 g/L c) 118.65 g/L
Analysed density = 1.0832 g/cm3
Appearance = amber liquid
Storage at Test Facility = ambient laboratory conditions
Sample expiry date = 16 February 2024

Toxic reference item = Dimethoate 400 EC
Formulation type = emulsifiable concentrate (EC)
Batch No. = H2006-005
Common name of a.s. = dimethoate
Nominal content a.s. = 400 g/L
Appearance = pale yellow liquid
Expiry date = 19 February 2024
Application rate in test = 48 mL product/ha in 400 L water/ha †
Dilution rate = 0.25 mL product diluted to 100 mL with deionised water (stock = 1000 mL/ha), followed by dilution of 9.6 mL stock to 200 mL with deionised water

CHR/H/CFF 250 EC was evaluated at a single application rate, equivalent to 0.5 L test item/ha. This treatment was compared to a water control. A toxic reference treatment of dimethoate (an EC formulation containing nominally 400 g a.s./L, applied at a rate of 48 mL product/ha) was also included in the study.
All treatments were applied to sweetcorn plants, (Zea mays L.), using a laboratory track-sprayer, at a volume rate equivalent to 400 L spray solution/ha. After treatment, the plants were placed under UV permeable rain protection and extended laboratory bioassays were carried out using leaves collected from the plants at 0 and 14 DAT (days after treatment).
For each bioassay, 5-cm leaf sections were cut from the treated leaves (n = 5 per treatment). These were each laid, with the treated upper (adaxial) surface exposed, onto a layer of water-saturated cotton wool lining a Petri dish. A line of a non-drying sticky gel was drawn around the edge of each leaf section, to serve as a barrier to mite dispersal. Twenty protonymphal mites were placed at the centre of each arena and untreated pollen and water were provided for nourishment. The survival of the mites was assessed after 7 days, by which time the mites in the control treatment were adult. The sex of the surviving mites was determined and they were then left in situ so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after initiation (DAI) of the test was calculated. These reproduction assessments were made for the control and for the test-item treatment only, in both bioassays since the test item treatment resulted in ≤ 50% corrected mortality at 7 DAI.
The testing programme was to be continued until residues no longer resulted in unacceptable effects (i.e. where corrected mortality was ≤ 50% and any reduction in reproduction was ≤ 50% when compared to the control), in two consecutive bioassays

Results

The results for bioassays initiated at 0 and 14 DAT are summarised below.

Bioassay initiated	Treatment	Test item rate (L/ha)	Mean % mortality at 7 DAI [a)]	Corrected % mortality at 7 DAI [b)]	Mean number eggs/female (7-14 DAI) [c)]	Reduction in reproduction [%] [d)]
0 DAT	Control	-	11.3	-	7.5	-
	CHR/H/CFF 250 EC	0.5	13.8	2.8	7.9	-5.8
	Toxic reference	-	100 *	100	~	-
14 DAT	Control	-	3.0	-	11.6	-
	CHR/H/CFF 250 EC	0.5	10.0 *	7.2	10.4	10.9

a) For each bioassay, treatment mortalities were compared to the respective control using either the chi^2 2x2 table test or Fisher's exact binomial test ($\alpha = 0.05$, one-sided, > respective control), a statistically significant effect is denoted by an asterisk (*).

b) Mortality corrected for respective control treatment deaths using Abbott's formula. A positive value indicates an increase.

c) Treatments were compared to the respective control using Student's t-test for homogenous variances ($\alpha = 0.05$, one-sided, < respective control), there were no significant differences.

d) Percentage reduction in numbers of eggs per female, relative to the respective control. A positive value indicates a decrease and a negative value indicates an increase in egg production, relative to the respective control.

~ indicates no assessments were made for this treatment.

Conclusions:
The effects of freshly-dried and field-aged foliar residues of CHR/H/CFF 250 EC on the predatory mite Typhlodromus pyri were evaluated in a series of extended laboratory tests. When applied to sweetcorn plants at a rate equivalent to 0.5 L test item/ha, fresh-dried residues and 14-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

Validity criteria
For a bioassay to be deemed valid (Blümel *et al*., 2000), it was considered that:
a) mortality in the control treatment over the initial 7 days of a bioassay should not exceed 20%.
b) corrected mortality in the toxic reference treatment should be 50-100%.
c) the mean cumulative number of eggs produced between 7 and 14 days should be equal to or exceed 4.0 per female in the control treatment.
All of these criteria, where relevant, were met in the 0 and 14 DAT bioassays.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 KCP 10.4 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

Comments of zRMS:

The study is acceptable as provisional*.
The following validity criteria were met during the study:
- each replicate produced from 78 to 104 juveniles (84.9 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 10.0% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

Deviation of the study:
As it is indicated in the SOP/G/122, the amount of calcium carbonate to adjust the pH should be in the range from 0.04 to 0.055%. In the study, the needed amount of calcium carbonate was equal to 0.15%, therefore it is a deviation from the SOP/G/122. According to the OECD Guideline the amount of CaCO3 should be less than 1.0%. The applied quantity of the calcium carbonate in the study was in line with OECD assumptions. The deviation did not affect the results of the study.
Deviation of the study:

Temporarily accepted endpoints:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of clopyralid /kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]	Value [mg of fluroxypyr acid/kg dry weight of artificial soil]
EC_{10}	183.5 (124.5 – 221.4)	19.93 (13.52 – 24.05)	1.66 (1.13 –2.01)	20.10 (13.64 – 24.25)
EC_{20}	222.8 (168.6 – 257.9)	24.20 (18.32 – 28.02)	2.02 (1.53 – 2.34)	24.40 (18.47 – 28.25)
EC_{50}	323.0 (284.4 – 366.2)	35.09 (30.89 – 39.78)	2.93 (2.58 – 3.32)	35.38 (31.15 – 40.11)
NOEC (reproduction)	180.0	19.55	1.63	19.72
LOEC (reproduction)	320.0	34.76	2.90	35.05
LC_{50}	498.7 (158.0 – >1000.0)	54.17 (17.16 – >108.63)	4.52 (1.43 – >9.07)	54.63 (17.31 – 109.54)
NOEC (survival)	320.0	34.76	2.90	35.05
LOEC (survival)	560.0	60.83	5.08	61.34

*The toxicity endpoints was based on nominal concentration. However, at the end on the study concentration of fluroxypyr-methyl was below 80%.

Results from analysis of fluroxypyr-meptyl in test sample

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr-meptyl [mg/kg d.w.]	Mean concentration of fluroxypyr-meptyl determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (13.10.2022)	control	---	< LoD	---
	1000	157.7	146.4	92.8 ± 1.6
Day 28 (10.11.2022)	control	---	< LoD	---
	1000	157.7	9.11	5.8 ± 2.7
Day 56 (08.12.2022)	control	---	< LoD	---
	1000	157.7	4.65	2.9 ± 1.9

LOQ = 1.0 mg fluroxypyr-meptyl /kg; LOD = 0.05 mg fluroxypyr-meptyl /kg;
--- not calculated; ND – not detected

Results of fluroxypyr in test sample recalculated form fluroxypyr-meptyl

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr [mg/kg d.w.]	Mean concentration of fluroxypyr determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (13.10.2022)	control	---	< LoD	---
	1000	109.5	101.7	92.9 ± 1.6
Day 28 (10.11.2022)	control	---	< LoD	---
	1000	109.5	6.32	5.8 ± 2.8
Day 56 (08.12.2022)	control	---	< LoD	---
	1000	109.5	3.23	2.9 ± 2.2

--- not calculated; ND – not detected

The geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Aplicant should complete the calculations of toxicity endpoints based on geometric mean measured concentration with a risk assessment for earthworms.

The reliability of the test should be considered by MSs level.

Reference: KCP 10.4/01

Report: CHR/H/CFF 250 EC Earthworm reproduction test (Eisenia andrei); P. Pieczka, 2023, Study code: G-01-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

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/CFF 250 EC**
batch no.: CHE2AC2001

Active substances: clopyralid – 117.67 g/L
florasulam – 9.82 g/L
fluroxypyr acid – 118.65 g/L

Artificial soil: 10% sphagnum peat, 20% kaolin clay, 69.85% air-dried quartz sand, 0.15% calcium carbonate;

Test organism: the earthworm, *Eisenia andrei* obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology

Test design:	test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate
Concentrations of the test item:	control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil
Test conditions:	temperature: 20.3 – 22.0°C; pH at the beginning of the experiment: 5.68 – 5.78; pH at the end of the experiment: 5.60 – 5.71; soil moisture content at the beginning of the experiment: 15.5 –16.4% (45.5 – 48.2% of the maximum water holding capacity); soil moisture content at the end of the experiment: 14.9 – 16.4% (43.8 – 48.2% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 658.7 – 709.2 lux light intensity at the end of the experiment: 621.5 to 732.7 lux
Statistical analysis:	EC10, EC20, EC50, LC50 – probit analysis using linear max. likelihood regression, NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software
Endpoint:	EC10, EC20, EC50, NOEC, LOEC (reproduction) LC50, NOEC, LOEC (survival)

Results and discussion

The aims of the study were to assess the impact of CHR/H/CFF 250 EC on reproduction of the earthworm, Eisenia andrei and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous emulsion was mixed with a suitable amount of the artificial soil. Ten concentrations of the test item were: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is equal to 498.7 mg/kg dry weight of the artificial soil (i.e. 54.17 mg of clopyralid + 4.52 mg of florasulam + 54.63 mg of fluroxypyr/kg dry weight of the artificial soil).
No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed.
After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.6 to 560.0 mg/kg dry weight of artificial soil, the body weight change was between 5.6 and 13.4%. As for the control group, the body weight increase was equal to 15.0%.
After 8 weeks of the experiment, the obtained results led to the following conclusions:
After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 0.0 and 83.3 per replicate. The mean number of juveniles in the control group was equal to 84.9 per replicate.
After 8 weeks of the experiment, it was concluded that CHR/H/CFF 250 EC had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 320.0 to 1000.0 mg/kg dry weight of the artificial soil..

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of clopyralid /kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]	Value [mg of fluroxypyr acid/kg dry weight of artificial soil]
EC_{10}	183.5 (124.5 – 221.4)	19.93 (13.52 – 24.05)	1.66 (1.13 –2.01)	20.10 (13.64 – 24.25)
EC_{20}	222.8 (168.6 – 257.9)	24.20 (18.32 – 28.02)	2.02 (1.53 – 2.34)	24.40 (18.47 – 28.25)
EC_{50}	323.0 (284.4 – 366.2)	35.09 (30.89 – 39.78)	2.93 (2.58 – 3.32)	35.38 (31.15 – 40.11)
NOEC (reproduction)	180.0	19.55	1.63	19.72
LOEC (reproduction)	320.0	34.76	2.90	35.05
LC_{50}	498.7 (158.0 – >1000.0)	54.17 (17.16 – >108.63)	4.52 (1.43 – >9.07)	54.63 (17.31 – 109.54)
NOEC (survival)	320.0	34.76	2.90	35.05
LOEC (survival)	560.0	60.83	5.08	61.34

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the controls:
• each replicate produced from 78 to 104 juveniles (84.9 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
• the coefficient of variation of reproduction was 10.0% (criterion: ≤ 30%),
• adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

Amendment no. 1:

Additionally, the endpoint values were corrected by the recovery factor, calculated based on the results of the analytical measurements during the study. The recovery factor was determined by the geometric mean of recovery of the active substances.

Updated April 2024

The Aplicant provided the calculations of toxicity endpoints for earthworms based on geometric mean measured concentration with a risk assessment for eartworms. The calculations were accepted by RMS. 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 for proposed use of the product **Turango 250 EC**.

The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
clopyralid	108.6	96.14	88.53
florasulam	9.1	7.64	83.96
fluroxypyr acid	109.5	12.76	11.65

d.w. – dry weight of the artificial soil

The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
clopyralid	108.6	96.14
florasulam	9.1	7.64
fluroxypyr acid	109.5	12.76
SUM:	227.20	116.54
Recovery factor*	0.513	

* Recovery factor = (sum of recovered values)/(sum of nominal values)

The endpoint values corrected by the recovery factor

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
EC_{10}	183.5 (124.5 – 221.4)	94.1 (63.9 – 113.6)
EC_{20}	222.8 (168.6 – 257.9)	114.3 (86.5 – 132.3)
EC_{50}	323.0 (284.4 – 366.2)	165.7 (145.9 – 187.9)
NOEC (reproduction)	180.0	92.3
LOEC (reproduction)	320.0	164.2
LC_{50}	498.7 (158.0 – >1000.0)	255.8 (81.1 – >513.0)
NOEC (survival)	320.0	164.2
LOEC (survival)	560.0	287.3

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1.1 Folsomia candida

Comments of zRMS:

The study is acceptable as provisional*.
The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 12.5% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 514.8 (criterion: ≥ 100 juveniles at the end of the test).

Deviation of the study:
- culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016),
- at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016).

The deviations did not affect the results of the study.

Temporarily accepted endpoints:

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of clopyralid/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]	Value [mg of fluroxypyr acid/kg dry weight of the artificial soil]
LC_{10}	58.4 (26.8 – 85.7)	6.34 (2.91 – 9.31)	0.53 (0.24 – 0.78)	6.39 (2.94 – 9.38)
LC_{20}	87.3 (50.1 – 118.1)	9.48 (5.44 – 12.83)	0.79 (0.45 – 1.07)	9.56 (5.48 – 12.93)
LC_{50}	160.4 (118.7 – 207.6)	17.43 (12.90 – 22.56)	1.45 (1.08 – 1.88)	17.57 (13.01 – 22.75)
NOEC	32.0	3.48	0.29	3.51

*The toxicity endpoints was based on nominal concentration. However, at the end on the study concentration of fluroxypyr-methyl was below 80%.

Results from analysis of fluroxypyr-meptyl in test sample

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr-meptyl [mg/kg d.w.]	Mean concentration of fluroxypyr-meptyl determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (11.10.2022)	control	---	< LoD	---
	1000	157.7	163.575	103.7 ± 5.5
Day 14 (25.10.2022)	control	---	< LoD	---
	1000	157.7	20.924	13.3 ± 1.4
Day 28 (08.11.2022)	control	---	< LoD	---
	1000	157.7	9.953	6.3 ± 7.5

LOQ = 1.0 mg fluroxypyr-meptyl /kg; LOD = 0.05 mg fluroxypyr-meptyl /kg;
--- not calculated; ND – not detected

Results of fluroxypyr in test sample recalculated form fluroxypyr-meptyl

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr [mg/kg d.w.]	Mean concentration of fluroxypyr determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (11.10.2022)	control	---	< LoD	---
	1000	109.5	113.6	103.7 ± 5.5
Day 14 (25.10.2022)	control	---	< LoD	---
	1000	109.5	14.5	13.2 ± 1.4
Day 28 (08.11.2022)	control	---	< LoD	---
	1000	109.5	6.91	6.3 ± 7.5

--- not calculated; ND – not detected

The geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Aplicant should complete the calculations of toxicity endpoints based on geometric mean measured concentration with a risk assessment for *Folsomia candida.*

The reliability of the test should be considered by MSs level.

Reference:	KCP 10.4/02
Report	CHR/H/CFF 250 EC Collembolan (Folsomia candida) Reproduction Test, A. Gierbuszewska, 2023, Study code: G-02-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Depart-ment of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
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/CFF 250 EC
batch no.: CHE2AC2001

Active substances: clopyralid – 117.67 g/L
florasulam – 9.82 g/L
fluroxypyr acid – 118.65 g/L

Artificial soil: 5% sphagnum peat, 20% kaolin clay, 74.96% air-dried industrial sand and 0.04% calcium carbonate,

Test organism: the collembolan, Folsomia candida obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The collembolans used in the study were between 9 to 12 days old

Test design:
test duration: 28 days

number of replicates: 4 replicates / concentration + 8
replicates / control; number of collembolans: 10 / replicate

Concentrations of the test item:
a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil

Test conditions: temperature: 20.3 – 22.0°C;
pH at the beginning of the test: 6.30 – 6.44;
pH at the end of the test: 6.32 – 6.44;
soil moisture content at the beginning of the test: 14.4 – 15.4% (45.0 – 48.2% of the maximum water holding capacity);
soil moisture content at the end of the test: 13.4 – 14.7% (41.9 – 46.0% of the maximum water holding capacity);
lighting: 16 h light and 8h dark;
light intensity at the beginning of the experiment: 593.4 – 664.2 lux;
light intensity at the end of the experiment: 577.4 – 621.1 lux;

Statistical analysis: EC10, EC20, EC50 – probit analysis using linear max. likelihood regression
LC10, LC20 and LC50 – Weibull analysis using linear max. likelihood regression
NOEC (number of juveniles):
- Shapiro-Wilk's Test on Normal Distribution,
- Levene's Test on Variance Homogeneity (with Residuals),
- Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm
NOEC (survival):
- Shapiro-Wilk's Test on Normal Distribution,
- Levene's Test on Variance Homogeneity (with Residuals),
- Williams Multiple Sequential t-test Procedure

.
Endpoints: EC10, EC20, EC50, NOEC
LC10, LC20, LC50, NOEC

Results and discussion
The aims of the study were to assess the impact of CHR/H/CFF 250 EC on reproduction of the collembolans, Folsomia candida and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These were 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous emulsion was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.
After the application of the test item at the concentrations ranging from 5.6 to 180.0 mg/kg dry weight of the artificial soil, the mortality of adults was between 10 and 50%. No survival collembolans after application of the test item at the concentrations: 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil were observed. As for the control group, mortality of collembolans was equal to 12.5%

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of clopyralid/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]	Value [mg of fluroxypyr acid/kg dry weight of the artificial soil]
LC_{10}	58.4 (26.8 – 85.7)	6.34 (2.91 – 9.31)	0.53 (0.24 – 0.78)	6.39 (2.94 – 9.38)
LC_{20}	87.3 (50.1 – 118.1)	9.48 (5.44 – 12.83)	0.79 (0.45 – 1.07)	9.56 (5.48 – 12.93)
LC_{50}	160.4 (118.7 – 207.6)	17.43 (12.90 – 22.56)	1.45 (1.08 – 1.88)	17.57 (13.01 – 22.75)
NOEC	32.0	3.48	0.29	3.51

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 180.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 82.8 and 695.8 per replicate. No juvenile collembolans after application of the test item at the concentrations 320.0, 560.0 and 1000.0 mg/kg dry weight of artificial soil were observed. As for the control group, the number of juveniles was equal to 514.8 per replicate.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of clopyralid/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]	Value [mg of fluroxypyr acid/kg dry weight of the artificial soil]
EC_{10}	67.2 (59.6 – 73.5)	7.30 (6.47 – 7.99)	0.61 (0.54 – 0.67)	7.36 (6.52 – 8.06)
EC_{20}	81.6 (74.7 – 87.4)	8.86 (8.11 – 9.50)	0.74 (0.68 – 0.79)	8.94 (8.18 – 9.58)
EC_{50}	118.3 (112.4 – 124.6)	12.86 (12.21 – 13.54)	1.07 (1.02 – 1.13)	12.96 (12.31 – 13.65)
NOEC	56.0	6.08	0.51	6.13

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the controls:
• mean adult mortality: 12.5% (criterion: ≤ 20%),
• the mean number of juveniles per vessel at the end of the test: 514.8 (criterion: ≥ 100 juveniles at the end of the test)
- the coefficient of variation calculated for the number of juveniles: 10.8 (criterion: ≤ 30%).

Amendment no. 1:

Additionally, the endpoint values were corrected by the recovery factor, calculated based on the results of the analytical measurements during the study (Table 15). The recovery factor was determined by the geometric mean of recovery of the active substances (Tables 13, 14).

Table 11. The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
clopyralid	108.6	100.72	92.74
florasulam	9.1	8.91	97.89
fluroxypyr acid	109.5	22.49	20.54

d.w. – dry weight of the artificial soil

Table 12. The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
clopyralid	108.6	100.72
florasulam	9.1	8.91
fluroxypyr acid	109.5	22.49
SUM:	227.20	132.12
Recovery factor*	0.582	

* Recovery factor = (sum of recovered values)/(sum of nominal values)

Table 13. The endpoint values corrected by the recovery factor

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
EC_{10}	67.2 (59.6 – 73.5)	39.1 (34.7 – 42.8)
EC_{20}	81.6 (74.7 – 87.4)	47.5 (43.5 – 50.9)
EC_{50}	118.3 (112.4 – 124.6)	68.9 (65.4 – 72.5)
NOEC (reproduction)	56.0	32.6
LC_{10}	58.4 (26.8 – 85.7)	34.0 (15.6 – 49.9)
LC_{20}	87.3 (50.1 – 118.1)	50.8 (29.2 – 68.7)
LC_{50}	160.4 (118.7 – 207.6)	93.4 (69.1 – 120.8)
NOEC (survival)	32.0	18.6

Updated April 2024

The Aplicant provided the calculations of toxicity endpoints for *Folsomia candida* based on geometric mean measured concentration with a risk assessment for *Folsomia candida.* The calculations were accepted by RMS. 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 *Folsomia candida* for proposed use of the product **Turango 250 EC**.

A 2.4.2.1.2 Hypoaspis aculeifer

Comments of zRMS:	The study is acceptable as provisional*. The results are considered valid because the following criteria were satisfied in the controls: • mean adult mortality: 2.5% (criterion: ≤ 20%), • the mean number of juveniles per vessel at the end of the test: 131.5 (criterion: ≥ 50 juveniles at the end of the test), • the coefficient of variation for the number of juveniles: 18.9% (criterion: ≤ 30%). **Deviation of the study:** Deviations from the OECD Guideline No. 226 (2016): 1. According to the OECD Guideline No. 226 (2016) the water content of the arti-

ficial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test.
2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution.
3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory.

Deviation from the SOP/G/122:
As it is indicated in the SOP/G/122, the amount of calcium carbonate to adjust the pH should be in the range from 0.02 to 0.04%. In the study, the needed amount of calcium carbonate was equal to 0.22%, therefore it is a deviation from the SOP/G/122. According to the OECD Guideline No. 226 the amount of $CaCO_3$ should be less than 1.0%. The applied quantity of the calcium carbonate in the study was in line with OECD assumptions.

The deviations did not affect the results of the study.
Temporarily accepted endpoints:

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of clopyralid/kg dry weight of the artificial soil]	Value [mg of florasulam /kg dry weight of the artificial soil]	Value [mg of fluroxypyr acid /kg dry weight of the artificial soil]
EC_{10}	325.0 (276.0 – 382.8)	35.31 (29.98 – 41.58)	2.95 (2.50 – 3.47)	35.60 (30.23 – 41.93)
EC_{20}	354.8 (302.5 – 415.4)	38.54 (32.86 – 45.13)	3.22 (2.74 – 3.77)	38.86 (33.13 – 45.50)
EC_{50}	419.7 (349.7 – 507.6)	45.59 (37.99 – 55.14)	3.80 (3.17 – 4.60)	45.97 (38.30 – 55.60)
NOEC (reproduction)	320.0	34.76	2.90	35.05
LC_{10}	358.9	38.99	3.25	39.31
LC_{20}	>1000.0	>108.63	>9.07	>109.54
LC_{50}	>1000.0	>108.63	>9.07	>109.54
NOEC (survival)	320.0	34.76	2.90	35.05

*The toxicity endpoints was based on nominal concentration. However, at the end on the study concentration of fluroxypyr-methyl was below 80%.

Results from analysis of fluroxypyr-meptyl in test sample

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr-meptyl [mg/kg d.w.]	Mean concentration of fluroxypyr-meptyl determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (11.10.2022)	control	---	< LoD	---
	1000	157.7	139.176	88.3 ± 0.9
Day 7 (18.10.2022)	control	---	< LoD	---
	1000	157.7	55.349	35.1 ± 1.6
Day 14 (25.10.2022)	control	---	< LoD	---
	1000	157.7	26.07	16.5 ± 0.2

LOQ = 1.0 mg fluroxypyr-meptyl /kg; LOD = 0.05 mg fluroxypyr-meptyl /kg;
--- not calculated; ND – not detected

Results of fluroxypyr in test sample recalculated form fluroxypyr-meptyl

Time/ date of analysis	Concentration of test item [mg/kg d.w.]	Nominal concentration of fluroxypyr [mg/kg d.w.]	Mean concentration of fluroxypyr determined (n=3) in samples [mg/kg d.w.]	% ± RSD of nominal concentration
Day 0 (11.10.2022)	control	---	< LoD	---
	1000	109.5	96.6	88.2 ± 0.8
Day 7 (18.10.2022)	control	---	< LoD	---
	1000	109.5	38.5	35.2 ± 1.8
Day 14 (25.10.2022)	control	---	< LoD	---
	1000	109.5	18.1	16.5 ± 0.2

--- not calculated; ND – not detected

The geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Aplicant should complete the calculations of toxicity endpoints based on geometric mean measured concentration with a risk assessment for *Hypoaspis aculeifer*.

The reliability of the test should be considered by MSs level.

Reference:	KCP 10.4/03
Report	CHR/H/CFF 250 EC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, P. Pieczka, 2023, Study code: G-03-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Depart-ment of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
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/CFF 250 EC
batch number: CHE2AC2001

Active substance:
clopyralid – 117.67 g/L
florasulam – 9.82 g/L
fluroxypyr acid – 118.65 g/L

Artificial soil:
5% sphagnum peat, 20% kaolin clay, and 74.78% air-dried industrial sand, 0.22% calcium carbonate

Test organism:

the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.

Test design:
Concentrations of the test item:
test duration: 14 days
number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate

Concentrations of the test item:
a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg test item/kg dry weight of the artificial soil..

Test conditions:
temperature: 20.3 – 22.0°C
pH at the beginning of the test: 6.28 – 6.41
pH at the end of the test: 6.24 – 6.36
soil moisture content at the beginning of the test: 14.1 – 15.3% (44.1 – 47.9% of the maximum water holding capacity)
soil moisture content in the middle of the test: 14.0 – 15.0% (43.8 – 46.9% of the maximum water holding capacity)
soil moisture content at the end of the test: 13.8 – 14.9% (43.2 – 46.6% of the maximum water holding capacity)
light-dark cycle: 16 h light and 8 h dark
light intensity at the beginning of the test: 472.7– 511.8 lux
light intensity at end of the test: 499.7 – 543.2 lux

Statistical analysis:
EC10, EC20, EC50: 3 – param. Normal CDF
LC10, LC20, LC50: probit analysis
NOEC:
- offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm
- survival – Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response), Chi2 2x2 Table Test with Bonferroni Correction

Endpoints:
EC10, EC20, EC50, NOEC
LC10, LC20, LC50, NOEC

Results and discussion
The aims of the study were to assess the impact of CHR/H/CFF 250 EC on reproduction of the predatory mite, Hypoaspis (Geolaelaps) aculeifer and to determine the EC10, EC20, EC50, and NOEC.
Ten concentrations of the test item were used. These included: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of aqueous emulsion was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.
Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil was between 0.0% and 20.0%. Mortality of the control group was

equal to 2.5%.
After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 0.8 – 143.0 per replicate. The mean number of juveniles in the control group was equal to 131.5 per replicate.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days		
		No.	%	
control	80	2	2.5	131.5
5.6	40	4	10.0	125.5
10.0	40	2	5.0	120.5
18.0	40	1	2.5	122.3
32.0	40	1	2.5	143.0
56.0	40	3	7.5	129.3
100.0	40	0	0.0	135.8
180.0	40	0	0.0	141.3
320.0	40	3	7.5	119.8
560.0	40	7	17.5	9.8
1000.0	40	8	20.0	0.8

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of clopyralid/kg dry weight of the artificial soil]	Value [mg of florasulam /kg dry weight of the artificial soil]	Value [mg of fluroxypyr acid /kg dry weight of the artificial soil]
EC_{10}	325.0 (276.0 – 382.8)	35.31 (29.98 – 41.58)	2.95 (2.50 – 3.47)	35.60 (30.23 – 41.93)
EC_{20}	354.8 (302.5 – 415.4)	38.54 (32.86 – 45.13)	3.22 (2.74 – 3.77)	38.86 (33.13 – 45.50)
EC_{50}	419.7 (349.7 – 507.6)	45.59 (37.99 – 55.14)	3.80 (3.17 – 4.60)	45.97 (38.30 – 55.60)
NOEC (reproduction)	320.0	34.76	2.90	35.05
LC_{10}	358.9	38.99	3.25	39.31
LC_{20}	>1000.0	>108.63	>9.07	>109.54
LC_{50}	>1000.0	>108.63	>9.07	>109.54
NOEC (survival)	320.0	34.76	2.90	35.05

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the control:
• mean adult mortality: 2.5% (criterion: ≤ 20%),
• the mean number of juveniles per vessel at the end of the test: 131.5 (criterion: ≥ 50 juveniles at the end

of the test),
• the coefficient of variation for the number of juveniles: 18.9% (criterion: ≤ 30%).

Amendment no. 1:

Additionally, the endpoint values were corrected by the recovery factor, calculated based on the results of the analytical measurements during the study (Table 15). The recovery factor was determined by the geometric mean of recovery of the active substances (Tables 13, 14).

Table 11. The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
clopyralid	108.6	98.07	90.30
florasulam	9.1	8.37	91.93
fluroxypyr acid	109.5	40.68	37.15

d.w. – dry weight of the artificial soil

Table 12. The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
clopyralid	108.6	98.07
florasulam	9.1	8.37
fluroxypyr acid	109.5	40.68
SUM:	227.20	147.11
Recovery factor*	0.647	

* Recovery factor = (sum of recovered values)/(sum of nominal values)

Table 13. The endpoint values corrected by the recovery factor

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
LC_{10}	358.9	232.2
LC_{20}	>1000.0	>647.0
LC_{50}	>1000.0	>647.0
NOEC (survival)	320.0	207.0
EC_{10}	325.0 (276.0 – 382.8)	210.3 (178.6 –247.7)
EC_{20}	354.8 (302.5 – 415.4)	229.6 (195.7 – 268.8)
EC_{50}	419.7 (349.7 – 507.6)	271.5 (226.3 – 328.4)
NOEC (reproduction)	320.0	207.0

Updated April 2024

The Aplicant provided the calculations of toxicity endpoints for *Hypoaspis aculeifer* based on geometric mean measured concentration with a risk assessment for *Hypoaspis aculeifer*. The calculations were accepted by RMS. 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 *Hypoaspis aculeifer* for proposed use of the product **Turango 250 EC**.

A 2.4.2.2 KCP 10.4.2.1 Species level testing

A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1.1.1 Nitrogen transformation

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: The coefficients of variation (CV) in the control group were 6.3, 6.9, 2.5 and 1.9%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

Deviation from the OECD Guideline No. 216 (2000), the EU Method C.21:
According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer (point 3.4.4.4.).
These deviation did not affect the results of the study.

Agreed toxicity endpoints:

Nitrate formation rate* [mg nitrate/kg dry weight of soil/day] for selected time intervals.

Time interval [d]	Control					PEC					5xPEC				
	Replicate			Mean	± SD	Replicate			Mean	± SD	Replicate			Mean	± SD
	I	II	III			I	II	III			I	II	III		
0 – 7	10.292	9.092	7.727	9.037	± 1.28	7.051	7.837	9.280	8.056	± 1.13	5.270	4.742	5.706	5.239+	± 0.48
0 – 14	7.681	8.267	7.781	7.909	± 0.31	11.064	10.693	10.453	10.737	± 0.31	9.602	9.749	9.792	9.714	± 0.10
0 – 28	6.629	6.316	6.359	6.435	± 0.17	7.006	7.147	7.189	7.114	± 0.10	7.740	7.883	7.820	7.814	± 0.07

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14 and 28 day
* - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller)

Reference:	KCP 10.5/01
Report	CHR/H/CFF 250 EC Soil Microorganisms: Nitrogen Transformation Test, A. Gierbuszewska, 2022, Study code: G-17-22, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	Organization for Economic Cooperation and Development (OECD), Guidelines for Testing of Chemicals, Guideline No. 216, "Soil Microorganisms: Nitrogen Transformation Test" adopted January 21, 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material: CHR/H/CFF 250 EC
batch no.: CHE2AC2001

Active substance: clopyralid – 117.67 g/L
florasulam – 9.82 g/L
fluroxypyr – 118.65 g/

Soil: Agricultural soil collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna

Test design: Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. After adding the deionized water, every portion was divided into three replicates (3 x 500 g). Exposure period: 28 days..

Concentrations of the test item:

control; - PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil),
- - 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil).

Test conditions:
temperature: 18.9 – 22.0°C,
soil moisture: 46.6 – 49.3% of the maximum water holding capacity, incubation in darkness

Endpoints: The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation.
The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28 days.

Statistical analysis: - Shapiro-Wilk's test on Normal Distribution
- Levene's Test on Variance Homogeneity (with Residuals)
- Williams Multiple Sequential t-test Procedure

Results and discussion

The aim of the study was to detect long-term adverse effects of CHR/H/CFF 250 EC on the processes of nitrogen transformation in aerobic surface soils.
The freshly collected agricultural soil was used in the experiment. It was manually cleared of large objects and sieved to a particle size of 2 mm.
Two concentrations of the test item were used, i.e.:
- PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil),
- 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil).
The treated and the control soils were divided into three replicates.
On days 0, 7, 14 and 28 of incubation, soil samples were collected to determine the quantities of nitrate.
The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using deionised water. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used.
The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated

Results:
The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil) and 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil) did not exceed 25% on 28 day of analysis.
Conclusions:
On the basis of the results, it was concluded that CHR/H/CFF 250 EC at the concentrations corresponding to the PEC: 0.72 mg test item/kg dry weight of soil (0.078 mg of clopyralid + 0.007 mg of florasulam + 0.079 mg of fluroxypyr/kg dry weight of soil) and 5 x PEC: 3.60 mg test item/kg dry weight of soil (0.391 mg of clopyralid + 0.033 mg of florasulam + 0.394 mg of fluroxypyr/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils..

VALIDITY CRITERION
The coefficients of variation (CV) in the control group were 6.3, 6.9, 2.5 and 1.9%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is 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

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1.1 Seedling Emergence

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Turango 250 EC 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: 100.0% – pea, 95.0% – flax, 85.0% – carrot, 85.0% – onion, 90.0% – perennial ryegrass, 90.0% – oats, - the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%); - the control seedlings did not exhibit any visible phytotoxic effects; - environmental conditions for all plants of the same species were identical. Deviation from OECD Guideline No. 208: According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 72.4 and 242.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. Deviation from the Study Plan: The volume of samples subjected to the chemical analysis was equal to 400 mL each. According to the study plan it should have been 100 mL each. All deviations did not affects the results of the study. **Agreed toxicity endpoints:** **Expressed as mL of the test item/ha:**

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	291.5	300.1	162.0	> 500.0	> 500.0	> 500.0
NOER	80.0	200.0	32.0	200.0	≥ 500.0	≥ 500.0
Shoot length						
ER_{50}	128.7	134.4	47.8	155.2	> 500.0	> 500.0
NOER	32.0	32.0	12.8	32.0	≥ 500.0	≥ 500.0
Plant dry weight						
ER_{50}	81.7	122.8	74.8	> 500.0	> 500.0	> 500.0
NOER	12.8	32.0	12.8	32.0	≥ 500.0	≥ 500.0
Plant Damage						
ER_{50}	83.1	69.7	62.2	154.3	> 500.0	> 500.0

Expressed as g of clopyralid/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	34.3	35.3	19.1	> 58.8	> 58.8	> 58.8
NOER	9.4	23.5	3.8	23.5	≥ 58.8	≥ 58.8
Shoot length						
ER_{50}	15.1	15.8	5.6	18.3	> 58.8	> 58.8
NOER	3.8	3.8	1.5	3.8	≥ 58.8	≥ 58.8
Plant dry weight						
ER_{50}	9.6	14.5	8.8	> 58.8	> 58.8	> 58.8
NOER	1.5	3.8	1.5	3.8	≥ 58.8	≥ 58.8
Plant Damage						
ER_{50}	9.8	8.2	7.3	18.2	> 58.8	> 58.8

Expressed as as g of florasulam/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	2.86	2.95	1.59	> 4.91	> 4.91	> 4.91
NOER	0.79	1.96	0.31	1.96	≥ 4.91	≥ 4.91
Shoot length						
ER_{50}	1.26	1.32	0.47	1.52	> 4.91	> 4.91
NOER	0.31	0.31	0.13	0.31	≥ 4.91	≥ 4.91
Plant dry weight						
ER_{50}	0.80	1.21	0.74	> 4.91	> 4.91	> 4.91
NOER	0.13	0.31	0.13	0.31	≥ 4.91	≥ 4.91
Plant Damage						
ER_{50}	0.82	0.68	0.61	1.52	> 4.91	> 4.91

Expressed as g of fluroxypyr acid/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	34.6	35.6	19.2	> 59.3	> 59.3	> 59.3
NOER	9.5	23.7	3.8	23.7	≥ 59.3	≥ 59.3
Shoot length						
ER_{50}	15.3	16.0	5.7	18.4	> 59.3	> 59.3
NOER	3.8	3.8	1.5	3.8	≥ 59.3	≥ 59.3
Plant dry weight						
ER_{50}	9.7	14.6	8.9	> 59.3	> 59.3	> 59.3
NOER	1.5	3.8	1.5	3.8	≥ 59.3	≥ 59.3
Plant Damage						
ER_{50}	9.9	8.3	7.4	18.3	> 59.3	> 59.3

Reference: KCP 10.6.1/01

Report: CHR/H/CFF 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, A. Wróbel, 2023, Study code: G-06-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Depart-ment of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s):	OECD Guideline 208, 2006
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	**CHR/H/CFF 250 EC** batch number: CHE2AC2001 active substances: clopyralid – 117.67 g/L, florasulam – 9.82 g/L and fluroxypyr acid – 118.65 g/L
Test species:	pea (Pisum sativum), flax (Linum usitatissimum), carrot (Daucus carota), onion (Allium cepa), perennial ryegrass (Lolium perenne), oats (Avena sativa)
Soil:	Sandy loam
Study design:	number of rates: 7 + control; number of replicates/rate: 7 (pea), 4 (flax, carrot, onion, perennial ryegrass and oats). The total number of seeds per application rate: 21 (pea) and 20 (flax, carrot, onion, perennial ryegrass and oats)
Application rates:	control, 2.0, 5.1, 12.8, 32.0, 80.0, 200.0 and 500.0 mL/ha
Volume of deionized water:	volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha.
Test conditions:	temperature: 19.4 – 23.8°C, humidity: 47.2 – 79.7%, lighting: 16 h light : 8 h dark; light intensity: 72.4 – 242.2 µE/m2/s; carbon dioxide concentration: 348 – 385 ppm;
Statistical analysis:	The ER10, ER25 and ER50 values for the final number of plants - probit analysis (carrot, perennial ryegrass, oats) or logit analysis (pea, flax onion) using linear max likelihood regression. The ER10, ER25 and ER50 values for shoot length were determined with the 3-param. normal CDF non-linear regression (pea, flax) or with the 4-param. normal CDF non-linear regression (carrot, onion) or probit analysis using linear max likelihood regression (perennial ryegrass, oats). The ER10, ER25 and ER50 values for shoot

	dry weight were determined with the 3-param. normal CDF non-linear regression (pea, carrot) or probit analysis using linear max likelihood regression (flax, onion, perennial ryegrass, oats). Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period. The probit (pea, flax, onion, perennial ryegrass, oats) or Weibull (carrot) analysis using linear max likelihood regression was used. NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on seedling emergence and seedling growth. In order to determine the NOER values, the following tests were used: The final number of plants: Multiple Sequentially-rejective Chi-2x2 Table Test After Bonferroni-Holm or Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm. The shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Multiple Sequentially-rejective Median (2x2-Table) Test After Bonferroni-Holm or Williams Multiple Sequential t-test Procedure or Step-down Jonkckheere-Terpstra Test Procedure or Dunnett's Multiple t-test Procedure. The plant dry weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Step-down Jonkckheere-Terpstra Test Procedure or Dunnett's Multiple t-test Procedure or Multiple Sequentially-rejective Welsh-t test After Bonferroni-Holm.
Endpoints:	ER25, ER50, NOER

Results and discuss:

The study, aimed at evaluating the effect CHR/H/CFF 250 EC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 3 dicotyledonous and 3 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 5 (flax, carrot, onion, perennial ryegrass and oats) or 3 (pea) seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day to the emergence of 50% of the control seedlings and after then every 1 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The exposure period

finished 14 days after the emergence of 50% of the control seedlings. At the end of the exposure, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed.
The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER10, ER25, ER50, and NOER.
Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period..

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 and ER50 values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	291.5	300.1	162.0	> 500.0	> 500.0	> 500.0
NOER	80.0	200.0	32.0	200.0	≥ 500.0	≥ 500.0
Shoot length						
ER_{50}	128.7	134.4	47.8	155.2	> 500.0	> 500.0
NOER	32.0	32.0	12.8	32.0	≥ 500.0	≥ 500.0
Plant dry weight						
ER_{50}	81.7	122.8	74.8	> 500.0	> 500.0	> 500.0
NOER	12.8	32.0	12.8	32.0	≥ 500.0	≥ 500.0
Plant Damage						
ER_{50}	83.1	69.7	62.2	154.3	> 500.0	> 500.0

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 and ER50 values for plant damages at the end of the exposure period expressed as g of clopyralid/ha for all test species are given below..

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	34.3	35.3	19.1	> 58.8	> 58.8	> 58.8
NOER	9.4	23.5	3.8	23.5	≥ 58.8	≥ 58.8
Shoot length						
ER_{50}	15.1	15.8	5.6	18.3	> 58.8	> 58.8
NOER	3.8	3.8	1.5	3.8	≥ 58.8	≥ 58.8
Plant dry weight						
ER_{50}	9.6	14.5	8.8	> 58.8	> 58.8	> 58.8
NOER	1.5	3.8	1.5	3.8	≥ 58.8	≥ 58.8
Plant Damage						
ER_{50}	9.8	8.2	7.3	18.2	> 58.8	> 58.8

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 and ER50 values for plant damages at the end of the exposure period expressed as g of florasulam/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	2.86	2.95	1.59	> 4.91	> 4.91	> 4.91
NOER	0.79	1.96	0.31	1.96	≥ 4.91	≥ 4.91
Shoot length						
ER_{50}	1.26	1.32	0.47	1.52	> 4.91	> 4.91
NOER	0.31	0.31	0.13	0.31	≥ 4.91	≥ 4.91
Plant dry weight						
ER_{50}	0.80	1.21	0.74	> 4.91	> 4.91	> 4.91
NOER	0.13	0.31	0.13	0.31	≥ 4.91	≥ 4.91
Plant Damage						
ER_{50}	0.82	0.68	0.61	1.52	> 4.91	> 4.91

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 and ER50 values for plant damages at the end of the exposure period expressed as g of fluroxypyr acid/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	34.6	35.6	19.2	> 59.3	> 59.3	> 59.3
NOER	9.5	23.7	3.8	23.7	≥ 59.3	≥ 59.3
Shoot length						
ER_{50}	15.3	16.0	5.7	18.4	> 59.3	> 59.3
NOER	3.8	3.8	1.5	3.8	≥ 59.3	≥ 59.3
Plant dry weight						
ER_{50}	9.7	14.6	8.9	> 59.3	> 59.3	> 59.3
NOER	1.5	3.8	1.5	3.8	≥ 59.3	≥ 59.3
Plant Damage						
ER_{50}	9.9	8.3	7.4	18.3	> 59.3	> 59.3

On the basis of the obtained results it was proved that the test item i.e. CHR/H/CFF 250 EC had impact on seedling emergence and seedling growth of pea, flax, carrot and onion.
Mortality of plants was not observed in case of pea, carrot, onion, perennial ryegrass and oats. One plant of flax was dead after application of the test item at the rate of 500.0 mL/ha.
On the basis of NOER, ER10, ER25 and ER50 values determined from the plant number it was proved that the test item inhibited process of seedling emergence of pea, flax, carrot and onion. Seedling emergence of perennial ryegrass and oats was not inhibited.
On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited process of the growth of pea, flax, carrot and onion. Process of growth of perennial ryegrass and oats was not inhibited.
On the basis of NOER, ER10, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited process of the growth of pea, flax, carrot and onion. Process of growth of perennial ryegrass and oats was not inhibited.
During the experiment phytotoxic symptoms in cultivation of pea, flax, carrot and onion were observed. Among phytotoxic symptoms stunted growth (pea, flax, carrot, onion), chlorosis (carrot) and defromations (pea, carrot) were observed. No phytotoxic symptoms in cultivation of perennial ryegrass and oats were 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 CHR/H/CFF 250 EC 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:
100.0% – pea,
95.0% – flax,

85.0% – carrot,
85.0% – onion,
90.0% – perennial ryegrass,
90.0% – oats,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- 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 Vegetative Vigour

Comments of zRMS:	The study is acceptable. The following validity criteria were met during the study: On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Turango 250 EC on vegetative vigour of terrestrial plants were met: - the seedling emergence of plants (validity criterion: at least 70%) was as follows: 85.7 – 95.2% – pea, 90.0 – 100.0% – flax, 90.0 – 97.5% – carrot, 92.5 – 97.5% – onion, 87.5 – 97.5% – perennial ryegrass, 90.0 – 100.0% – oats, - the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%), - the control plants did not exhibit any visible phytotoxic symptoms, - environmental conditions for all plants belonging to the same species were identical. Deviation from OECD Guideline No. 227: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 95.5 – 168.0 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the experiment. **Agreed toxicity endpoints:** **Expressed as mL of the test item/ha:**

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 500.00	182.27	> 500.00	> 500.00	> 500.00	> 500.00
NOER	≥ 500.00	80.00	≥ 500.00	≥ 500.00	≥ 500.00	≥ 500.00
Shoot length						
ER_{50}	75.79	49.71	55.18	> 500.00	> 500.00	> 500.00
NOER	12.80	12.80	5.10	32.00	200.00	≥ 500.00
Plant dry weight						
ER_{50}	49.76	33.90	19.30	149.21	> 500.00	> 500.00
NOER	2.00	5.10	5.10	32.00	80.00	200.00
Plant Damage						
ER_{50}	49.65	34.89	32.31	475.33	> 500.00	> 500.00

Expressed as g of clopyralid/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 58.84	21.45	> 58.84	> 58.84	> 58.84	> 58.84
NOER	≥ 58.84	9.41	≥ 58.84	≥ 58.84	≥ 58.84	≥ 58.84
Shoot length						
ER_{50}	8.92	5.85	6.49	> 58.84	> 58.84	> 58.84
NOER	1.51	1.51	0.60	3.77	23.53	≥ 58.84
Plant dry weight						
ER_{50}	5.86	3.99	2.27	17.56	> 58.84	> 58.84
NOER	0.24	0.60	0.60	3.77	9.41	23.53
Plant Damage						
ER_{50}	5.84	4.11	3.80	55.93	> 58.84	> 58.84

Expressed as as g of florasulam/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 4.91	1.79	> 4.91	> 4.91	> 4.91	> 4.91
NOER	≥ 4.91	0.79	≥ 4.91	≥ 4.91	≥ 4.91	≥ 4.91
Shoot length						
ER_{50}	0.74	0.49	0.54	> 4.91	> 4.91	> 4.91
NOER	0.13	0.13	0.05	0.31	1.96	≥ 4.91
Plant dry weight						
ER_{50}	0.49	0.33	0.19	1.47	> 4.91	> 4.91
NOER	0.02	0.05	0.05	0.31	0.79	1.96
Plant Damage						
ER_{50}	0.49	0.34	0.32	4.67	> 4.91	> 4.91

Expressed as g of fluroxypyr acid/ha:

	Pea *Pisum sativum*	Flax *Linum usitatissimum*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 59.33	21.63	> 59.33	> 59.33	> 59.33	> 59.33
NOER	≥ 59.33	9.49	≥ 59.33	≥ 59.33	≥ 59.33	≥ 59.33
Shoot length						
ER_{50}	8.99	5.90	6.55	> 59.33	> 59.33	> 59.33
NOER	1.52	1.52	0.61	3.80	23.73	≥ 59.33
Plant dry weight						
ER_{50}	5.90	4.02	2.29	17.70	> 59.33	> 59.33
NOER	0.24	0.61	0.61	3.80	9.49	23.73
Plant Damage						
ER_{50}	5.89	4.14	3.83	56.40	> 59.33	> 59.33

Reference: KCP 10.6.1/02

Report: CHR/H/CFF 250 EC Terrestial Plant Test: Vegetative Vigour Test, A. Gierbuszewska, 2023, Study code: G-05-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Depart-ment of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland

Guideline(s):	OECD Guideline 227, 2006
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods
Test item:
CHR/H/CFF 250 EC
batch number: CHE2AC2001
active substances: clopyralid – 117.67 g/L, florasulam – 9.82 g/L, fluroxypyr – 118.65 g/L

Test species:
pea (Pisum sativum), flax (Linum usitatissimum), carrot (Daucus carota), onion (Allium cepa), perennial ryegrass (Lolium perenne), oats (Avena sativa)
Soil: Sandy loam

Study design: number of rates: 8 + control; number of replicates/rate: 7 (pea), 4 (flax, carrot, onion, perennial ryegrass, oats). The total number of plants per application rate: 21 (pea) or 20 (flax, carrot, onion, perennial ryegrass, oats)

Application rates:
- 2.0 mL of the test item /ha (0.24 g of clopyralid + 0.02 g of florasulam + 0.24 g of fluroxypyr/ha),
- 5.1 mL of the test item /ha (0.60 g of clopyralid + 0.05 g of florasulam + 0.61 g of fluroxypyr/ha),
- 12.8 mL of the test item /ha (1.51 g of clopyralid + 0.13 g of florasulam + 1.52 g of fluroxypyr/ha),
- 32.0 mL of the test item /ha (3.77 g of clopyralid + 0.31 g of florasulam + 3.80 g of fluroxypyr/ha),
- 80.0 mL of the test item /ha (9.41 g of clopyralid + 0.79 g of florasulam + 9.49 g of fluroxypyr/ha),
- 200.0 mL of the test item /ha (23.53 g of clopyralid + 1.96 g of florasulam + 23.73 g of fluroxypyr/ha),
- 500.0 mL of the test item /ha (58.84 g of clopyralid + 4.91 g of florasulam + 59.33 g of fluroxypyr/ha),
In case of each species, there was one untreated control group.
volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha.
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Test conditions:
Because no change in mortality of plants was to be observed, no computations in plant number have been performed for pea, carrot, onion, perennial ryegrass and oats.
In order to determine ER10, ER25, ER50 the following test were used:
- plant number: probit analysis using linear max. likelihood regression,
- shoot length: probit analysis using linear max. likelihood regression, 3-param. Normal CDF
- shoot dry weight: 3-param. Normal CDF
ER50 (plant damages) – probit analysis using linear max. likelihood regression, logit analysis using linear max. likelihood regression, 3-param. Normal CDF.
In order to determine the NOER values, the following tests were used:
- for the plant number –Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure;
- for the shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Dunnett's Multiple t-test Procedure or Williams Multiple Sequential t-test Procedure or Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm or Step-down Jonckheere-Terpstra Test Procedure;
- for the plant shoot dry weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Step-down Jonckheere-Terpstra Test Procedure or Williams Multiple Sequential t-test Procedure or Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm.

Endpoints: ER25, ER50, NOER

Results and discussion

The study, aimed at evaluating the effect of CHR/H/CFF 250 EC on vegetative vigour of 6 terrestrial plants, was conducted on 3 dicotyledonous and 3 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea; 10 seeds/pot for flax, carrot, onion, perennial ryegrass and oats). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. As a result, the number of plants per pot as well as the total number of plants per rate were:
- pea: 3 plants/pot – 21 plants/ application rate (7 pots/ application rate);
- flax: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- perennial ryegrass: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate),
- oats: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate).

The pot is defined as a replicate. The test item was sprayed onto the plants. For each species, seven application rates were used. Untreated control group was conducted simultaneously. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The exposure period finished 21 days after the spraying. At the end of the exposure, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed.
The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER10, ER25, ER50 and NOER.
Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results after 21 days of the experiment.

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 and ER50 values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 500.00	182.27	> 500.00	> 500.00	> 500.00	> 500.00
NOER	≥ 500.00	80.00	≥ 500.00	≥ 500.00	≥ 500.00	≥ 500.00
Shoot length						
ER_{50}	75.79	49.71	55.18	> 500.00	> 500.00	> 500.00
NOER	12.80	12.80	5.10	32.00	200.00	≥ 500.00
Plant dry weight						
ER_{50}	49.76	33.90	19.30	149.21	> 500.00	> 500.00
NOER	2.00	5.10	5.10	32.00	80.00	200.00
Plant Damage						
ER_{50}	49.65	34.89	32.31	475.33	> 500.00	> 500.00

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 and ER50 values for plant damages at the end of the exposure period expressed as g of clopyralid/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 58.84	21.45	> 58.84	> 58.84	> 58.84	> 58.84
NOER	≥ 58.84	9.41	≥ 58.84	≥ 58.84	≥ 58.84	≥ 58.84
Shoot length						
ER_{50}	8.92	5.85	6.49	> 58.84	> 58.84	> 58.84
NOER	1.51	1.51	0.60	3.77	23.53	≥ 58.84
Plant dry weight						
ER_{50}	5.86	3.99	2.27	17.56	> 58.84	> 58.84
NOER	0.24	0.60	0.60	3.77	9.41	23.53
Plant Damage						
ER_{50}	5.84	4.11	3.80	55.93	> 58.84	> 58.84

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 and ER50 values for plant damages at the end of the exposure period expressed as g of florasulam/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 4.91	1.79	> 4.91	> 4.91	> 4.91	> 4.91
NOER	≥ 4.91	0.79	≥ 4.91	≥ 4.91	≥ 4.91	≥ 4.91
Shoot length						
ER_{50}	0.74	0.49	0.54	> 4.91	> 4.91	> 4.91
NOER	0.13	0.13	0.05	0.31	1.96	≥ 4.91
Plant dry weight						
ER_{50}	0.49	0.33	0.19	1.47	> 4.91	> 4.91
NOER	0.02	0.05	0.05	0.31	0.79	1.96
Plant Damage						
ER_{50}	0.49	0.34	0.32	4.67	> 4.91	> 4.91

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 and ER50 values for plant damages at the end of the exposure period expressed as g of fluroxypyr acid/ha for all test species are given below.

	Pea *Pisum sativum*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Allium cepa*
Plant number at the end of the experiment						
ER_{50}	> 59.33	21.63	> 59.33	> 59.33	> 59.33	> 59.33
NOER	≥ 59.33	9.49	≥ 59.33	≥ 59.33	≥ 59.33	≥ 59.33
Shoot length						
ER_{50}	8.99	5.90	6.55	> 59.33	> 59.33	> 59.33
NOER	1.52	1.52	0.61	3.80	23.73	≥ 59.33
Plant dry weight						
ER_{50}	5.90	4.02	2.29	17.70	> 59.33	> 59.33
NOER	0.24	0.61	0.61	3.80	9.49	23.73
Plant Damage						
ER_{50}	5.89	4.14	3.83	56.40	> 59.33	> 59.33

The test item, i.e. CHR/H/CFF 250 EC, applied at rates ranging from 2.0 to 500.0 mL/ha, had a varied impact on vegetative vigour of all tested plant species.
On the basis of NOER, ER10, ER25 and ER50 values determined from the plant number at the end of the experiment it was proved that the test item did not inhibit the process of growth of pea, carrot, onion, perennial ryegrass and oats. The test item inhibited the process of growth flax.
On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of growth of pea, flax, carrot and onion. The test item slightly inhibited process of growth of perennial ryegrass and it did not inhibited process of growth of oats.
On the basis of NOER, ER10, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of pea, flax, carrot, onion and perennial ryegrass. The test item slightly inhibited the process of growth oats.
During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of pea, flax, carrot, onion and perennial ryegrass.
In the study, the most sensitive plant to influence of the test item was flax.
The most resistant species was oats.

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 CHR/H/CFF 250 EC on vegetative vigour of terrestrial plants were met:
- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
85.7 – 95.2% – pea,
90.0 – 100.0% – flax,
90.0 – 97.5% – carrot,
92.5 – 97.5% – onion,
87.5 – 97.5% – perennial ryegrass,
90.0 – 100.0% – oats,

- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical..

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

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

A 2.8 KCP 10.8 Monitoring data