FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: CHR/H/PENDIF 599.5 SC

Product name(s): Cevino Trio 599.5 SC/ Trivino 599.5 SC

Chemical active substance(s):

Penoxsulam, 37.5 g/kg
Diflufenican, 250 g/kg
Flufenacet, 312 g/kg

Central Zone

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date:

MS Finalisation date: 24/08/2022

Version history

When	What
October 2021	Submission to the Polish Ministry of Agriculture and Rural Development
February 2022	Submission to the evaluation unit Merit Mark
March 2022	Updated version of dRR
April 2022	Updated version of dRR
April 2022	zRMS evaluation of dRR
August 2022	Final version prepared by zRMS after Commenting period

Table of Contents

9 Ecotoxicology (KCP 10)

Review Comments:

This application was submitted by Innvigo Sp. z o.o. for approval of the formulation Cevino Trio 599.5 SC/ Trivino 599.5 SC (Product code: CHR/H/PENDIF 599.5 SC) which containing: penoxsulam, 37.5 g/kg; diflufenican, 250 g/kg and flufenacet, 312 g/kg for use as a herbicide in cereals.

This dRR report Part B reviews only ecotoxicological data (Annex III) and additional information that has not previously been considered within the EU review process.

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations, and conclusions of the zRMS are presented in grey commenting boxes. 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.

In the following document, data for active substances - penoxsulam, diflufenican and flufenacet - was described during its inclusion on Annex 1 process in respectively 2010, 2009 and 2004 . 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

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

Appendix 1 ALL intended uses

GAP rev. , date: 2018-07-02day

PPP (product name/code):	CHR/H/PENDIF 599.5 SC*	Formulation type:	150 WG (a, b)
Active substance 1:	Jodosulfuron-methyl	Conc. of as 1:	50g (c)
Active substance 2:	Tribenuron - methyl	Conc. of as 2:	75g (c)
Active substance.3:	Penoxsulam	Conc. of as ….:	25g (c)
Safener:	n/a	Conc. of safener:	conc. (c)
Synergist:	n/a	Conc. of synergist:	conc. (c)
Applicant:	PUH Chemirol Sp. z o.o.	Professional use:	☒
Zone(s):	Central	Non professional use:	☐
Verified by MS:			
Field of use:	herbicide		

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Use-No. *	Member state(s)	Crop and/ or situation (crop destination / purpose of crop)	F, Fn, Fnp G, Gn, Gnp 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, other dose rate expression, dose range (min-max)	zRMS Conclusion (efficacy)
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L/kg product / ha a) max. rate per appl. b) max. total rate per crop/season	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	F	dicotyledonous weeds	Spray, medium sprayer	autumn BBCH 11-25	a)1 b)1	n/a	a) 0.4 l/ha b) 0.4 l/ha	a) 0.2398 kg a.s./ha (0.1248 FLU + 0.1 D + 0.015 P) b) 0.2398 kg a.s./ha (0.1248 FLU + 0.1 D + 0.015 P)	200-400	n/a	CHR/H/PENDIF 599.5 SC: J 9 + T 13,5 + F 4,5	

		~~(TTLWI),~~												
							~~a) —~~		~~a) —~~	~~b) —~~				
~~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)~~														
~~Minor uses according to Article 51 (field uses)~~														
~~Minor uses according to Article 51 (interzonal uses)~~														

GAP rev. 1, date: 2022-04

PPP (product name/code):	CHR/H/PENDIF 599.5 SC*	Formulation type:	599.5 SC (a, b)
Active substance 1:	Flufenacet	Conc. of as 1:	312 g/L (c)
Active substance 2:	Diflufenican	Conc. of as 2:	250 g/L (c)
Active substance.3:	Penoxsulam	Conc. of as ….:	37.5 g/L (c)
Safener:	n/a	Conc. of safener:	conc. (c)
Synergist:	n/a	Conc. of synergist:	conc. (c)
Applicant:	PUH Chemirol Sp. z o.o.	Professional use:	☒
Zone(s):	Central	Non professional use:	☐
Verified by MS:			
Field of use:	Herbicide		

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	**Member state(s)**	**Crop and/ or situa-tion** (crop destination / purpose of crop)	**F, Fn, Fnp G, Gn, Gnp or I ****	**Pests or Group of pests con-trolled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/ synergist per ha, other dose rate expression, dose range (min-max)						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter-val between applications (days)	L/kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter wheat (TRZAW), Winter triticale (TTLWI),	F	dicotyle-donous weeds	Spray, medium sprayer	autumn BBCH 11-25	a)1 b)1	n/a	a) 0.4 l/ha b) 0.4 l/ha	a) 0.2398 kg a.s./ha (0.1248 FLU + 0.1 D + 0.015 P) b) 0.2398 kg a.s./ha (0.1248 FLU + 0.1 D + 0.015 P)	200-400	n/a	CHR/H/PENDIF 599.5 SC: J-9 + T-13,5 + F-4,5							
							~~b)~~		~~c)~~	~~d)~~										
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)																				

Minor uses according to Article 51 (field uses)																			
Minor uses according to Article 51 (interzonal uses)																			

CHR/H/PENDIF 599.5 SC* brand names: Mepengo 150 WG, Herpende 150 WG, Galaxo 150 WG

(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

Review Comments:

Critical GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

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)

The risk assessment for birds and mammals was carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

CHR/H/PENDIF 599.5 SC pose no unacceptable risk to birds and mammals used according to the label.

There were also no negative effects regarding to drinking water exposure and effect of secondary poisoning (diflufenican, flufenacet). There is no influence to evaluated organism regarding to dangerous to food poisoning. Furthermore, for mixture toxicity acceptable risk could be demonstrated.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

CHR/H/PENDIF 599.5 SC pose no unacceptable risk to aquatic organisms according to the label with appropriate buffer zone:

- 20 ~~10~~ meters vegetative and no-spray buffer zone

Risk for mixture is acceptable following proposed uses of the product CHR/H/PENDIF 599.5 SC / Cevino Trio 599.5 SC/ Trivino 599.5 SC.
Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

9.1.1.3 Effects on bees (KCP 10.3.1)

The evaluation of the risk for bees has been performed in line with SANCO/10329/2002 rev 2 final.
CHR/H/PENDIF 599.5 SC pose no unacceptable risk to bees according to the label label

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. Only Test No. 245: Honey Bee (*Apis Mellifera* L.), Chronic Oral Toxicity Test (10-Day Feeding) was performed. Therefore, for Poland, the deficiencies need to be fill till EFSA bee guidance will come in to the force.

Nevertheless, such studies were deemed not necessary to finalize the risk assessment. Since the risk assessment was performed according to SANCO/10329/2002 rev 2.
Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

CHR/H/PENDIF 599.5 SC 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/PENDIF 599.5 SC 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/PENDIF 599.5 SC 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

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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).

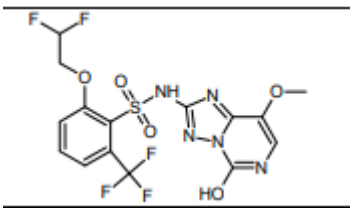
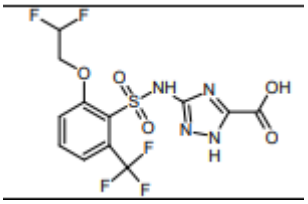
Table 9.1-2: Critical use pattern of CHR/H/PENDIF 599.5 SC grouped according to criterion

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**
1	Winter Cereals BBCH 11-25 489.72 ~~439.72~~ 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/PENDIF 599.5 SC is indicated in the table.

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

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
5-OH-penoxsulam			Soil/Water/Sediment: 41/-/19	Yes
BSTCA			Soil/Water/Sediment: 53/-/24	Yes

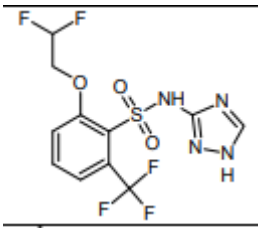
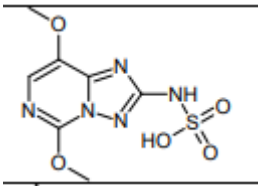
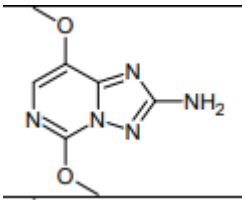
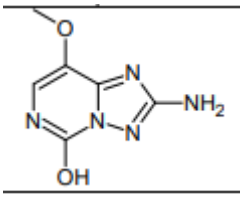
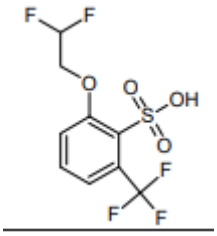
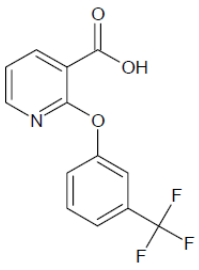
Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
BST			Soil/Water/Sediment: 8/-/-	Yes
TPSA			Soil/Water/Sediment: -/56/-	Yes
2-amino-TP			Soil/Water/Sediment: -/18/-	Yes
5-OH-2-amino-TP			Soil/Water/Sediment: -/23/-	Yes
BSA			Soil/Water/Sediment: -/36/-	Yes

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

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
AE B107137	283		Soil: 16.8% Water: 32.6% Sed: 13.3%	Yes

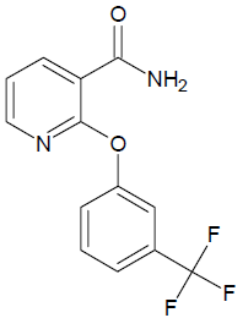
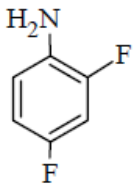
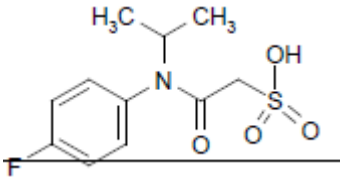
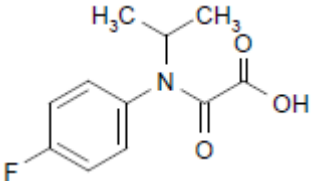
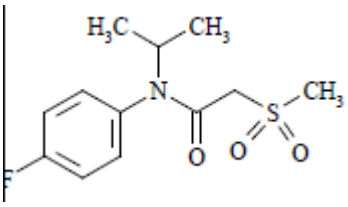
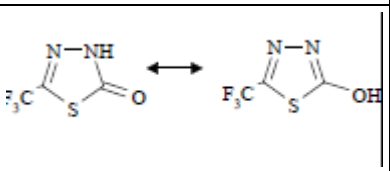
Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
AE 0542291	282		Soil: 26.3% Water: 6.1% Sed: 1.0%	Yes
AE C522392	129.11		Soil: 26.3% Water: 6.1% Sed: 1.0%	Yes

Table 9.1-5: Metabolites of flufenacet potentially relevant for exposure assessment

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
FOE sulfonic acid	275.3g/mol		Soil (lab): max 26.3% AR	Yes
FOE oxalate	225.2g/mol		Soil (Lab): max 15.6 % AR	Yes
FOE methyl-sulfone	273.3g/mol		Water/sediment max. 8 % in water, 3.4 % in sediment on day 157	Yes
FOE-thiadone	170.1g/mol		Maximum occurrence observed in sediment/ water studies: 82 % in water (55 d)	Yes

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with penoxsulam, diflufenican, flufenacet and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on birds of CHR/H/PENDIF 599.5 SC were not evaluated as part of the EU assessment of penoxsulam, diflufenican and Flufenacet.
However, the provision of further data on the CHR/H/PENDIF 599.5 SC is not considered essential, because studies from Annex I inclusion can be used in Annex I inclusion.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Mallard duck	Penoxsulam	Acute oral	LD50 > 2000 mg/ kg bw/d	EFSA Scientific Report (2009) 343, 84-90
Bobwhite quial	Penoxsulam	Reproduction	80.1 mg/kg bw/d	EFSA Scientific Report (2009) 343, 84-90
Bobwhite quail	Diflufenican	Oral 1 d Acute	LD50 >2150 mg a.s./kg bw	EFSA Scientific Repor EFSA Scientific Report (2007) 122, 1-84
Bobhwite quail	Diflufenican	Dietary Reproductive toxicity	NOAEL= 91.84 mg a.s./kg bw per day	EFSA Scientific Repor EFSA Scientific Report (2007) 122, 1-84
Bobwhite quail	Flufenacet	Oral Acute	LD50 = 1608 mg a.s./kg bw	7469/VI/98-Final 3 July 2003
Mallard duck	Flufenacet	Dietary Short-term	LC50 > > 4970 ppm	7469/VI/98-Final 3 July 2003
Japanese quail	Flufenacet	Dietary Reproductive toxicity	NOEC (mallard duck) = 88 ppm, equivalent to 9.87 mg/kg bw/day	7469/VI/98-Final 3 July 2003

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 screening and first-tier risk assessments are summarised in the following tables.

Table 9.2-2: **Screening ~~First tier~~ assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/PENDIF 599.5 SC in cereals winter for the penoxsulam**

Intended use	**Cereals**				
Active substance/product	Penoxsulam				
Application rate (g/ha)	1 x 15				
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	2.38	839.6
Reprod. toxicity (mg/kg bw/d)	80.1				
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	0.52	155.5

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 9.2-3: **Screening ~~First tier~~ assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/PENDIF 599.5 SC in cereals winter/spring for the diflufenican**

Intended use	**Cereals**				
Active substance/product	Diflufenican				
Application rate (g/ha)	1 x 100				
Acute toxicity (mg/kg bw)	2150 ~~2250~~				
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	15.88	135.4
Reprod. toxicity (mg/kg bw/d)	91.84				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omnivorous bird	64.8	0.53	3.43	26.7

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

Table 9.2-4: **Screening and First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/PENDIF 599.5 SC in cereals winter/spring for the flufenacet**

Intended use	**Cereals**				
Active substance/product	Flufenacet				
Application rate (g/ha)	1 x 124.8				
Acute toxicity (mg/kg bw)	1608				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small omnivorous bird	158.8	1.0	19.83	81.1
Reprod. toxicity (mg/kg bw/d)	9.87 ~~9.9~~				
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	4.29	2.3
First Tier					
Cereals BBCH 10 - 29	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	0.53	0.72	13.7
~~Cereals BBCH 10 - 29~~	~~Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods~~	~~10.9~~			~~13.7~~
Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose" Grass + cereals 100% cereal shoots	16.2	0.53	1.07	9.2

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

Selection of the endpoint used for acute risk assessment:
To justify the use of the EU agreed endpoints, the assessment of combined toxicity was performed.

Theoretical toxicity approach
The theoretical toxicity of the product was calculated using the formula given below and compared with the empirical value.
where:
X (a.s.i) = fraction of the active substance [i] in the mixture (the sum Σ X(a.s.i) must be 1).
LD_{50} (a.s.i) = acute toxicity for the active substance [i]

The values used for the calculation of acute combination toxicity effects are the following:

Compound in CHR/H/PENDIF 599.5 SC	**Content (%)**	**Proportion**	**LD_{50} (mg as/kg)**	**Fraction a.s./ LD_{50} a.s.**	**Theoretical LD_{50} of the formulation (mg tot as/kg)**	**Tox per fraction (a.s.)**	**Tox per fraction (mix)**	**Deviation tox per fraction (a.s.) to tox per fraction**

								(mix) [%]
Penoxsulam	3.75	0.06	2000	0.000031	1821.9	33333.3	1821.9	1730
Diflufenican	25	0.42	2150	0.0002		5119.0		181
Flufenacet	31.2	0.52	1608	0.00032		3092.3		69
Sum	59.95	1	-	0.00055		-		-

Performed evaluation indicates that based on the assumption of dose additivity. surrogate **LD_{50} = 1821.9 mg/kg bw/d.**
A combined acute risk assessment is not required if for one active substance the deviation between 'tox per fraction (a.s.)' and 'tox per fraction (mix)' is ≤ 10%. In that case the risk for mixture would not be covered by the assessment for that active substance.

Acute mixture toxicity need to be performed.

Product	CHR/H/PENDIF 599.5 SC				
Application rate (g/ha)	1 x (15 + 100 + 124.8= 239.8)				
Acute toxicity (mg/kg bw)	1821.9				
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	38.11	47.78

Performed acute mixture toxicity show low risk for birds for use of CHR/H/PENDIF 599.5 SC according to critical GAP.

Mixture toxicity – chronic

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance *i*

Crop	TER_{LT} 1)			1/TER (p)	1/TER (d)	1/TER (f)	$TER_{LTcombi}$	Trigger
	Penoxsulam (p)	Diflufenican (d)	Flufenacet (f)					
Cereals	155.5	26.7	2.3	0.0064	0.037	0.43	2.12	5
	155.5	26.7	9.2	0.0064	0.037	0.11	6.53	

Performed long-term mixture toxicity show low risk for birds for use of CHR/H/PENDIF 599.5 SC according to critical GAP.

~~**Combined risk assessment for CHR/H/PENDIF 599.5 SC mixture**~~
~~**A LD50 (mix) was calculated with the following formula:**~~

$$LD_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

$LD50_{mix\ birds\ acute}$	Worst case DDD	TERa	Trigger value
1850.9	19.83	93.2	10
$LD50_{mix\ birds\ reproduction\ acute}$	Worst case DDD	TERlt	Trigger value
17.3	4.29	4.03	5

The calculated LD_{50}mix and TER for mixture 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/PENDIF 599.5 SC does not posses unacceptable acute and long-term risk for mammals.

Risk refinement is needed:

Combined risk assessment for CHR/H/PENDIF 599.5 SC mixture

A TER_{mix} was calculated with the following formula:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance *i*

TER_A Penoxsulam	TER_A Diflufenican	TER_A Flufenacet	$TER_{mix\ birds\ acute}$	Trigger value
839.6	135.4	81.1	104.5	10
TER_{LT} Penoxsulam	TER_{LT} Diflufenican	TER_{Lt} Flufenacet	$TER_{mix\ birds\ acute}$	Trigger value
155.5	26.7	9.2	13.8	5

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/PENDIF 599.5 SC does not posses unacceptable acute and long-term risk for mammals.
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/PENDIF 599.5 SC 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 16, Penoxsulam 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)	=	15		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.0075
Reprod. toxicity (mg/kg bw/d)	=	80.1	quotient =	0.1873

With a K(f)oc of 3417, diflufenican 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)	=	100		
Acute toxicity (mg/kg bw)	=	2150	quotient =	0.0465
Reprod. toxicity (mg/kg bw/d)	=	91.84	quotient =	1.089

With a K(f)oc of 349, Flufenacet 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)	=	124.8		
Acute toxicity (mg/kg bw)	=	1608	quotient =	0.078
Reprod. toxicity (mg/kg bw/d)	=	9.87	quotient =	12.64

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Penoxsulam amounts to -0.605 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 Diflufenican amounts to 4.2 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on winter cereals.

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure to Diflufenican via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals

Parameter	Diflufenican	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1318	Escape ver 2.
log P_{ow} / P_{ow}	4.2	EFSA Scientific Report (2007) 122, 1-84
Koc	3417	EFSA Scientific Report (2007) 122, 1-84
foc	Organic carbon content of soil (0.02 taken as a default value)	Default

Parameter	Diflufenican	comments
BCF_{worm}	2.8	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.3609	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.3875	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	91.8	EFSA Scientific Report (2007) 122, 1-84
TER_{lt}	236.9	Above trigger 5

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Diflufenican in water.

Table 9.2-6: Assessment of the risk for fish-eating birds due to exposure to diflufenican via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	Flufenacet	comments
PEC_{sw} (initial) (mg/L)	0.00479	Focus STEP 2
BCF_{fish}	1596	EFSA Scientific Report (2007) 122, 1-84
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	7.65	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.22	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	91.8	EFSA Scientific Report (2007) 122, 1-84
TER_{lt}	75	Trigger 5

The log P_{ow} of Flufenacet amounts to 3.2 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on winter cereals.

Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to Flufenacetvia bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals

Parameter	Flufenacet	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1459	Escape ver 2.
log P_{ow} / P_{ow}	3.2/1585	7469/VI/98-Final 3 July 2003
Koc	349	7469/VI/98-Final 3 July 2003
foc	Organic carbon content of soil (0.02 taken as a default	Default

Parameter	Flufenacet	comments
	value)	
BCF_{worm}	2.85	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.4158	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.4366	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	9.87	7469/VI/98-Final 3 July 2003
TER_{lt}	22.6	Above trigger 5

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Flufenacet in water.

Table 9.2-8: Assessment of the risk for fish-eating birds due to exposure to flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	Flufenacet	comments
PEC_{sw} (initial) (mg/L)	0.01284	Focus STEP 2
BCF_{fish}	71.4	7469/VI/98-Final 3 July 2003
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.9168	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.146	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	9.87	7469/VI/98-Final 3 July 2003
TER_{lt}	67.7	Trigger 5

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 penoxsulam, diflufenican, flufenacet was found acceptable. CHR/H/PENDIF 599.5 SC 750 WG pose no unacceptable risk to birds with according to the label

Review comments:

The acute and long-term risk assessment for birds performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed end-

points. No formulation study was required.

TER_A and TER_{LT} in the acute and long-term risk assessment indicated acceptable risk assessment for all active substances at screening (penoxsulam, diflufenican) and first-tier step (flufenacet).

Provided acute and long-term risk assessment for the mixture indicated acceptable risk.
CHR/H/PENDIF 599.5 SC presents no unacceptable risk to birds resulting from exposure via drinking water. Presented secondary poisoning for diflufenican, flufenacet presents no unacceptable risk to birds.

Overall, acceptable acute and reproductive risk to birds may be concluded for application of CHR/H/PENDIF 599.5 SC in compliance with proposed GAP.

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 penoxsulam, diflufenican, flufenacet 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/PENDIF 599.5 SC 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	Penoxsulam	Acute	LD50>5000 mg a.s./kg bw	EFSA Scientific Report (2009) 343, 84-90
Rat	Penoxsulam	Long term	NOEL>25 mg a.s./kg bw/d	EFSA Scientific Report (2009) 343, 84-90
Rat	Diflufenican	Acute	LD50 rat oral >5000 mg a.s./kg bw	EFSA Scientific Repor EFSA Scientific Report (2007) 122, 1-84
Rat	Diflufenican	Long-term	NOAEL = 35.5 mg a.s./kg bw per day	EFSA Scientific Repor EFSA Scientific Report (2007) 122, 1-84
Rat	Flufenacet	Acute	LD_{50} = 589 mg/kg bw	7469/VI/98-Final 3 July 2003
Rat	Flufenacet	Long-term*	NOAEL = 500 ppm (37.4 mg/kg bw/d) – 2 generation rat	7469/VI/98-Final 3 July 2003 *According to the Toxicology section of the EU review report (2003) as there is no mammalian reproductive endpoint listed in the Ecotoxicoloy section

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 screening ~~first-tier~~ risk assessments are summarised in the following tables.

Table 9.3-2: Screening ~~First-tier~~ assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/PENDIF 599.5 SC in winter cereals for penoxsulam

Intended use	cereals				
Active substance/product	Penoxsulam				
Application rate (g/ha)	1 × 15				
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	1.78	2815.3
Reprod. toxicity (mg/kg bw/d)	25				
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	0.38	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 9.3-3: Screening ~~First-tier~~ assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/PENDIF 599.5 SC in winter cereals for diflufenican

Intended use	cereals				
Active substance/product	Diflufenican				
Application rate (g/ha)	1 × 100				
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	11.84	422.3
Reprod. toxicity (mg/kg bw/d)	35.5				
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	2.56	13.87

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

Table 9.3-4: **Screening ~~First tier~~ assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/PENDIF 599.5 SC in winter cereals for flufenacet**

Intended use	cereals				
Active substance/product	Flufenacet				
Application rate (g/ha)	1 × 124.8				
Acute toxicity (mg/kg bw)	589				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	118.4	1.0	14.78	39.9
Reprod. toxicity (mg/kg bw/d)	37.4				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small herbivorous mammal	48.3	0.53	3.19	11.71

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.

Selection of the endpoint used for acute risk assessment:
To justify the use of the EU agreed endpoints, the assessment of combined toxicity was performed.

Theoretical toxicity approach
The theoretical toxicity of the product was calculated using the formula given b8elow and compared with the empirical value.

where:
X (a.s.i) = fraction of the active substance [i] in the mixture (the sum Σ X(a.s.i) must be 1).
LD_{50} (a.s.i) = acute toxicity for the active substance [i]

Compound in CHR/H/PENDIF 599.5 SC	**Content (%)**	**Proportion**	**LD_{50} (mg as/kg)**	**Fraction a.s./ LD_{50} a.s.**	**Theoretical LD_{50} of the formulation (mg tot as/kg)**	**Tox per fraction (a.s.)**	**Tox per fraction (mix)**	**Deviation tox per fraction (a.s.) to tox per fraction (mix) [%]**
Penoxsulam	3.75	0.06	5000	0.000012	1021.6	83333	1021.6	80.61
Diflufenican	25	0.42	5000	0.000084		11904		10.65
Flufenacet	31.2	0.52	589	0.000883		1132		**0.11**
Sum	59.95	1	-	0.000979		-		-

Performed evaluation indicates that based on the assumption of dose additivity. surrogate LD_{50} = 1021.6 mg/kg bw/d.
A combined acute risk assessment is not required if for one active substance the deviation between 'tox per fraction (a.s.)' and 'tox per fraction (mix)' is ≤ 10%. As in that case the risk for mixture would be

covered by the assessment for flufenacet. No combined acute toxity is required.

Combined reproductive toxicity

The combined TER value is calculated according to the following formula:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance *i*

Mixture toxicity - long term

Crop	TER_{LT} 1)			1/TER (p)	1/TER (d)	1/TER (f)	$TER_{LTcombi}$	Trigger
	Penoxsulam (p)	Diflufenican **(d)**	Flufenacet (f)					
Cereals	65.11	13.87	11.71	0.01	0.07	0.08	6.25	5

Performed long-term mixture toxicity show low risk for mammals for use of CHR/H/PENDIF 599.5 SC according to critical GAP.

~~**Combined risk assessment for CHR/H/PENDIF 599.5 SC mixture**~~

~~**A LD50 (mix) was calculated with the following formula:**~~

$$LD_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1} \frac{\quad _i)}{.s._i)}^{-1}$$

~~-~~

~~LD50mix birds acute~~	~~Worst case DDD~~	~~TERa~~	~~Trigger value~~
~~1020.9~~	~~14.78~~	~~69.07~~	~~10~~
~~LD50mix birds acute~~	~~Worst case DDD~~	~~TERlt~~	~~Trigger value~~
~~35.5~~	~~3.19~~	~~11.1~~	~~5~~

~~The calculated LD50mix and TER for mixture 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/PENDIF 599.5 SC does not posses unacceptable acute and long term risk for mammals.~~

~~No further risk refinement is needed~~

~~**Combined risk assessment for CHR/H/PENDIF 599.5 SC mixture**~~

~~A TER_{mix} was calculated with the following formula:~~

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance *i*

~~TER_A Penoxsulam~~	~~TER_A~~	~~TER_A Flufe~~	~~TER_{mix} mammals~~	~~Trigger value~~

	~~**Diflufenican**~~	~~**nacet**~~	~~acute~~	
~~2815.3~~	~~422.3~~	~~**39.9**~~	~~71.16~~	~~10~~
~~**TER_{LT} Penoxsulam**~~	~~**TER_{LT} Diflufenican**~~	~~**TER_{Lt} Flufe-nacet**~~	~~**TER_{mix} mammals acute**~~	~~**Trigger value**~~
~~65.11~~	~~13.87~~	~~11.71~~	~~13.25~~	~~5~~

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/PENDIF 599.5 SC does not posses unacceptable acute and long-term risk for mammals. 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 16, Penoxsulam 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)	=	15			
Acute toxicity (mg/kg bw)	=	5000	quotient	=	0.003
Reprod. toxicity (mg/kg bw/d)	=	25	quotient	=	0.6

With a K(f)oc of 3417, diflufenican 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)	=	100			
Acute toxicity (mg/kg bw)	=	5000	quotient	=	0.02
Reprod. toxicity (mg/kg bw/d)	=	35.5	quotient	=	2.67

With a K(f)oc of 349, Flufenacet 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)	=	124.8			
Acute toxicity (mg/kg bw)	=	589	quotient	=	0.21
Reprod. toxicity (mg/kg bw/d)	=	37.4	quotient	=	3.34

9.3.2.4 Effects of secondary poisoning

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

The log Pow of Diflufenican amounts to 4.2 and thus exceeds the trigger value of 3. A risk as-sessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.
To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to Flufenacet via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter wheat.

Parameter	Diflufenican	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1318	Escape ver 2 calculations
log P_{ow} / P_{ow}	4.2	7469/VI/98-Final 3 July 2003
Koc	3417	7469/VI/98-Final 3 July 2003
foc	0.02	Default
BCF_{worm}	2.80	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.369	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.47	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	35.5	7469/VI/98-Final 3 July 2003
TER_{lt}	75.53	Trigger value 5

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Flufenacet in water.

Table 9.3-6: Assessment of the risk for fish-eating mammals due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in winter cereals

Parameter	Diflufenican	comments
PEC_{sw} (initial) (mg/L)	0.00479	Focus STEP 2
BCF_{fish}	1596	7469/VI/98-Final 3 July 2003

PEC_{fish}	7.65	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.08	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	35.5	7469/VI/98-Final 3 July 2003
TER_{lt}	32.87	Trigger value 5

The log Pow of Flufenacet amounts to 3.2 and thus exceeds the trigger value of 3. A risk as-sessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.
To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to Flufenacet via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter wheat.

Parameter	Flufenacet	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1459	Escape ver 2 calculations
log P_{ow} / P_{ow}	3.2/1585	7469/VI/98-Final 3 July 2003
Koc	349	7469/VI/98-Final 3 July 2003
foc	0.02	Default
BCF_{worm}	2.85	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.4158	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.5322	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.87	7469/VI/98-Final 3 July 2003
TER_{lt}	18.55	Trigger value 5

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on pre-dicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Flufenacet in water.

Table 9.3-8: Assessment of the risk for fish-eating mammals due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in win-ter cereals

Parameter	Flufenacet	comments
PEC_{sw} (initial) (mg/L)	0.01284	Focus STEP 2
BCF_{fish}	71.4	7469/VI/98-Final 3 July 2003
PEC_{fish}	0.9168	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.13	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	37.4	7469/VI/98-Final 3 July 2003
TER_{lt}	287,69	Trigger value 5

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 penoxsulam, diflufenican, flufenacet was found acceptable. CHR/H/PENDIF 599.5 SC 750 WG pose no unacceptable risk to mammals with according to the label

Review comments:

The acute and long-term risk assessment for mammals performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.

TER_A and TER_{LT} in the acute and long-term risk assessment indicated acceptable risk assessment for all active substances already at screening step.

Provided risk assessment for the mixture indicated acceptable risk.
CHR/H/PENDIF 599.5 SC presents no unacceptable risk to mammals resulting from exposure via drinking water. Presented secondary poisoning for diflufenican, flufenacet presents no unacceptable risk to mammals.
Overall, acceptable acute and reproductive risk to mammals may be concluded for application of CHR/H/PENDIF 599.5 SC in compliance with proposed GAP.

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

~~N/A~~

Review comments:

This issue is not assessed at the product level.

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 penoxsulam, diflufenican, flufenacet 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/PENDIF 599.5 SC were not evaluated as part of the EU assessment of penoxsulam, diflufenican, flufenacet. 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 – Penoxsulam and relevant metabolites

Species	Substance	Exposure System	Results mg/L	Reference
Lepomis macrochirus	Penoxsulam	Acute static	96-h LC50 >100 mg/L	EFSA Scientific Report (2009) 343, 76-90
Oncorhynchis mykiss	Penoxsulam	Chronic flow through (juveniles)	36-d NOEC= 10 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	Penoxsulam	Acute static	48-h EC50 >100 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	Penoxsulam	Chronic semi-static	21-d NOEC= 3.33 mg/L	EFSA Scientific Report (2009) 343, 76-90
Chironomus riparius	Penoxsulam	Chronic semi-static	28 day NOEC= 61 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	Penoxsulam	Static	96-h ErC50= 0.0864 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna gibba	Penoxsulam	Chronic	14 EC50=0.00329 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	5-OH-penoxsulam	Acute static	48-h EC50 > 100 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	5-OH-penoxsulam	Static	72-h ErC50= 10 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna Gibba	5-OH-penoxsulam	Chronic	14d EC50>10 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	BSTCA	Acute static	48-h EC50 >100 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	BSTCA	Static	72-h EyC50=10 mg/:	EFSA Scientific Report (2009) 343, 76-90
Lemna gibba	BSTCA	Chronic	14-d EC50>10 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	BSA	Acute static	48-h EC50 = 1.6 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	BSA	Static	72 h ErC50>1.6 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna Gibba	BSA	Chronic	14 d EC50>1.6 mg//L	EFSA Scientific

Species	Substance	Exposure System	Results mg/L	Reference
				Report (2009) 343, 76-90
Daphnia magna	TPSA	Acute	48 EC50 > 1.4 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	TPSA	Static	72-h ErC50>1.4 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna Gibba	TPSA	Chronic	14d EC50>1.4 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	2-amino-TP	Acute	48-h EC50 > 1 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	2-amino-TP	Static	72-h ErC50>1 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna Gibba	2-amino-TP	Chronic	14 d EC50>1 mg/L	EFSA Scientific Report (2009) 343, 76-90
Daphnia magna	5-OH-2-amino-TP	Acute	48h-h EC50>1 mg/L	EFSA Scientific Report (2009) 343, 76-90
Pseudokirchneriella subcapitata	5-OH-2-amino-TP	Static	72-h ErC50 > 1 mg/L	EFSA Scientific Report (2009) 343, 76-90
Lemna gibba	5-OH-2-amino-TP	Chronic	14d EC50>1.25 mg/L	EFSA Scientific Report (2009) 343, 76-90
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 – Diflufenican and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Ciprinus carpio	Diflufenican	96 h, s	LC50 > * mg/L m	EFSA Scientific Report (2007) 122, 1-84
O. mykiss	AE B107137	96 h, s	LC50 > 17.3* mg/L, m	EFSA Scientific Report (2007) 122, 1-84
Pimephales promelas [(a)]	Diflufenican	35 d	NOEC = 0.015 mg/L	EFSA Scientific Report (2007) 122, 1-

Species	Substance	Exposure System	Results	Reference
				84
D. magna	Diflufenican	48 h, s	EC50 > 0.24* mg/L, m	EFSA Scientific Report (2007) 122, 1-84
D. magna	AE B107137	48 h, s	EC50 > 20.4* mg/L, m	EFSA Scientific Report (2007) 122, 1-84
D. magna	AE 0542291	48 h, s	EC50 > 10 mg/L nom	EFSA Scientific Report (2007) 122, 1-84
D. magna	Diflufenican	21 d	NOEC = 0.052 mg/L	EFSA Scientific Report (2007) 122, 1-84
Chironomus riparius (spiked water)	Diflufenican	28 d,s	NOEC = 0.10 mg/L, nom	EFSA Scientific Report (2007) 122, 1-84
C. riparius (spiked sediment)	Diflufenican	28 d, s	NOEC = 2.0 mg/kg sed nom	EFSA Scientific Report (2007) 122, 1-84
C. riparius (spiked sediment)	AE C522392	28 d, s	NOEC = 1.0 mg/kg sed nom	EFSA Scientific Report (2007) 122, 1-84
S. subspicatus (Without sediment)	Diflufenican	72 h, s	EbC50 = 0.00025 mg/L E_rC_{50} = 0.00045 mg/L NOEC = 0.0009 mg/L**(a)** m	EFSA Scientific Report (2007) 122, 1-84
S. subspicatus (With sediment)	Diflufenican	72 h, s	EbC50 = 0.0024 mg/L E_rC_{50} = 0.0047 mg/L NOEC = 0.00076 mg/L nom	EFSA Scientific Report (2007) 122, 1-84
S. subspicatus (Without sediment)	Diflufenican	72 h, s	EbC50 = 0.00046 mg/L E_rC_{50} = 0.00122 mg/L Maximum concentration from which recovery possible = 0.0042 mg/L NOEC = 0.00015 mg/L m	EFSA Scientific Report (2007) 122, 1-84
S. subspicatus (Without sediment)	AE B107137	72 h, s	EbC50 > 20.4* mg/L E_rC_{50} > 20.4* mg/L m	EFSA Scientific Report (2007) 122, 1-84
S. subspicatus	AE 0542291	72 h, s	EbC50 = 36.0 mg/L	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
(Without sediment)			E_rC_{50} = 66.0 mg/L	Report (2007) 122, 1-84
Pseudokirchneriella sub-capitata	AE 592370	72 h, s	E_bC_{50} > 39.0 mg/L**(a)** E_rC_{50} > 58.0 mg/L**(a)**	EFSA Scientific Report (2007) 122, 1-84
P. subcapitata	AE C522392	72 h, s	EbC50 = 3.4 mg/L E_rC_{50} = 16.0 mg/L m	EFSA Scientific Report (2007) 122, 1-84
L. gibba	Diflufenican	14 d, ss	EbC50 = 0.056 mg/L EC_{50} frond density = 0.039 mg/L m	EFSA Scientific Report (2007) 122, 1-84
Higher-tier studies (micro- or mesocosm studies)				
Higher tier data are available, but insufficient information is currently available to derive an endpoint.				

Table 9.5-3: **Endpoints and effect values relevant for the risk assessment for aquatic organisms – Flufenacet**

Species	Substance	Exposure System	Results	Reference
Lepomis macrochirus	Flufenacet	96 h	LC_{50} = 2.13 mg a.s./L	SANCO 7469/VI/98-Final 3 July 2003
Oncorhynchus mykiss	flufenacet-sulfonic acid	96 h	LC_{50} = 86.7 mg/ L	SANCO 7469/VI/98-Final 3 July 2003
Oncorhynchus mykiss	FOE thiadone	96 h	LC_{50} = 9.1 mg/L mm	SANCO 7469/VI/98-Final 3 July 2003
Oncorhynchus mykiss	Flufenacet	97 days	LC_{50} > 0.2 mg a.s./L mm	SANCO 7469/VI/98-Final 3 July 2003
Daphnia magna	Flufenacet	48 h	EC_{50} = 30.9 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Daphnia magna	flufenacet-sulfonic acid	48 h	EC_{50} = 87.3 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Daphnia magna	FOE thiadone	48 h	EC_{50}= 31.7 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Daphnia magna	Flufenacet	21 d	NOEC=3.26 mg a.s./L	SANCO 7469/VI/98-Final 3 July 2003
Selenastrum capricornutum	Flufenacet	72 h	EbC50 = 0.00204 mg/l	SANCO 7469/VI/98-Final 3 July 2003
Scenedesmus subspicatus	flufenacet-sulfonic acid	120 h	ErC50 > 86.7 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Selenastrum capricornutum	FOE thiadone	72 h	EbC50 = 4.1 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Selenastrum capricornutum	flufenacet-methylsulfide	72 h (static)	ErC50 = 83.8 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Lemna gibba	Flufenacet	14 d	EC50 = 0.00243 mg /l	SANCO 7469/VI/98-Final 3 July 2003

Species	Substance	Exposure System	Results	Reference
Lemna gibba	flufenacet-sulfonic acid	14 d	EC50 > 86.7 mg/L	SANCO 7469/VI/98-Final 3 July 2003
Higher-tier studies (micro- or mesocosm studies) SANCO 7469/VI/98-Final 3 July 2003				

NOEC = 0.012 mg a.s./l (WG 60, macrophyte, duckweed and periphyton)

The fate and biological effects of Flufenacet WG 60 in aquatic microcosms.

Reference: Foekema E.M. and Jak R.G., 1999, TNO-MEP – R 99/423
Test guideline: OECD (1996), SETAC (1991)
GLP compliance: yes

An indoor microcosm test was performed to investigate the effect of a concentration series of FOE 5043 WG 60 (flufenacet: 61.5 %) on an aquatic biocoenosis.

Each microcosm consisted of a polyethylene container with a diameter of approximately 100 cm and a height of 80 cm. Fourteen of these containers were used in this study. In order to simulate a natural mixing regime, the water column was gently aerated throughout the study period. The microcosms contained a 10 cm deep layer of sediment, covered by a 50 cm deep water column.

Four weeks before application of the test substance, the microcosms were filled with natural sediment and water. Some days later submerged macrophytes were introduced. Duckweed and periphyton substrate were introduced two weeks before application.

The test substance was applied just under the water surface as a stock solution in water. The concentration series was: 0.75, 1.5, 3, 6, 12 and 24 microg as/l. All tests concentrations were duplicated, with the exception of the highest one, which was not replicated. Untreated reference systems were triplicated. The test period was 84 d.
Analysis of flufenacet in the water column of the microcosms 4 h after application confirmed nominal concentrations. The concentrations declined thereafter with a DT50 for the active ingredient of 18.8 d.

Overall, in the current microcosm experiment with the herbicide flufenacet significant treatment related effects could not be observed at any treatment level, although some slight differences in community metabolism (O2 and pH) were noted in the highest treatment level (24 microg as/l) as was a slightly reduced growth of some macrophytes and periphyton. All other measured parameters were unaffected. All the observations at the highest treatment level were slight and transient only, with a recovery before the end of the study. The fact that treatment related effects were only observed at the highest concentration as well as the observed recovery of even the most sensitive endpoints (community metabolism) is in accordance with the short half-life of flufenacet in the water column.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/H/PENDIF 599.5 SC

Species	Substance	Exposure System	Results	Reference
Daphnia magna	CHR/H/PENDIF 599.5 SC	48 h, s	EC_{50} > 100 mg formulation /L $_{nom}$	E. Malada, Study code: W-44-20
Pseudokirchneriella subcapitata	CHR/H/PENDIF 599.5 SC	72 h, s	E_rC_{50} = 3.267 µg formulation/L $_{nom}$ E_yC_{50} = 0.636 µg formulation /L $_{nom}$	M. Czarnecka, Study code: W-45-20
Anabaena flos-aquae	CHR/H/PENDIF 599.5 SC	72 h, static	ErC50= 11.07 mg test item/L EyC50 = 2.01 mg test item/L	M. Czarnecka, Study code: W-47-20

Species	Substance	Exposure System	Results	Reference
Lemna Gibba	CHR/H/PENDIF 599.5 SC	7d, ss	EyC_{50} (7-day) Yield (frond number)= 0.019 [mg test item/L] ErC_{50} (7-day) Growth rate (frond number)= 0.056[mg test item/L] EyC_{50} (7-day) Yield (dry weight)= 0.024 [mg test item/L] ErC_{50} (7-day) Growth rate (dry weight)= 0.162 [mg test item/L]	M. Czarnecka, Study code: W-46-20
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 Penoxsulam for each organism group based on FOCUS Steps 1, 2, 3 and 4 calculations for the use of CHR/H/PENDIF 599.5 SC in winter 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)		100 000	10 000	100 000	3330	86.4	61 000	3.29
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	100	1000	333	8.64	6100	0.329
Exposure	**PEC $_{gl-max}$(µg/L)**							
Step 1								
pH > 6.5	5.03	0.00503	0.05030	0.00503	0.01511	0.58218	0.00082	15.2888
pH <6.5	4.63	0.00463	0.04630	0.00463	0.01390	0.53588	0.00076	14.0729
Step 2								
pH > 6.5	2.35	0.00235	0.02350	0.00235	0.00706	0.27199	0.00039	7.1429
pH <6.5	2.18	0.00218	0.02180	0.00218	0.00655	0.25231	0.00036	6.6261
Step 3 pH > 6.5								
D3/ditch	0.1664	0.00017	0.00166	0.00017	0.00050	0.01926	0.00003	0.5058
D4/pond	0.2745	0.00027	0.00275	0.00027	0.00082	0.03177	0.00005	0.8343
D4/stream	0.2705	0.0002705	0.00271	0.000271	0.000812	0.03131	0.000044	0.8222
D5/pond	0.2289	0.0002289	0.00229	0.000229	0.000687	0.02649	0.000038	0.6957
D5/stream	0.1669	0.0001669	0.00167	0.000167	0.000501	0.01932	0.000027	0.5073

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Sed. dwell. pro-longed	Aquatic plants
R1/pond	0.009001	0.0000090	0.00009	0.000009	0.000027	0.00104	0.000001	0.0274
R1/stream	0.5833	0.0005833	0.00583	0.000583	0.001752	0.06751	0.000096	**1.7729**
R3/stream	1.095	0.0010950	0.01095	0.001095	0.003288	0.12674	0.000180	**3.3283**
R4/stream	0.2161	0.0002161	0.00216	0.000216	0.000649	0.02501	0.000035	0.6568
Step 3 pH< 6.5								
D3/ditch	0.09474	0.00009	0.00095	0.00009	0.00028	0.01097	0.00002	0.2880
D4/pond	0.1022	0.00010	0.00102	0.00010	0.00031	0.01183	0.00002	0.3106
D4/stream	0.1419	0.0001419	0.00142	0.000142	0.000426	0.01642	0.000023	0.4313
D5/pond	0.1020	0.0001020	0.00102	0.000102	0.000306	0.01181	0.000017	0.3100
D5/stream	0.1573	0.0001573	0.00157	0.000157	0.000472	0.01821	0.000026	0.4781
R1/pond	0.008377	0.0000084	0.00008	0.000008	0.000025	0.00097	0.000001	0.0255
R1/stream	0.5333	0.0005333	0.00533	0.000533	0.001602	0.06172	0.000087	1.6210
R3/stream	0.7044	0.0007044	0.00704	0.000704	0.002115	0.08153	0.000115	2.1410
R4/stream	0.4042	0.0004042	0.00404	0.000404	0.001214	0.04678	0.000066	1.2286
Step 4 pH>6.5	**10 meters vegetative buffer zone and 10 meters no-spray buffer zone**							
D3/ditch	0.08541	0.00009	0.00085	0.00009	0.00026	0.00989	0.00001	0.2596
D4/pond	0.2742	0.00027	0.00274	0.00027	0.00082	0.03174	0.00004	0.8334
D4/stream	0.2705	0.0002705	0.00271	0.000271	0.000812	0.03131	0.000044	0.8222
D5/pond	0.2287	0.0002287	0.00229	0.000229	0.000687	0.02647	0.000037	0.6951
D5/stream	0.1669	0.0001669	0.00167	0.000167	0.000501	0.01932	0.000027	0.5073
R1/pond	0.004166	0.0000042	0.00004	0.000004	0.000013	0.00048	0.000001	0.0127

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Sed. dwell. pro-longed	Aquatic plants
R1/stream	0.2616	0.0002616	0.00262	0.000262	0.000786	0.03028	0.000043	0.7951
R3/stream	0.4942	0.0004942	0.00494	0.000494	0.001484	0.05720	0.000081	**1.5021**
R4/stream	0.09756	0.0000976	0.00098	0.000098	0.000293	0.01129	0.000016	0.2965
Step 4 pH > 6.5	10 meters vegetative buffer zone and 10 metersno-spray buffer zone							
D3/ditch	0.01377	0.00001	0.00014	0.00001	0.00004	0.00159	0.00000	0.0419
D4/pond	0.1019	0.00010	0.00102	0.00010	0.00031	0.01179	0.00002	0.3097
D4/stream	0.1419	0.0001419	0.00142	0.000142	0.000426	0.01642	0.000023	0.4313
D5/pond	0.1018	0.0001018	0.00102	0.000102	0.000306	0.01178	0.000017	0.3094
D5/stream	0.1573	0.0001573	0.00157	0.000157	0.000472	0.01821	0.000026	0.4781
R1/pond	0.003899	0.0000039	0.00004	0.000004	0.000012	0.00045	0.000001	0.0119
R1/stream	0.2391	0.0002391	0.00239	0.000239	0.000718	0.02767	0.000039	0.7267
R3/stream	0.3179	0.0003179	0.00318	0.000318	0.000955	0.03679	0.000052	0.9663
R4/stream	0.1825	0.0001825	0.00183	0.000183	0.000548	0.02112	0.000030	0.5547
Step 4 pH > 6.5	20 meters vegetative buffer zone and 20 meter no-spray buffer zone							
R3/stream	0.00026	0.00258	0.00026	0.00078	0.02990	0.00004	0.7851	0.00026

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-Penoxsulam of Penoxsulam for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

5-OH Penoxsulam				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***

5-OH Penoxsulam				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		100 000	10 000	10 000
AF		100	10	10
RAC (µg/L)		1000	1000	1000
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
PEC/RAC	3.42	0.00342	0.00342	0.00342

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite BSTCA of Penoxsulam for each organism group based on FOCUS Steps 1-2 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

BSTCA				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		100 000	10 000	10 000
AF		100	10	10
RAC (µg/L)		1000	1000	1000
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
PEC/RAC	2.72	0.00272	0.00272	0.00272

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite BSA of Penoxsulam for each organism group based on FOCUS Steps 1,2 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

BSA				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		1600	1600	1600
AF		100	10	10
RAC (µg/L)		16	160	160
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
pH >6.5 ~~PEC/RAC~~	1.15	0.07188	0.00719	0.00719
pH < 6.5	1.06	0.06625	0.00663	0.00663

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite TPSA of Penoxsulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

TPSA				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		1400	1400	1400
AF		100	10	10
RAC (µg/L)		14	140	140
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
pH >6.5 ~~PEC/RAC~~	1.60	0.01600	0.16000	0.16000

TPSA				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
pH < 6.5	1.47	0.10500	0.01050	0.01050

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 2-amino-TP of Penoxsulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

2-amino-TP				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		1000	1000	1000
AF		100	10	10
RAC (µg/L)		10	100	100
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
pH >6.5 ~~PEC/RAC~~	0.37	0.03700	0.00370	0.00370
pH < 6.5	0.34	0.03400	0.00340	0.00340

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 5-OH-2-amino-TP of Penoxsulam for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter.

5-OH-2-amino-TP				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Pseudokirchneriella***	***Lemna Gibba***
Endpoint		EC_{50}	EbC50	EC50
(µg/L)		1000	1000	1250
AF		100	10	10

5-OH-2-amino-TP				
Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
RAC (µg/L)		10	100	125
Exposure	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
pH >6.5 ~~PEC/RAC~~	0.44	0.04400	0.00440	0.00352
pH < 6.5	0.40	0.04000	0.00400	0.00320

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Test species		*Cipri-nus carpio*	*Pimephales promelas*	***Daphnia magna***	***Daphnia magna***	***Selenastrum***	***Chironomus riparius***	***Lemna Gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EbC50	NOEC	EC50
(µg/L)		98.5	15	240	5.2	4.2	2000	39
AF		100	10	100	10	10	10	10
RAC (µg/L)		0.985	1.5	2.4	5.2	0.42	200	3.9
Exposure	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	10.05	10.20305	6.70000	4.18750	1.93269	23.92857	0.05025	2.5769
Step 2								
	4.79	4.86294	3.19333	1.99583	0.92115	11.40476	0.02395	1.2282
Step 3								
D3/ditch	0.6299	0.63949	0.41993	0.26246	0.12113	1.49976	0.00315	0.1615

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Sed. dwell. pro-longed	Aquatic plants
D4/pond	0.04667	0.04738	0.03111	0.01945	0.00898	0.11112	0.00023	0.0120
D4/stream	0.5464	0.5547208	0.36427	0.227667	0.105077	1.30095	0.002732	0.1401
D5/pond	0.02189	0.0222234	0.01459	0.009121	0.004210	0.05212	0.000109	0.0056
D5/stream	0.5896	0.5985787	0.39307	0.245667	0.113385	1.40381	0.002948	0.1512
R1/pond	0.06265	0.0636041	0.04177	0.026104	0.012048	0.14917	0.000313	0.0161
R1/stream	0.4154	0.4217259	0.27693	0.173083	0.079885	0.98905	0.002077	0.1065
R3/stream	0.5829	0.5917766	0.38860	0.242875	0.112096	1.38786	0.002915	0.1495
R4/stream	0.5912	0.6002030	0.39413	0.246333	0.113692	1.40762	0.002956	0.1516
Step 4			10 meters vegetative buffer zone and 10 meters no-spray buffer zone					
D3/ditch	0.09049	0.09187	0.06033	0.03770	0.01740	0.21545	0.00045	0.0232
D4/pond	0.04452	0.04520	0.02968	0.01855	0.00856	0.10600	0.00022	0.0114
D4/stream	0.1656	0.1681218	0.11040	0.069000	0.031846	0.39429	0.000828	0.0425
D5/pond	0.01363	0.0138376	0.00909	0.005679	0.002621	0.03245	0.000068	0.0035
D5/stream	0.1140	0.1157360	0.07600	0.047500	0.021923	0.27143	0.000570	0.0292
R1/pond	0.02718	0.0275939	0.01812	0.011325	0.005227	0.06471	0.000136	0.0070
R1/stream	0.1749	0.1775635	0.11660	0.072875	0.033635	0.41643	0.000875	0.0448
R3/stream	0.1949	0.1978680	0.12993	0.081208	0.037481	0.46405	0.000975	0.0500
R4/stream	0.2667	0.2707614	0.17780	0.111125	0.051288	0.63500	0.001334	0.0684

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 9.5-13: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite IN-AE B10737 of Diflufenican for each organism based on FOCUS Steps 1, 2 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter**

Group		**Fish acute**	**Invertebrate acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus sub-spicatus***
Endpoint		LC_{50}	EC50	EC_{50}
(µg/L)		17300	20400	20400
AF		100	100	10
RAC (µg/L)		173	204	2040
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	12.59	0.07277	0.06172	0.00617

Table 9.5-14: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE 0542291 of Diflufenican for each organism based on FOCUS Steps 1, 2 calculations for the use of CHR/H/PENDIF 599.5 SC in cereals winter**

Group		**Invertebrate acute**	**Algae**
Test species		***Daphnia magna***	***Scenedesmus sub-spicatus***
Endpoint		EC50	EC_{50}
(µg/L)		10000	66000
AF		100	10
RAC (µg/L)		100	6600
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	5.34	0.05340	0.00081

-

Table 9.5-15: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Flufenacet for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CHR/H/PENDIF 599.5 SC 500 SC in winter cereals**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Selenastrum capricornutum***	***Lemna Gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EbC50	EC50
(µg/L)		2130	200	30900	3260	2.04	2.43
AF		100	10	100	10	10	10
RAC (µg/L)		21.3	20	309	326	0.204	0.243
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	29.54	1.38685	1.47700	0.09560	0.09061	144.80392	121.56379
Step 2							
	12.84	0.60282	0.64200	0.04155	0.03939	62.94118	52.83951
Step 3							
D3/ditch	0.7881	0.03700	0.03941	0.00255	0.00242	3.86324	3.24321
D4/pond	0.02724	0.00128	0.00136	0.00009	0.00008	0.13353	0.11210
D4/stream	0.6839	0.0321080	0.03420	0.002213	0.002098	3.35245	2.814403
D5/pond	0.3578	0.0167981	0.01789	0.001158	0.001098	1.75392	1.472428
D5/stream	0.7378	0.0346385	0.03689	0.002388	0.002263	3.61667	3.036214
R1/pond	0.08545	0.0040117	0.00427	0.000277	0.000262	0.41887	0.351646
R1/stream	2.046	0.0960563	0.10230	0.006621	0.006276	10.02941	8.419753
R3/stream	2.602	0.1221596	0.13010	0.008421	0.007982	12.75490	10.707819
R4/stream	2.429	0.1140376	0.12145	0.007861	0.007451	11.90686	9.995885

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

For the intended uses not, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for Selenastrum capricornutum as characterised by an EC_{50} for species of 2.04 □g/L and form Lemna 2.43□g/L in connection with an assessment factor of 10) in FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on risk mitigation in FOCUS Step 4 PECSW considering reduced exposure of surface water bodies.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Flufenacet based on FOCUS Step 4 calculations and toxicity data for most sensitive species. with mitigation of spray drift and run-off for the use of CHR/H/PENDIF 599.5 SC 500 SC in winter cereals

Intended use		Winter cereals
Active substance		Flufencet
Application rate (g/ha)		1 × 124.8
Nozzle reduction	**No-spray buffer (m)**	10
	Vegetated filter strip (m)	10
None	D3/ditch	0.1134
None	D4/pond	0.01696
None	D4/stream	0.1325
None	D5/pond	0.3550
None	D5/stream	0.4940
None	R1/pond	0.03775
None	R1/stream	0.9162
None	R3/stream	1.173
None	R4/stream	1.096
RAC (µg/L) Selenastrum capricornutum		
0.204		**PEC/RAC ratio**
None	D3/ditch	0.55588
None	D4/pond	0.08314
None	D4/stream	0.64951

Intended use **Active substance** **Application rate (g/ha)**		Winter cereals Flufencet 1 × 124.8
Nozzle reduction	**No-spray buffer (m)**	10
	Vegetated filter strip (m)	10
None	D5/pond	**1.74020**
None	D5/stream	**2.42157**
None	R1/pond	0.18505
None	R1/stream	**4.49118**
None	R3/stream	**5.75000**
None	R4/stream	**5.37255**
RAC (µg/L) Lemna Gibba 0.243		**PEC/RAC ratio**
None	D3/ditch	0.46667
None	D4/pond	0.06979
None	D4/stream	0.545267
None	D5/pond	**1.460905**
None	D5/stream	**2.032922**
None	R1/pond	0.155350
None	R1/stream	**3.770370**
None	R3/stream	**4.827160**
None	R4/stream	**4.510288**

For the intended uses not, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for Selenastrum capricornutum as characterised by an EC_{50} for species of 2.04 □g/L and form Lemna 2.43□g/L in connection with an assessment factor of 10) in FOCUS Step4 with 10 m buffer zone scenarios. Therefore, higher tier study is necessary. Such study was performed on Annex I inclusion and was used in risk refinement and presented below.

Risk Refinement:

An indoor microcosm test was performed to investigate the effect of a concentration series of FOE 5043 WG 60 (flufenacet: 61.5 %) on an aquatic biocoenosis.

Each microcosm consisted of a polyethylene container with a diameter of approximately 100 cm and a height of 80 cm. Fourteen of these containers were used in this study. In order to simulate a natural mixing regime, the water column was gently aerated throughout the study period. The microcosms contained a 10 cm deep layer of sediment, covered by a 50 cm deep water column.

Four weeks before application of the test substance, the microcosms were filled with natural sediment and water. Some days later submerged macrophytes were introduced. Duck-weed and periphyton substrate were introduced two weeks before application.

The test substance was applied just under the water surface as a stock solution in water. The concentration series was: 0.75, 1.5, 3, 6, 12 and 24 microg as/l. All tests concentrations were duplicated, with the exception of the highest one, which was not replicated. Untreated reference systems were triplicated. The test period was 84 d.

Analysis of flufenacet in the water column of the microcosms 4 h after application confirmed nominal concentrations. The concentrations declined thereafter with a DT50 for the active ingredient of 18.8 d.

Overall, in the current microcosm experiment with the herbicide flufenacet significant treatment related effects could not be observed at any treatment level, although some slight differences in community metabolism (O2 and pH) were noted in the highest treatment level (24 microg as/l) as was a slightly reduced growth of some macrophytes and periphyton. All other measured parameters were unaffected. All the observations at the highest treatment level were slight and transient only, with a recovery before the end of the study. The fact that treatment related effects were only observed at the highest concentration as well as the observed recovery of even the most sensitive endpoints (community metabolism) is in accordance with the short half-life of flufenacet in the water column.

Agreed NOEC = 0.012 mg a.s./l

Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Flufenacet based on FOCUS Step 4 calculations and toxicity data for microcosm study. with mitigation of spray drift and run-off for the use of CHR/H/PENDIF 599.5 SC 500 SC in winter cereals

Intended use **Active substance** **Application rate (g/ha)** **RAC 1.2 µg/L (AF=10 and NOEC microcosm=12 µg/L)**		Winter cereals Flufencet 1 × 124.8	PEC/RAC ratio (trigger <1)
Nozzle reduction	**No-spray buffer (m)**	10	

	Vegetated filter strip (m)	10	
None	D3/ditch	0.1134	0.0945
None	D4/pond	0.01696	0.0141
None	D4/stream	0.1325	0.1104
None	D5/pond	0.3550	0.2958
None	D5/stream	0.4940	0.4117
None	R1/pond	0.03775	0.0315
None	R1/stream	0.9162	0.7635
None	R3/stream	1.173	0.9775
None	R4/stream	1.096	0.9133

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite FOE sulfonic acid for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in winter cereals

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenastrum capricornutum***	***Lemna Gibba***
Endpoint		LC_{50}	EC_{50}	EbC50	EC50
(µg/L)		86700	87300	86700	86700
AF		100	100	10	10
RAC (µg/L)		867	873	8670	8670
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
PEC/RAC	8.12	0.00937	0.00930	0.00094	0.00094

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite FOE thiadone of Flufenacet for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in winter cereals

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenestrum capricornutum***
Endpoint		LC_{50}	EC_{50}	EbC50
(µg/L)		9100	31700	4100
AF		100	100	10
RAC (µg/L)		91	317	410
Exposure	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
PEC/RAC	16.41	0.18033	0.05177	0.04002

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite FOE methylsulfide of flufenacet for each organism based on FOCUS Steps 1 calculations for the use of CHR/H/PENDIF 599.5 SC in winter cereals

Group		**Algae**
Test species		***S. capricornutum***
Endpoint		EC50
(µg/L)	**PEC $_{gl\text{-}max}$ (µg/L)**	83800
AF		10
RAC (µg/L)		838
Exposure		
Step 1		
PEC/RAC	3.67	0.00438

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

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of CHR/H/PENDIF 599.5 SC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/PENDIF 599.5 SC in winter cereals

Intended use **Formulation** **Application rate (g[prod]/ha)**	Winter cereals CHR/H/PENDIF 599.5 SC 1 x 180
Entry into surface water via spraydrift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L]**
1	1.1564
16	0.2906
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 =EC50 100 000 µg/L** **RAC=1000(AF=100)**
1	0.0012000
Buffer zone (m)	**PEC/RAC ratio** **Pseudokirchmeirella subcapitata =ErC50 3.267µg/L** **RAC=0.3267 (AF=10)**
1	3.54
16	0.89
Buffer zone (m)	**PEC/RAC ratio** **Anabaena flos-aquae =ErC_{50} 11070 µg/L** **RAC=1107 (AF=10)**
1	0.001
Buffer zone (m)	**PEC/RAC ratio** **Lemna Gibba =ErC_{50} (based on frond number) 56 µg/L** **RAC=5.6 (AF=10)**
1	0.2071

Based on the calculated concentrations of the formulation CHR/H/PENDIF 599.5 SC (spray drift) respectively its active ingredients Penoxsulam, Diflufenican and Flufenacet (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/PENDIF 599.5 SC according to the GAP of the formulation achieve the acceptability criterium <1 for run-off exposure~~, therefore no risk mitigations are required.~~

Review comment:

According to National requirements in PL the mitigation measures for the formulation would be: unsprayed buffer zone of 20 m not 16 as is stated in the table above.

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.
-

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/PENDIF 599.5 SC**~~

~~**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. (ECxa.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. As these are the most sensitive aquatic organisms, it is justified to conduct the mixture toxicity risk assessment only for these two organism groups. → Go to 2~~

~~**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/PENDIF 599.5 SC**~~

~~Name/code of the product~~	~~CHR/H/PENDIF 599.5 SC~~	~~-~~
~~Name of the active substance A~~	~~Flufenacet~~	~~-~~

Name of the active substance B	Diflufenican	-	
Name of the active substance C	Penoxsulam	-	
Density [g product/cm^3]	1.2243	-	-
-	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 Flufenacet in the product	312	25.5%	52.0%
Concentrations of the active substance Diflufenican in the product	250	20.4%	41.7%
Concentrations of the active substance Penoxsulam in the product	37.5	3.1%	6.3%

Table 2. Toxicity of CHR/H/PENDIF 599.5 SC and active substance

-

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\,PPP}$) [mg a.s./L]	Toxicity of the a.s. Flufenacet (ECx A) [mg a.s./L]	Toxicity of the a.s. Diflufenican (ECx B) [mg a.s./L]	Toxicity of the a.s. Penoxulam ($EC_{x\,C}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
EC_{50} daphnids	100	48.967	30.9	0.24	100	0.01
E_rC_{50} algae	0.003267	0.002	0.00204	0.0042	0.0864	0.1
E_rC_{50} higher plant	0.086	0.042	0.00243	0.039	0.00329	0.1

Table 3. Calculation of toxicity exposure in CHR/H/PENDIF 599.5 SC

Toxicity per fraction of the Tribenuron-methyl ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the Metsulfuron-methyl ($1/TU_B$) [mg a.s./L]	Toxicity per fraction of the Florasulam ($1/TU_C$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum(TU_i)$) [mg a.s./L]	Model deviation ratio (MDR = $EC_{x\ mix\text{-}CA}/EC_{x\ PPP}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
59.37355769	0.57552	1598.666667	0.570	0.012	0.582
0.003919808	0.0100716	1.381248	0.003	1.760	1.052
0.004669183	0.093522	0.052596133	0.004	0.097	1.256

Answer: MDRs for dpahnias and lemnas are below <0.2 Therefore , go to Step 9 MDRs for algaes are betewn 0.2-5. Therefore, go to Step 3

Step 9. Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?

Yes (measured mixture toxicity not plausible):	Go to step 8
No (measured mixture toxicity plausible):	Go to step 3

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: $\Sigma\, p_i$ must be 1)
ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

Answer: NO. → 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 daphnias	**0.196**		**Yes**
ErC50 algae	**0.131**		**Yes**
ErC50 lemna	**1.184**	**Yes**	

Answer: Calculated factors for algae and daphnias gives results outside 0.8 1.2 Therefore, go to step 5.

Calculated factor for higher plants is between 0.8 1.2. Therefore, got to step 4.

STEP 4. Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.

Exposure	-	**(lower exposure tier)**	**(higher exposure tier)**	-	-	-	-					
Exposure tier (FOCUS step) - Penoxsulam	-	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (10 m, R3 stream)	Step 4 (10 m, R4 stream)	
PECsw [mg a.s./L]	-	0.002350	0.000085	0.000274	0.000271	0.000229	0.000167	0.000004	0.000262	0.000494	0.000098	
Exposure tier (FOCUS step) - Diflufeni-	-	Step 2	Step 4 (20 m bz vfs, D3	Step 4 (20 m bz vfs, D4	Step 4 (20 m bz vfs, D4 stream	Step 4 (20 m bz vfs, D5	Step 4 (20 m bz vfs, D5 stream	Step 4 (20 m bz vfs, R1	Step 4 (20 m bz vfs, R1 stream	Step 4 (20 m bz vfs, R3 stream	Step 4 (20 m bz vfs, R4 stream	

can			ditch)	pond)	)	pond)	)	pond)	)	)	)	
PECsw [mg a.s./L]	-	0.004790	0.000047	0.000043	0.000166	0.000012	0.000064	0.000009	0.000052	0.000059	0.000042	
Exposure tier (FOCUS step) Flufenacet	-	Step 2	Step 4 (20 m bz vfs, D3 ditch)	Step 4 (20 m bz vfs, D4 pond)	Step 4 (20 m bz vfs, D4 stream)	Step 4 (20 m bz vfs, D5 pond)	Step 4 (20 m bz vfs, D5 stream)	Step 4 (20 m bz vfs, R1 pond)	Step 4 (20 m bz vfs, R1 stream)	Step 4 (20 m bz vfs, R3 stream)	Step 4 (20 m bz vfs, R4 stream)	
PECsw [mg a.s./L]	-	0.012840	0.000059	0.000014	0.000069	0.000354	0.000494	0.000011	0.000052	0.000074	0.000052	
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	-	0.019980	0.000191	0.000331	0.000505	0.000594	0.000724	0.000025	0.000366	0.000626	0.000191	
-	-			-								
End-point/Test species	Toxicity of the product (a.s. based) ($EC_{x\ ppp}$) [mg a.s./L	$ETR_{mix} = PEC_{mix}/EC_{x\ ppp}$										Triggers

	]											
ErC50 higher plant	0.027	0.729	0.007	0.012	0.018	0.022	0.026	0.001	0.013	0.023	0.007	0.10

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

		Penoxsulam		Diflufenican		Flufenacet		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.
EC50 daphnids	0.570	1598.667	0.0%	0.576	99.00%	59.374	1.0%	Yes	
ErC50 algae	0.003	1.381	0.2%	0.010	28.0%	0.004	71.8%		Yes

Equation 14:

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

Answer: No toxicity drivers were found for algae. Therefore, got to Step 8. Toxicity drivers for daphnias is Diflufenican.

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	Penoxsulam	Step 2	Step 4 (10 m,	Step 4 (10 m,	Step 4 (10 m,	Step 4 (10	Step 4 (10	Step 4 (10	Step 4 (10	Step 4 (10	Step 4 (10	

step)			D3 ditch)	D4 pond)	D4 stream)	m, D5 pond)	m, D5 stream)	m, R1 pond)	m, R1 stream)	m, R3 stream)	m, R4 stream)	
PECsw [mg a.s./L]		0.002350	0.000085	0.000274	0.000271	0.0000229	0.0000167	0.0000004	0.0000262	0.0000494	0.0000098	
Exposure tier (FOCUS step)	Diflufenican	Step 2	Step 4 (20 m bz vfs, D3 ditch)	Step 4 (20 m bz vfs, D4 pond)	Step 4 (20 m bz vfs, D4 stream)	Step 4 (20 m bz vfs, D5 pond)	Step 4 (20 m bz vfs, D5 stream)	Step 4 (20 m bz vfs, R1 pond)	Step 4 (20 m bz vfs, R1 stream)	Step 4 (20 m bz vfs, R3 stream)	Step 4 (20 m bz vfs, R4 stream)	
PECsw [mg a.s./L]		0.004790	0.000047	0.000043	0.000166	0.0000012	0.0000064	0.0000009	0.0000052	0.0000059	0.0000042	
Exposure tier (FOCUS step)	Flufenacet	Step 2	Step 4 (20 m bz vfs, D3 ditch)	Step 4 (20 m bz vfs, D4 pond)	Step 4 (20 m bz vfs, D4 stream)	Step 4 (20 m bz vfs, D5 pond)	Step 4 (20 m bz vfs, D5 stream)	Step 4 (20 m bz vfs, R1 pond)	Step 4 (20 m bz vfs, R1 stream)	Step 4 (20 m bz vfs, R3 stream)	Step 4 (20 m bz vfs, R4 stream)	
PECsw [mg a.s./L]		0.012840	0.000059	0.000014	0.000069	0.0000354	0.0000494	0.0000011	0.0000052	0.0000074	0.0000052	
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	-	0.019980	0.000191	0.000331	0.000505	0.0000594	0.0000724	0.0000025	0.0000366	0.0000626	0.0000191	
-	-			-								
End-point/Test species	-	Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix,CA} = \sum (p_{i,PEC}/EC_{x,i})$) [mg a.s./L] -										
ErC50 algae	-	0.003	0.005	0.016	0.007	0.003	0.003	0.003	0.009	0.011	0.005	
End-point/Test species	-	$ETR_{mix} = PEC_{mix}/EC_{x,PPP}$										Trig-gers
ErC50 algae	-	7.462	0.041	0.020	0.076	0.179	0.259	0.008	0.041	0.056	0.036	0.10

Answer: ETRmix for higher exposure tier are below the triggers. Therefore, CHR/H/PENDIF 599.5 SC no poses unacceptable mixture toxicity to aquatic species in Poland relevant scenario

For risk refinement mixture toxicity for all scenario in Central Zone used endpoint of microcosm study for Flufenacet with assessment factor 10. Therefore, mentioned endpoint for higher study on Flufenacet replaced the endpoint for algae and lemna. New calculation are presented below:

Decision scheme for mixture toxcitiy risk assessment for CHR/H/PENDIF 599.5 SC

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. (ECxa.s.): Go to 7
For both formulation (ECxPPP) and a.s. (ECxa.s.): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for daphnia, algae and macrophytes. As these are the most sensitive aquatic organisms, it is justified to conduct the mixture toxicity risk assessment only for these two organism groups. → Go to 2

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/PENDIF 599.5 SC

Name/code of the product	CHR/H/PENDIF 599.5 SC	-
Name of the active substance A	Flufenacet	-
Name of the active substance B	Diflufenican	-
Name of the active substance C	Penoxsulam	-

Density [g product/cm^3]	1.2243	-	-
-	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 Flufenacet in the product	312	25.5%	52.0%
Concentrations of the active substance Diflufenican in the product	250	20.4%	41.7%
Concentrations of the active substance Penoxsulam in the product	37.5	3.1%	6.3%

Table 2. Toxicity of CHR/H/PENDIF 599.5 SC and active substance

-

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\,PPP}$) [mg a.s./L]	Toxicity of the a.s. Flufenacet (ECx A) [mg a.s./L]	Toxicity of the a.s. Diflufenican (ECx B) [mg a.s./L]	Toxicity of the a.s. Penoxulam ($EC_{x\,C}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
EC_{50} daphnids	100	48.967	30.9	0.24	100	0.01
E_rC_{50} algae	0.003267	0.002	0.00204	0.0042	0.0864	0.1
E_rC_{50} higher plant	0.086	0.042	0.00243	0.039	0.00329	0.1

Table 3. Calculation of toxicity exposure in CHR/H/PENDIF 599.5 SC

Toxicity per fraction of the Penoxsulam ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the Diflufenican ($1/TU_B$) [mg a.s./L]	Toxicity per fraction of the Flufenacet ($1/TU_C$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\,mix\text{-}CA} = 1/\sum(TU_i)$) [mg a.s./L]	Model deviation ratio ($MDR = EC_{x\,mix\text{-}CA}/EC_{x\,ppp}$)	$EC_{x\,mix\text{-}CA}$ (a.s. in product)/$EC_{x\,mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
1598.666667	0.57552	59.37355769	0.570	0.012	0.582
1.381248	0.0100716	2.305769231	0.010	6.223	0.587
0.052596133	0.134288	2.305769231	0.037	1.356	1.508

Answer: MDRs for daphnias are below <0.2 Therefore , go to Step 9
MDRs for algaes are higher than 5. Therefore, go to Step 10
MDRs for higher plant are between 0.2-5. Therefore, go to Step 3.

Step 9. Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?

Yes (measured mixture toxicity not plausible):	Go to step 8
No (measured mixture toxicity plausible):	Go to step 3

Answer: NO. → Go to step 3

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?

Yes:	Go to 3
If measured data are not available or if the assessment in point 3 indicates that the mixtures are not similar (use modified ETR trigger values, see section 10.3.4):	Go to 8
No:	Go to 3

Answer: NO. → 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).

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

- n: number of mixture components
- i: index from 1…n mixture components
- p_i: the i[th] component as a relative fraction of the mixture composition (note: $\Sigma\, p_i$ must be 1)
- ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

~~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 daphnias~~	~~0.196~~		~~Yes~~
~~ErC50 algae~~	~~0.131~~		~~Yes~~
~~ErC50 lemna~~	~~1.184~~		~~YES~~

~~Answer: Calculated factors for daphnias, algae and higher plants gives results outside 0.8-1.2 Therefore, go to step 5.~~

~~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~~

		~~Penoxsulam~~		~~Diflufenican~~		~~Flufenacet~~		~~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.~~
~~EC50 daphnids~~	~~0.570~~	~~1598.667~~	~~0.0%~~	~~0.576~~	~~99.00%~~	~~59.374~~	~~1.0%~~	~~Yes~~	
~~ErC50 algae~~	~~0.010~~	~~1.381~~	~~0.7%~~	~~0.010~~	~~98.8%~~	~~2.306~~	~~0.4%~~	~~Yes~~	
~~ErC50 higher plant~~	~~0.037~~	~~0.053~~	~~70.7%~~	~~0.134~~	~~27.7%~~	~~2.306~~	~~1.6%~~		~~Yes~~

Equation 14:

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

Answer: No toxicity drivers were found for higher plan. Therefore, got to Step 8. Toxicity drivers for daphnias and algae is Diflufenican.

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)	Penoxsulam	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (20 m, R3 stream)	Step 4 (10 m, R4 stream)	
PECsw [mg a.s./L]		0.002350	0.000085	0.000274	0.000271	0.0000229	0.0000167	0.0000004	0.0000262	0.0000258	0.0000098	
Exposure tier (FOCUS step)	Diflufenican	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (10 m, R3 stream)	Step 4 (10 m, R4 stream)	
PECsw [mg a.s./L]		0.004790	0.000090	0.000045	0.000166	0.0000014	0.0000114	0.0000027	0.0000175	0.0000195	0.0000267	
Exposure tier (FOCUS step)	Flufenacet	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (20 m, R3 stream)	Step 4 (10 m, R4 stream)	
PECsw [mg a.s./L]		0.012840	0.000113	0.000017	0.000133	0.0000355	0.0000494	0.0000038	0.0000916	0.0000477	0.0001096	
Total exposure concentration of the mix-	-	0.019980	0.000289	0.000336	0.000569	0.0000597	0.0000775	0.0000069	0.0001353	0.0000931	0.0001460	

ture (a.s. based) (PECmix) [mg/L]												
-	-			-								
End-point/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] -										
ErC50 algae	-	0.025	0.010	0.004	0.007	0.009	0.015	0.039	0.016	0.011	0.041	
End-point/Test species	-	$ETR_{mix} = PEC_{mix}/EC_{x\,PPP}$										Trig-gers
ErC50 algae	-	0.811	0.028	0.084	0.085	0.070	0.053	0.002	0.083	0.082	0.035	0.10

Answer: ETRmix for higher exposure tier are below the triggers. Therefore, CHR/H/PENDIF 599.5 SC no poses unacceptable mixture toxicity to aquatic species.

Decision scheme for mixture toxcitiy risk assessment for CHR/H/PENDIF 599.5 SC

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. (ECxa.s.): Go to 7
For both formulation (EC_{xPPP}) and a.s. ($EC_{xa.s.}$): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for daphnia, algae and macrophytes. As these are the most sensitive aquatic organisms, it is justified to conduct the mixture toxicity risk assessment only for these two organism groups. → Go to 2

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/PENDIF 599.5 SC

Name/code of the product	CHR/H/PENDIF 599.5 SC	-	
Name of the active substance A	Flufenacet	-	
Name of the active substance B	Diflufenican	-	
Name of the active substance C	Penoxsulam	-	
Density [g product/cm^3]	1.2243	-	-
-	Nominal [g a.s./kg or L product]	Fraction considering density [%]	$\rho_{i\,mix}$ = Fraction of active substance i in the mixture with $\sum \rho_{i\,mix}$ = 100 [%]
Concentrations of the active substance Flufenacet in the product	312	25.5%	52.0%
Concentrations of the active substance Diflufenican in the product	250	20.4%	41.7%
Concentrations of the active substance Penoxsulam in the product	37.5	3.1%	6.3%

Table 2. Toxicity of CHR/H/PENDIF 599.5 SC and active substance

-

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\,PPP}$) [mg a.s./L]	Toxicity of the a.s. Flufenacet (ECx A) [mg a.s./L]	Toxicity of the a.s. Diflufenican (ECx B) [mg a.s./L]	Toxicity of the a.s. Penoxulam ($EC_{x\,C}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
EC_{50} daphnids	100	48.967	30.9	0.24	100	0.01
E_rC_{50} algae	0.003267	0.002	0.00204	0.0042	0.0864	0.1
E_rC_{50} higher plant	0.086	0.042	0.00243	0.039	0.00329	0.1

Table 3. Calculation of toxicity exposure in CHR/H/PENDIF 599.5 SC

Toxicity per fraction of the Flufenacet ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the Diflufenican ($1/TU_B$) [mg a.s./L]	Toxicity per fraction of the Penoxsulam ($1/TU_C$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum(TU_i)$) [mg a.s./L]	Model deviation ratio (MDR = $EC_{x\ mix\text{-}CA}/EC_{x\ ppp}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
59.37355769	0.57552	1598.666667	0.570	0.012	0.582
0.003919808	0.0100716	1.381248	0.003	1.760	1.052
0.004669183	0.093522	0.052596133	0.004	0.097	1.256

Answer: MDRs for dpahnias and lemnas are below <0.2 Therefore , go to Step 9 MDRs for algaes are betewn 0.2-5. Therefore, go to Step 3

Step 9. Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?

Yes (measured mixture toxicity not plausible):	Go to step 8
No (measured mixture toxicity plausible):	Go to step 3

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: $\Sigma\, p_i$ must be 1)
ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

Answer: NO. → 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 daphnias	0.441		**Yes**
ErC50 algae	1.149	**YES**	
ErC50 lemna	1.369		**Yes**

Answer: Calculated factors for lemna and daphnias gives results outside 0.8-1.2 Therefore, go to step 5.

Calculated factor for algae is between 0.8-1.2. Therefore, got to step 4.

STEP 4. Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.

Exposure	-	**(lower exposure tier)**	**(higher exposure tier)**	-	-	-	-					
Exposure tier (FOCUS step) Penoxsulam	-	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (10 m, R3 stream)	Step 4 (10 m, R4 stream)	
PECsw [mg a.s./L]	-	0.002350	0.000085	0.000274	0.000271	0.000229	0.000167	0.000004	0.000262	0.000494	0.000098	
Exposure tier (FOCUS step) - Diflufenican	-	Step 2	Step 4 (20 m bz-vfs, D3 ditch)	Step 4 (20 m bz-vfs, D4 pond)	Step 4 (20 m bz-vfs, D4 stream)	Step 4 (20 m bz-vfs, D5 pond)	Step 4 (20 m bz-vfs, D5 stream)	Step 4 (20 m bz-vfs, R1 pond)	Step 4 (20 m bz-vfs, R1 stream)	Step 4 (20 m bz-vfs, R3 stream)	Step 4 (20 m bz-vfs, R4 stream)	
PECsw [mg a.s./L]	-	0.004790	0.000047	0.000043	0.000166	0.000012	0.000064	0.000009	0.000052	0.000059	0.000042	
Exposure tier (FOCUS step) Flufenacet	-	Step 2	Step 4 (20 m bz-vfs, D3 ditch)	Step 4 (20 m bz-vfs, D4 pond)	Step 4 (20 m bz-vfs, D4 stream)	Step 4 (20 m bz-vfs, D5 pond)	Step 4 (20 m bz-vfs, D5 stream)	Step 4 (20 m bz-vfs, R1 pond)	Step 4 (20 m bz-vfs, R1 stream)	Step 4 (20 m bz-vfs, R3 stream)	Step 4 (20 m bz-vfs, R4 stream)	

PECsw [mg a.s./L]	-	0.012840	0.000059	0.000014	0.000069	0.000354	0.000494	0.000011	0.000052	0.000074	0.000052	
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	-	0.019980	0.000191	0.000331	0.000505	0.000594	0.000724	0.000025	0.000366	0.000626	0.000191	
-	-			-								
End-point/Test species	**Toxicity of the product (a.s. based) (EC_x PPP) [mg a.s./L]**	$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$										Triggers
ErC50 algae	12.489	0.119	0.207	0.316	0.372	0.453	0.015	0.229	0.392	0.120	0.10	12.489

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

		Penoxsulam		Diflufenican		Flufenacet		**Triggers**	
Endpoint/Test species	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA}$) [mg a.s./L]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	>=90% for one a.s.	>=90% for no a.s.
EC50 daphnids	0.570	1598.667	0.0%	0.576	99.00%	59.374	1.0%	Yes	
ErC50 lemna	0.004	0.053	7.8%	0.094	4.4%	0.005	87.8%		Yes

Equation 14:

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

Answer: No toxicity drivers were found for algae. Therefore, got to Step 8. Toxicity drivers for daphnias is Diflufenican.

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)	Penoxsulam	Step 2	Step 4 (10 m, D3 ditch)	Step 4 (10 m, D4 pond)	Step 4 (10 m, D4 stream)	Step 4 (10 m, D5 pond)	Step 4 (10 m, D5 stream)	Step 4 (10 m, R1 pond)	Step 4 (10 m, R1 stream)	Step 4 (10 m, R3 stream)	Step 4 (10 m, R4 stream)
PECsw [mg a.s./L]		0.002350	0.000085	0.000274	0.000271	0.000229	0.000167	0.000004	0.000262	0.000494	0.000098
Exposure tier (FOCUS step)	Diflufenican	Step 2	Step 4 (20 m bz vfs, D3 ditch)	Step 4 (20 m bz vfs, D4 pond)	Step 4 (20 m bz vfs, D4 stream)	Step 4 (20 m bz vfs, D5 pond)	Step 4 (20 m bz vfs, D5 stream)	Step 4 (20 m bz vfs, R1 pond)	Step 4 (20 m bz vfs, R1 stream)	Step 4 (20 m bz vfs, R3 stream)	Step 4 (20 m bz vfs, R4 stream)
PECsw [mg a.s./L]		0.004790	0.000047	0.000043	0.000166	0.000012	0.000064	0.000009	0.000052	0.000059	0.000042
Exposure tier (FOCUS step)	Flufenacet	Step 2	Step 4 (20 m bz vfs, D3 ditch)	Step 4 (20 m bz vfs, D4 pond)	Step 4 (20 m bz vfs, D4 stream)	Step 4 (20 m bz vfs, D5 pond)	Step 4 (20 m bz vfs, D5 stream)	Step 4 (20 m bz vfs, R1 pond)	Step 4 (20 m bz vfs, R1 stream)	Step 4 (20 m bz vfs, R3 stream)	Step 4 (20 m bz vfs, R4 stream)
PECsw [mg a.s./L]		0.012840	0.000059	0.000014	0.000069	0.000354	0.000494	0.000011	0.000052	0.000074	0.000052
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	-	0.019980	0.000191	0.000331	0.000505	0.000594	0.000724	0.000025	0.000366	0.000626	0.000191
-	-			-							
End-point/Test species	-	Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\ CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L] -									
ErC50 algae	-	0.003	0.004	0.004	0.004	0.003	0.003	0.004	0.004	0.003	0.004	
End-point/Test species	-	$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$									Triggers
ErC50 lemna	-	6.121	0.051	0.090	0.115	0.210	0.256	0.006	0.102	0.182	0.052	0.10

Answer: ETRmix for higher exposure tier exceed trigger value. Therefore, please see presented risk refinment for mixture toxicity:

For risk refinement mixture toxicity for all scenario in Central Zone used endpoint of microcosm study for Flufenacet and Penoxsulam. Therefore, mentioned endpoint for higher study on Flufenacet replaced the endpoint for algae and higher study on Penoxsulam replaced the endpoint for lemna. New calculation are presented below:

Decision scheme for mixture toxcitiy risk assessment for CHR/H/PENDIF 599.5 SC

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. (ECxa.s.): Go to 7
For both formulation (EC_{xPPP}) and a.s. ($EC_{xa.s.}$): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for daphnia, algae and macrophytes. As these are the most sensitive aquatic organisms, it is justified to conduct the mixture toxicity risk assessment only for these two organism groups. → Go to 2

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

CHR/H/PENDIF 599.5 SC / Cevino Trio 599.5 SC/ Trivino 599.5 SC containing three active ingredients. Studies with the product have been conducted for: daphnia (acute), algae and aquatic plants.

Table 1. Composition of CHR/H/PENDIF 599.5 SC

Name/code of the product	CHR/H/PENDIF 599.5 SC		
Name of the active substance A	penoxsulam		
Name of the active substance B	diflufenican		
Name of the active substance C	flufenacet		
Density [g product/cm^3]	1.2243		
	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 penoxsulam in the product	37.5	3.1%	6.3%
Concentrations of the active substance diflufenican in the product	250	20.4%	41.7%
Concentrations of the active substance flufenacet in the product	312	25.5%	52.0%

Table 2. Toxicity of CHR/H/PENDIF 599.5 SC and active substance

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\ PPP}$) [mg a.s./L]	Toxicity of the a.s. penoxsulam (ECx A) [mg a.s./L]	Toxicity of the a.s. diflufenican (ECx B) [mg a.s./L]	Toxicity of the a.s. flufenacet (ECx C) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
EC_{50} daphnids	100	48.967	100	0.24	30.9	0.01
E_rC_{50} algae	0.003267	0.002	0.086	0.0042	0.00204	0.1
~~NOEC algae~~	~~0.00019~~	~~0.000~~	~~0.02~~	~~0.00015~~	~~0.012~~	~~0.1~~
E_rC_{50} higher plant	0.056	0.027	0.00329 ~~0.0126~~	0.039	0.00243	0.1

Table 3. Calculation of toxicity exposure in CHR/H/PENDIF 599.5 SC

Toxicity per fraction of the a.s. penoxsulam (1/TUA) [mg a.s./L]	Toxicity per fraction of the a.s. diflufenican (1/TUB) [mg a.s./L]	Toxicity per fraction of the a.s. flufenacet (1/TUC) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum(TU_i)$) [mg a.s./L]	Model deviation ratio (MDR = $EC_{x\ mix\text{-}CA}/EC_{x\ PPP}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
1598.666667	0.57552	59.37355769	0.570	0.012	0.85 ~~0.582~~
~~0.319733333~~	~~0.0003597~~	~~0.023057692~~	~~0.000~~	~~3.803~~	~~0.586~~
~~0.201432~~	~~0.093522~~	~~0.004669183~~	~~0.004~~	~~0.159~~	~~1.218~~
1.375	0.010	0.004	0.003	1.76	0.26
0.053	0.094	0.005	0.004	0.15	0.83

Answer: MDRs for dpahnias and lemnas are below <0.2 Therefore , go to Step 9 MDRs for algaes are betewn 0.2-5. Therefore, go to Step 3

Step 9. Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?

Yes (measured mixture toxicity not plausible):	Go to step 8
No (measured mixture toxicity plausible):	Go to step 3

Answer: NO. → 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).

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: $\Sigma\, p_i$ must be 1)
ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

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 daphnias	0.85 ~~0.582~~	**Go to step 4**	~~**Yes**~~
NOEC algae	0.26 ~~.586~~		**Go to step 5** ~~**Yes**~~
ErC50 lemna	0.83 ~~1.218~~	**Go to step 4**	~~**Yes**~~

Answer: Calculated factors for daphnia and lemna are in the range 0.8-1.2 go to step 4

Answer: Calculated factors for ~~lemna,~~ algae ~~and daphnias~~ gives results outside 0.8-1.2 Therefore, go to step 5.

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

Endpoint/Test species	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA}$) [mg a.s./L]	Penoxsulam: Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Penoxsulam: Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	Diflufenican: Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Diflufenican: Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	Flufenacet: Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Flufenacet: Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x ($1/EC_{x\ mix\text{-}CA}$-TU_i) [%]	Triggers: >=90% for one a.s.	Triggers: >=90% for no a.s.
EC50 daphnids	0.570	1598.667	0.0%	0.576	99.00%	59.374	1.0%	Yes	
ErC_{50} NOEC algae	0.003	1.375	0.2%	0.010	28.0%	0.004	71.8%		Go to step 4
ErC50 higher plant	0.004	0.053	7.8%	0.094	4.4%	0.005	87.8%		Go to step 4
~~NOEC algae~~	~~0.003~~	~~0.320~~	~~0.1%~~	~~0.000~~	~~98.4%~~	~~0.023~~	~~1.5%~~	~~Yes~~	
~~ErC50 higher plant~~	~~0.004~~	~~0.201~~	~~2.2%~~	~~0.094~~	~~4.7%~~	~~0.005~~	~~93.2%~~	~~Yes~~	

Equation 14:

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

Answer: Toxicity drivers for daphnias ~~and algae~~ is Diflufenican~~, but for lemna gibba is Flufenacet~~

For algae and higher plants toxicity drivers were not found go to step 4.

Step 4. Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.

$$ETR_{mix} = \frac{PEC_{mix}}{EC_{X_{mix-CA}}}$$

If ETRmix-CA < trigger: Low risk

If ETRmix-CA > trigger: Low risk not demonstrated, check single-substance refinement options.

Mixture risk assessment- cereals for worst case

Substance	**PEC values (mg a.s/L) worst case scenarios**	**Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA}$ = ∑ ($p_{i\ PEC}$/$EC_{x\ i}$)) [mg a.s./L]**	**ETR_{mix} = PEC_{mix}/$EC_{x\ PPP}$**	**Triggers**
Penoxsulam	Step 4 scenario R3 (20 m BZ + 20 VFS m)			
	0.000258	1.378	0.000187	0.01
Diflufenican	Step 4 scenario D4 (20 m BZ + 20 VFS m)			

	0.000166	0.003	0.075384	0.10
Flufenacet	Step 4 scenario D5 (20 m BZ + 20 VFS m)			
	0.000494	0.003	0.080374	0.10
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.000978			

Answer: ETRmix for higher exposure tier are below the triggers. Therefore, CHR/H/PENDIF 599.5 SC 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/PENDIF 599.5 SC .
The use CHR/H/PENDIF 599.5 SC according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1).

Review comments:

The evaluation of the risk for aquatic was performed in accordance with 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 (EFSA Journal 2013;11(7):3290).

Risk assessment for active substances and its metabolites:

Calculated PEC/RAC values for penoxsulam are below the trigger value of 1 at step 3 for almost all scenarios when 10 meters vegetative buffer zone and 10 meters no-spray buffer zone is used.
For scenario R3 stream PEC/RAC values for penoxsulam are below the trigger value of 1 at step 4 with 20 meters vegetative buffer zone and 20 meter no-spray buffer zone, indicating acceptable risk to aquatic organisms. Calculated PEC/RAC values for penoxsulam metabolites (5-OH-Penoxsulam, BSTCA, BSA, TPSA, 2-amino-TP, 5-OH-2-amino-TP) were below the trigger value of 1 already at step 1, indicating low risk to aquatic organisms.

Calculated PEC/RAC values for diflufenican are below the trigger value of 1 at step 4 for when 10 meters vegetative buffer zone and 10 meters no-spray buffer zone is used.
Calculated PEC/RAC values for diflufenican metabolites (IN-AE B10737, AE 0542291) were below the trigger value of 1 already at step 1, indicating low risk to aquatic organisms.

For the intended uses calculated PEC/RAC ratios for flufenacet did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for *Selenastrum capricornutum* as characterised by an EC_{50} for species of 2.04 µg/L and form Lemna 2.43µg/L in connection with an assessment factor of 10) in FOCUS Step 4 with 10 m buffer zone scenarios. Therefore, higher tier study was necessary. Such study was performed on Annex I inclusion and was used in risk refinement. For flufenacet, the higher tier risk assessment is based on the NOEC of 12 µg a.s./L from the microcosm study (ma-cophyte, duckweed and periphyton).

Calculated PEC/RAC values for flufenacet are below the trigger value of 1 at step 4, indicating low risk to aquatic organisms when 10 meters vegetative buffer zone and 10 meters no-spray buffer zone is used.
Calculated PEC/RAC values for flufenacet metabolites (FOE sulfonic acid, FOE thiadone, FOE methylsulfide) are below the trigger value of 1 at step 1, indicating low risk to aquatic organisms.

Calculated PEC/RAC values for the formulation indicated acceptable risk when 20 meters vegetative

buffer zone and 20 meters no-spray buffer zone is used.

Based on mix-tox calculations the toxicity driver was found for daphnias. For algae and higher plants toxicity driwers were not found. Thus, further assessment was need since active substances did not cover risk for mixture.

The mixture RA is conducted by taking into account worst case FOCUS PECsw from step 4 values for all three substances. No unacceptable risk to aquatic organisms is expected from the exposure to the combined active substances following proposed uses of the product when 20 meters vegetative buffer zone and 20 meters no-spray buffer zone is used.

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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 Penoxsulam, Diflufenican and Flufenacet. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of CHR/H/PENDIF 599.5 SC were not evaluated as part of the EU assessment of Penoxsulam, ~~Tribenuron methy~~ Diflufenican and Flufenacet. New data submitted with this application are listed in 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	Penoxsulam	Oral	LD_{50} > 100 µg a.s/bee	EFSA Scientific Report (2009) 343, 84-90
Apis mellifera	Penoxsulam	Contact	LD_{50} >100 µg a.s/bee	EFSA Scientific Report (2009) 343, 84-90
Apis mellifera	Diflufenican	Oral	LD_{50} > 112.3 µg a.s./bee	EFSA Scientific Report (2007) 122, 1-84 ~~EFSA Scientific Report (2004) 15, 1-52 EFSA Scientific Repor~~
Apis mellifera	Diflufenican	Contact	LD_{50} > 100 µg a.s./bee	EFSA Scientific Report (2007) 122, 1-84 ~~EFSA Scientific Report (2004) 15, 1-52 EFSA Scientific~~

Species	Substance	Exposure System	Results	Reference
				~~Repor~~
Apis mellifera	Flufenacet ~~sodium~~	Oral	‘LD_{50}> 170 µg a.s./bee	SANCO/7469/VI/98-Final 7469/VI/98-Final 3 July 2003 ~~EFSA Journal 2016;14(4):4453~~
Apis mellifera	Flufenacet ~~sodium~~	Contact	LD_{50} > 194 µg /bee	SANCO/7469/VI/98-Final 7469/VI/98-Final 3 July 2003 ~~EFSA Journal 2016;14(4):4453~~
Apis mellifera	CHR/H/PENDIF 599.5 SC	Acute Oral	LD_{50} > 200 µg/bee	P. Holewik, Study code: B-63-20
Apis mellifera	CHR/H/PENDIF 599.5 SC	Acute Contact	LD_{50} > 200 µg/bee	P. Holewik, Study code: B-64-20
Apis mellifera	CHR/H/PENDIF 599.5 SC	Chronic Oral	LC_{50} > 666.7 mg/kg LDD_{50} > 17.7 µg/bee/day	P. Holewik, Study code: B-62-20
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 risk for bees due to the use of CHR/H/PENDIF 599.5 SC in winter/spring cereals

Intended use	Cereals winter/spring		
Active substance	Penoxsulam		
Application rate (g/ha)	1 × 15		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	15	0.15
Contact toxicity	100		0.15
Intended use	Cereals winter/spring		
Active substance	Diflufenican		
Application rate (g/ha)	1 × 100		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	112.3	100	0.89
Contact toxicity	100		1
Intended use	Cereals winter/spring		
Active substance	Flufenacet		
Application rate (g/ha)	1 × 124.8		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	170	124.8	0.73
Contact toxicity	194		0.64
Product	CHR/H/PENDIF 599.5 SC		
Application rate (g/ha)	1 × 489.72		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	489.72	2.45
Contact toxicity	200		2.45

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

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

Not relevant.

Review Comments:

Since acceptable acute risk have been concluded for bees exposed to CHR/H/PENDIF 599.5 SC at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of CHR/H/PENDIF 599.5 SC.

9.6.3 Effects on bumble bees

Not available

Review Comments:

According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.4 Effects on solitary bees

Not available

Review Comments:

According to SANCO/10329/2002 rev 2 final, the risk assessment for solitary bees is not required.

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that CHR/H/PENDIF 599.5 SC applied at the maximum use rate in cereals winter/spring poses low risk to bees.

Review comments:

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final. The risk assessment performed for all active substances and the formulated product CHR/H/PENDIF 599.5 SC is agreed by the zRMS.

The acute hazard quotients for all the active substances and for the formulation are below the trigger value of 50 with large margins of safety, indicating an acceptable acute risk to bees from exposure to CHR/H/PENDIF 599.5 SC in cereals.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. Only Test No. 245: Honey Bee (*Apis Mellifera* L.), Chronic Oral Toxicity Test (10-Day Feeding) was performed. Therefore, for Poland, the deficiencies need to be fill till EFSA bee guidance will come in to the force.

Nevertheless, such studies were deemed not necessary to finalize the risk assessment. Since the risk assessment was performed according to SANCO/10329/2002 rev 2.

Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national 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 penoxsulam, diflufenican, flufenacet 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/PENDIF 599.5 SC were not evaluated as part of the EU assessment of Penoxsulam, Diflufenican and Flufenacet. 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/PENDIF 599.5 SC	Extended Laboratory test glass plates (2D)	LR_{50} = 0.128 L formulation//ha which is equivalent to 156.71 g/ha $ER_{50=}$ 0.067 L formulation/ha	P. Holewik, Study code: B-59-20
Aphidius rhopalosiphi (adults)	CHR/H/PENDIF 599.5 SC	Extended Laboratory test glass plates (3D)	LR50 > 0.4L/ha which is equicalent to > 489.72 g/ha	P. Holewik, Study code: B-60-20
Chrysoperla Carnea	CHR/H/PENDIF 599.5 SC	Extended Laboratory test glass plates (2D)	LR50 > 0.4 L/ha which is equicalent to > 489.72 g/ha	P. Holewik, Study code: B-02-21
Coccinella septempunctata	CHR/H/PENDIF 599.5 SC	Extended Laboratory test glass plates (2D)	LR > 0.4L/ha which is equivalent to > 489.72 g/ha	P. Holewik, Study code: B-01-21
Field or semi-field tests				
Aged-residue study Typhlodromus pyri	CHR/H/PENDIF 599.5 SC	Aged-residue Extended Laboratory Tests	The effects of freshly-dried and field-aged foliar residues of CHR/H/PENDIF 599.5 SC 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 0.4 L test item/ha, fresh-dried 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	L. Fallowfield, Study code: CHR-21-07, 2021

Species	Substance	Exposure System	Results	Reference
			to the control).	

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/PENDIF 599.5 SC in cereals winter/spring

Intended use	Cereals winter/spring
Active substance/product	CHR/H/PENDIF 599.5 SC
Application rate (g/ha)	1 × 489.72
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	156.71	489.72	3.12
Aphidius rhopalosiphi	>489.72		1
Chrysoperla Carneo	>489.72		1
Coccinella septempunctata	>489.72		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.

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/PENDIF 599.5 SC in cereals winter

Intended use	Cereals winter
Active substance/product	CHR/H/PENDIF 599.5 SC
Application rate (g/ha)	1 x 489.72
MAF	1
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	156.71	2.77	13.56	5	0.006
Aphidius rhopalosiphi	>489.72				0.002

Chrysoperla Carneo	>489.72				0.002
Coccinella septempunctata	>489.72				0.002

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.

9.7.2.3 Additional higher-tier risk assessment

According to GL aged residue study provided on the most sensitive species from laboratory studies – T.pyri the effects of freshly-dried and field-aged foliar residues of CHR/H/PENDIF 599.5 SC 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 0.4 L test item/ha, fresh-dried 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 control).

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	-	13	-	10.4	-
	CHR/H/PENDIF 599.5 SC	0.4	49 *	41.4	5.8 *	44.0
	Toxic reference	-	100 *	100	~	-
14 DAT	Control	-	5	-	9.9	-
	CHR/H/PENDIF 599.5 SC	0.4	16 *	11.6	10.1	-2.2

a) For each bioassay, treatment mortalities were compared to the control using chi^2 2x2 table test ($\alpha = 0.05$, one-sided, > 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 by Student's t-test for homogenous variances ($\alpha = 0.05$, one-sided, < control), a statistically significant effect is denoted by an asterisk (*).

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

~ indicates no assessments were made for this treatment.

Review comment:

Since, using of CHR/H/PENDIF 599.5 SC according to calculations may indicate risk for *Typhlodromus pyri*, aged-residue study was performed to estimate appropriate time of recolonization.
CHR/H/PENDIF 599.5 SC was evaluated at a single application rate, equivalent to 0.4 L test item/ha. Which covers maximum application rate in the critical GAP.
When applied to sweetcorn plants at a rate equivalent to 0.4 L test item/ha, fresh-dried 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 control).
Based on the results it can be stated that for CHR/H/PENDIF 599.5 SC / Cevino Trio 599.5 SC/ Trivino 599.5 SC the LR_{50} and ER_{50} is over 0.4 L/ha of CHR/H/PENDIF 599.5 SC, what indicate safe use of product according to critical GAP.

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/PENDIF 599.5 SC applied at the maximum use rate in cereals winter/spring poses no risk to non-target arthropods. No risk mitigation needed.

Review Comments:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the guidance document ESCORT 2.
Overall acceptable risk for in-field and off-field habitats may be concluded with no need for risk mitigation measures.

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 penoxsulam, diflufenican, flufenacet 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/PENDIF 599.5 SC were not evaluated as part of the EU assessment of penoxsulam, diflufenican and flufenacet. 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	Penoxsulam	Acute (14d) Incorporated into soil / 10% OM	LC50 > 1000 mg a.s./kg d.w.soil	EFSA Scientific Report (2009) 343, 83-90
Eisenia fetida	Diflufenican	14 d, acute 10 % peat content	$LC_{50, corr}$ > 500 mg/kg dw *	EFSA Scientific Report (2007) 122, 1-84
Eisenia fetida	Metabolite AE B107137	14 d, acute 10 % peat content	$LC_{50, corr}$ > 500 mg/kg dw *	EFSA Scientific Report (2007) 122, 1-84
Eisenia fetida	Metabolite AE 0542291	14 d, acute 10 % peat content	$LC_{50, corr}$ > 500 mg/kg dw *	EFSA Scientific Report (2007) 122, 1-84

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Diflufenican	56 d, chronic 10 % peat content	$NOEC_{corr}$ = 500 mg/kg dw *	EFSA Scientific Report
Eisenia fetida	Flufenacet	Mixed into substrate 14 d, acute 10 % peat content	LC50 = 219 mg /kg soil LC50 = 109.5 mg /kg soil corrected*	7469/VI/98-Final 3 July 2003
Eisenia fetida	Flufenacet	Mixed into substrate 56 d, chronic 10 % peat content	NOEC > 4 mg /kg soil NOEC > 2 mg /kg soil corrected*	7469/VI/98-Final 3 July 2003
Eisenia fetida	flufenacet-sulfonic acid	Mixed into substrate 14 d, acute 10 % peat content	LC50 > 1000 mg/kg LC50 > 500 mg/kg corrected *	7469/VI/98-Final 3 July 2003
Eisenia fetida	flufenacet oxalate	Mixed into substrate 14 d, acute 10 % peat content	LC50 > 1000 mg/kg LC50 > 500 mg/kg corrected *	7469/VI/98-Final 3 July 2003
Eisenia andrei	CHR/H/PENDIF 599.5 SC	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10.00 mg/kg dw (day 56 reproduction) NOEC = 5 mg/kg dw*	P. Pieczka, Study code: G-39-20
Folsomia candida	CHR/H/PENDIF 599.5 SC	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 180 mg/kg dw NOEC = 90 mg/kg dw*	A. Gierbuszewska, Study code: G-40-20
Hypoaspis aculeifer	CHR/H/PENDIF 599.5 SC	Mixed into substrate 14 d, chronic 5 % peat content	NOEC > 320 mg/kg dw NOEC= 160 mg/kg dw*	A. Arendarczyk, Study code: G-41-20
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.

zRMS comments:

Since the acute toxicity to earthworms is no longer a data requirement, the acute toxicity endpoints were not considered in performed evaluation. New studies were evaluated and accepted by zRMS. For details please, see Appendix 2.

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 penoxsulam, diflufenican and flufenacet.

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/PENDIF 599.5 SC 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)**
Penoxsulam	-	0.0200	-
Diflufenican	500	0.3985	1 255
Flufenacet ~~sodium~~	2	0.1680	12
CHR/H/PENDIF 599.5 SC	5	0.6530	7.7
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)**
Penoxsulam	-	0.0200	-
Diflufenican	-	0.3985	-
Flufenacet	-	0.1680	-
CHR/H/PENDIF 599.5 SC	90	0.6530	138
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)**
Penoxsulam	-	0.0200	-
Diflufenican	-	0.3985	-
Flufenacet	-	0.1680	-
CHR/H/PENDIF 599.5 SC	160	0.6530	245

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

Review comments:
A higher tier assessment is not required based on the low risk indicated in the chronic assessment on earthworms, collembolan, and soil mite.

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/PENDIF 599.5 SC in a first-tier risk assessment.

Review comments:

The risk assessment for earthworms and other soil macro-organisms exposed to PRL OD 75 was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002) and accepted by the zRMS.
The relevant PECsoil for risk assessments is taken from Section 8 (Environmental Fate), for details please, refer to Section 8.
TERlt values calculated for CHR/H/PENDIF 599.5 SC were above the respective trigger indicating acceptable long-term risk to earthworms and other soil macro-organisms. No further evaluation is deemed necessary.
Overall, acceptable risk could be concluded for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CHR/H/PENDIF 599.5 SC in cereals.

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 penoxsulam, diflufenican, flufenacet ~~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/PENDIF 599.5 SC were not evaluated as part of the EU assessment of penoxsulam, diflufenican and flufenacet. 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	Penoxsulam	28 d, aerobic soil type	penoxsulam 0.0% (Day 42) at 2 x 50 g a.s./ha penoxsulam 7.7% (Day 42) at 10 x 50 g a.s./ha No long term effects on nitrogen transformation.	EFSA Scientific Report (2009) 343, 84-90
N-mineralisation	Diflufenican	28 d, aerobic soil	Nitrate formation rate	EFSA Scientific

Endpoint	Substance	Exposure System	Results	Reference
		type	1.25 mg/kg soil dw < ± 25 %	Repor EFSA Scientific Report (2007) 122, 1-84
N-mineralisation	Metabolite AE B107137	28 d, aerobic soil type	Nitrate formation rate 0.36 mg/kg soil dw < ± 25 %	EFSA Scientific Report (2007) 122, 1-84
N-mineralisation	Metabolite AE 0542291	28 d, aerobic soil type	Nitrate formation rate 0.36 mg/kg soil dw < ± 25 %	EFSA Scientific Report (2007) 122, 1-84
N-mineralisation C-mineralisation	Flufenacet	28 d/14d, aerobic soil type	0.8 and 4 mg /kg soil: no significant effect	7469/VI/98-Final 3 July 2003
N-mineralisation	CHR/H/PENDIF 599.5 SC	28 d, aerobic soil type	On the basis of the results, it was concluded that CHR/H/PENDIF 599,5 SC at the concentrations corresponding to the PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/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-42-20

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/PENDIF 599.5 SC 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?**
Penoxsulam	penoxsulam 0.0% (Day 42) at 2 X 50 g a.s./ha penoxsulam 7.7% (Day 42) at 10 X 50 g a.s./ha No long term effects on nitrogen transformation.	0.0200	YES
Diflufenican	Nitrate formation rate 1.25 mg/kg soil dw < ± 25 %	0.3985	YES
Metabolite AE B107137	Nitrate formation rate 0.36 mg/kg soil dw < ± 25 %	0.0008	YES
Metabolite AE 0542291	Nitrate formation rate 0.36 mg/kg soil dw < ± 25 %	0.0030	YES
Flufenacet	0.8 and 4 mg /kg soil: no significant effect	0.1680	YES
CHR/H/PENDIF 599.5 SC	On the basis of the results, it was concluded that CHR/H/PENDIF 599,5 SC at the concentrations corresponding to the PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	0.6530	YES
C-mineralisation: Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/H/PENDIF 599.5 SC and its active substances Penoxsulam, Diflufenican, Flufenacet 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/PENDIF 599.5 SC poses an acceptable risk to soil microorganisms.

Review comments:

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) and was accepted by the zRMS.

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), for details please, refer to Section 8.

Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that CHR/H/PENDIF 599.5 SC had no significant impact on soil microorganisms when applied at test item concentrations up to 16.30 mg formulation/kg dw soil, did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

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 penoxsulam, diflufenican and flufenacet. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of CHR/H/PENDIF 599.5 SC were not evaluated as part of the EU assessment of penoxsulam, diflufenican and flufenacet ~~iodosulfuron-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	Results	Reference
Pisum sativum	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50}= 0.2131 L test item/ha which is eqivalent to 260.9 g prod/ha	~~ER_{50} = 0.7146 L test item/ha which is eqivalent to 874.9 g prod/ha~~	A. Arendarczyk, Study code: G-44-20
Helianthus annuus	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50}= 0.3374 L test item/ha which is eqivalent to 413.1 g prod/ha	~~ER_{50} = 0.1211 L test item/ha which is eqivalent to 148.3 g prod/ha~~	
Daucus carota	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50}= 0.0358 L test item/ha which is eqivalent to 43.83 g prod/ha	~~ER_{50} = 0.2514 L test item/ha which is eqivalent to 307.8 g prod/ha~~	
Linum usitatissimum	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50}= 0.1101 L test item/ha which is eqivalent to 134.8 g prod/ha	~~ER_{50} = 0.0358 L test item/ha which is eqivalent to 43.8 g prod/ha~~	
Allium cepa	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50} = 0.0882 L test item/ha which is eqivalent 108 g prod/ha		
Zea mays	CHR/H/PENDIF 599.5 SC	21 d Seedling emergence	ER_{50} > 0.400 L test item/ha which is eqivalent to 489.72 g prod/ha		

Species	Substance	Exposure System	Results	Results	Reference
Pisum sativum	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50} = 0.1L test item/ha which is eqivalent to 122.43 g prod/ha		A. Gierbuszewska, Study code: G-43-20
Helianthus annuus	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50}= 0.067 L test item/ha which is eqivalent to 82.03 g prod/ha	~~ER_{50} = 0.081 L test item/ha, which is eqivalent to 99.2 g prod/ha~~	
Daucus carota	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50} = 0.027 L test item/ha, which is eqivalent to 33.1 g prod/ha		
Linum usitatissimum	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50} = 0.030 L test item/ha which is eqivalent to 36.7 g prod/ha		
Allium cepa	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50}= 0.123 L test item/ha which is eqivalent to 150.60 g prod/ha	~~ER_{50} = 0.192 L test item/ha, which is eqivalent to 235.1 g prod/ha~~	
Zea mays	CHR/H/PENDIF 599.5 SC	21 d Vegetative vigour	ER_{50} > 400 L test item/ha which is eqivalent to 489.72 g prod/ha		

m: monocotyledonous; d: dicotyledonous

Review comments:

The lowest endpoints from the studies were used for RA purposes. zRMS updated table with lowest endpoints.

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/PENDIF 599.5 SC in winter cereals

Intended use	Winter cereals
Active substance/product	CHR/H/PENDIF 599.5 SC
Application rate (g/ha)	1 x 489.72
MAF	1

Test species	ER_{50} (g product/ha)	Drift rate	$PER_{off\text{-}field}$ (g/ha)	TER criterion: TER ≥ 5	
Pisum sativum	260.9 ~~874.9 g prod/ha~~	0.0277	13.56	19.24 ~~64~~	21 d Seedling emergence
Helianthus annuus	413.1 ~~148.3 g prod/ha~~	0.0277	13.56	30.46 ~~10.9~~	21 d Seedling emergence
Daucus carota	43.83 ~~307.8 g prod/ha~~	0.0277	13.56	3.23 ~~22.7~~	21 d Seedling emergence
Linum usitatissimum	134.8 ~~43.8 g prod/ha~~	0.0277	13.56	9.94 ~~3.23~~	21 d Seedling emergence
Allium cepa	108 ~~g prod/ha~~	0.0277	13.56	7.96	21 d Seedling emergence
Zea mays	489.72 ~~g prod/ha~~	0.0277	13.56	36.1	21 d Seedling emergence
Pisum sativum	122.43 ~~g prod/ha~~	0.0277	13.56	9.03	21 d Vegetative vigour
Helianthus annuus	82.03 ~~99.2 g prod/ha~~	0.0277	13.56	7.32	21 d Vegetative vigour
Daucus carota	**33.1 g ~~prod/ha~~**	**0.0277**	**13.56**	**2.44**	**21 d Vegetative vigour**
Linum usitatissimum	36.7 ~~g prod/ha~~	0.0277	13.56	2.7	21 d Vegetative vigour
Allium cepa	150.60 ~~235.1 g prod/ha~~	0.0277	13.56	11.10 ~~17.3~~	21 d Vegetative vigour
Zea mays	489.72 g prod/ha	0.0277	13.56	36.1	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.
In bold- Lowest value

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/PENDIF 599.5 SC 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/PENDIF 599.5 SC
Application rate (g/ha)	1 × 489.72
MAF	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	13.56	6.78	3.39	1.36
5	0.57	2.79	-	-	-
Toxicity value ER_{50} = 33.1 g/ha		**TER** **criterion: TER ≥ 5** ~~1~~			
1		2.44	4.88	**9.76**	24.3
5		11.9	-	-	-

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/PENDIF 599.5 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/PENDIF 599.5 SC according to the GAP of the formulation CHR/H/PENDIF 599.5 SC achieve the acceptability criteria TER ≥ 5 ~~1~~ based on SSD risk refinement, with applying:

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

Review comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was updated and accepted. Based on the predicted rates of CHR/H/PENDIF 599.5 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to formulation according to the GAP achieve the acceptability criteria TER ≥ 5. Following risk mitigation measures should be applied:

When using in non-crop areas.
- 1 m unsprayed buffer zone with 75 % drift reduction nozzle or,
- 5 m unsprayed buffer zone to non-agricultural land

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

CHR/H/PENDIF 599.5 SC was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC

and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Classification according tu CLP Regulation:

CLASSIFICATION	
Hazard classes, categories:	Aquatic Acute 1 Aquatic Chronic 1,
LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statements:	H400: Very toxic to aquaitic life. H410: Very toxic to aquatic life with long lasting effects
Precautionary statements:	P391 – Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.
EUH401	To avoid risks to man and the environment, comply with the instructions for use.

Standard phrases under Regulation (EU) No 547/2011

SP 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
SPe3	To protect aquatic organisms respect a: - 20 m unsprayed buffer zone and 20 m vegetative strip To protect non target terrestrial plants respect a: - 1 m unsprayed buffer zone with 75 % drift reduction nozzle or, - 5 m unsprayed buffer zone to non-agricultural land

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1	K. Florynski	2018	CHR/H/PENDIF 599.5 SC - TER Calculations for Terrestial Verterbrates Chemirol GLP No Unpublished	N	Chemirol
KCP 10.1.2	K. Florynski	2018	CHR/H/PENDIF 599.5 SC - TER Calculations for Terrestial Verterbrates Chemirol GLP No Unpublished	N	Chemirol
KCP 10.2/01	E. Malada	2021	CHR/H/PENDIF 599.5 SC Daphnia magna, Acute Immobilisation Test W-44-20 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	M. Czarnecka	2021	CHR/H/PENDIF 599.5 SC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test W-45-20 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 10.2/03	M. Czarnecka	2021	CHR/H/PENDIF 599.5 SC Anabaena flos-aquae UTEX B 1444 Growth inhibition test W-47-20 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 10.2/04	M. Czarnecka	2021	CHR/H/PENDIF 599.5 SC Lemna gibba CPCC 310, Growth inhibition test W-46-20 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 10.3/01	P. Holewik	2021	CHR/H/PENDIF 599.5 SC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test B-63-20 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	P. Holewik	2021	CHR/H/PENDIF 599.5 SC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test B-64-20 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 10.3/03	P. Holewik	2021	CHR/H/PENDIF 599.5 SC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test B-62-20 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	P. Holewik	2021	An extended laboratory test for evaluating the effects of CHR/H/PENDIF 599,5 SC on the predatory mite, Typhlodromus pyri (Sch.) B-59-20 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 10.3/05	P. Holewik	2021	An extended laboratory test for evaluating the effects of CHR/H/PENDIF 599,5 SC on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez)	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**
			B-60-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP10.3/06	P. Holewik	2021	An extended laboratory test for evaluating effects of CHR/H/PENDIF 599,5 SC on the green lacewing, Chrysoperla carnea (Steph.) B-02-21 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	P. Holewik	2021	An extended laboratory test for evaluating effects of CHR/H/PENDIF 599,5 SC on the ladybird beetle, Coccinella septempunctata (L.) B-01-21 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 10.3/09	L. Fallowfield	2021	CHR/H/PENDIF 599.5 SC – Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae) CHR-21-07 Mambo-Tox A Division of Cawood Scientific Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, UK GLP Unpublished	N	Chemirol
KCP 10.4/01	P. Pieczka	2021	CHR/H/PENDIF 599.5 SC Earthworm reproduction test (Eisenia andrei) G-39-20 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 10.4/02	A. Gierbuszewska	2021	CHR/H/PENDIF 599.5 SC Collembolan (Folsomia candida) Reproduction Test G-40-20 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	A. Arendarczyk	2021	CHR/H/PENDIF 599,5 SC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil G-41-20 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 10.5/01	A. Gierbuszewska	2021	CHR/H/PENDIF 599,5 SC Soil Microorganisms: Nitrogen Transformation Test G-42-20 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 10.6/01	A. Arendarczyk	2021	CHR/H/PENDIF 599,5 SC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test G-44-20 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	2021	CHR/H/PENDIF 599,5 SC Terrestial Plant Test: Vegetative Vigour Test G-43-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemirol

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1/01	XXXX	1999	XDE-638: Avian Acute Oral Toxicity Test with Northern Bobwhite (Colinus virginianus) Dr-0357-6203-025 Genesis Laboratories, Inc. 10122 N.E. Frontage Road, Wellington, Colorado 80549. U.S.A. GLP Unpublished	Y	Dow Agro-Sciences
KCP 10.1/02	XXXX	2001	XDE-638: Avian Acute Oral Toxicity Test with Mallard Ducks (Anas platyrhynchos) DECO HET DR-0357-6203-073 Genesis Laboratories, Inc. Wellington, CO, USA GLP Unpublished	Y	Dow Agro-Sciences
KCP 10.1/03	XXXXX	2000	XDE-638: Avian Acute Dietary Toxicity Test with Northern Bobwhite (Colinus Virginianus) DECO HET DR-0357-6203-026R Genesis Laboratories, Inc. 10122 N.E Frontage Road, Wellington Colorado, 80549. U.S.A GLP Unpublished.	Y	Dow Agro-Sciences
KCP 10.1/04	XXXXX	2000	XDE-638: Avian Acute Dietary Toxicity Test with the Mallard (Anus Platryhynchos) DECO HET DR-0357-6203-024R Genesis Laboratories, Inc. 10122 N.E Frontage Road, Wellington Colorado 80549. U.S.A. GLP Unpublished	Y	Dow Agro-Science
KCP 10.1/05	XXXXX	2002	XDE-638: Avian Reproduction Study with Northern Bobwhite (Colinus Virginianus) DECO HET DR-0357-06203-072 Genesis Laboratories, Inc. Wellington, CO, USA GLP	Y	Dow Agro-Science

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 10.2/01	XXXXX	2002	XDE-638: An Acute Toxicity Study with the Rainbow Trout, Oncorhynchus Mykiss Walbaum DECO HET DR-0357-6203-058 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	Y	Dow Agro-Science
KCP 10.2/02	XXXXX	2000	XDE-638: An Acute Toxicity Study with the Bluegill Sunfish, Lepomis Macrochirus Rafinesque DECO HET DR-0357-6203-059 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	Y	Dow Agro-Science
KCP 10.2/03	XXXXX	2001	Revised Report for XDE-638: An Acute Toxicity Study with the Common Carp, Cyprinus carpio Linneaus DECO HET DR-0357-6203-077R The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	Y	Dow Agro-Science
KCP 10.2/04	XXXXXX	2000	XDE-638: Acute Toxicity to the Silverside, Menidia beryllina DECO HET DR 0357-6203-070 T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLp Unpublished	Y	Dow Agro-Science
KCP 10.2/05	XXXXX	2002	XDE-638: Toxicity to the Early Life Stages of the Fathead Minnow, Pimephales promelas Rafinesque., DECO HET DR-0357-6203-078	Y	Dow Agro-Sciences

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**
			The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished		
KCP 10.2/06	Marino, T.A., McClymo nt, E.L., Henry, K.S.	2000	XDE-638: An Acute Toxicity Study with the Daphnia, Daphnia Magna Straus DECO HET DR-0357-6203-060 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/07	Putt, A.E.	2002	5-Hdroxy-XDE-638 – Acute Toxicity to Daphnids (Daphnia magna) Under Static Conditions DR-0370-5235-002 Springborn Laboratories, Inc Wareham,MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/08	Putt, A.E.	2002	XDE-638 Metabolite (BSTCA) – Acute Toxicity to Daphnids (Daphnia magna) Under Static Conditions DR-0370-5417-002 Springborn Laboratories, Inc Wareham,MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/09	Putt, A.E.	2002	XDE-638 Metabolite (BST) – Acute Toxicity to Daphnids (Daphnia magna) Under Static Conditions. DR-0370-5246-002 Springborn Laboratories, Inc Wareham,MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/10	Marino, T.A	2002	XDE-638 Metabolite BSA – An Acute Toxicity Study with the Daphnid, Daphnia magna	N	Dow Agro-

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**
			Straus. DR-0374-7577-001 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished 48674 USA		Sciences
KCP 10.2/11	Marino, T.A.	2002	XDE-638 Metabolite TPSA– An Acute Toxicity Study with the Daphnid, Daphnia magna Straus. DR-0374-7566-001 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/12	Marino, T.A.	2002	XDE-638 Metabolite 2-Amino-TP– An Acute Toxicity Study with the Daphnid, Daphnia magna Straus. DR-0374-7555-001 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/13	Marino, T.A.	2002	XDE-638 Metabolite 5-OH-2-Amino-TP– An Acute Toxicity Study with the Daphnid, Daphnia magna Straus. DR-0374-7588-001 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/14	Boeri, R.L., Ward, T.L.	2000	XDE-638: Acute Toxicity to the Gammarid, Gammarus Pseudolimnaeus DECO HET DR-0357-6203-038 T R Wilbury Laboratories Inc Marblehead Mass GLP Unpublished	N	Dow Agro-Sciences

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 10.2/15	Boeri, R.L., Magazu, J.P., Ward, T.J.	2001	Revised Report For XDE-638: Acute Toxicity to the Ramshorn Snail, Planorbella Trivolvis DECO HET DR-0357-6203-039R T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/16	Boeri, R.L., Magazu, J.P., Ward, T.J.	2000	XDE-638: Flow-Through Mollusc Shell Deposition Test DECO HET DR-0357-6203-037 T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLP Unpublished	N	Dow Agro-Sciences
kCP 10.2/17	Ward, T.J., Wyskiel, D.C. , Boeri, R.L.	2000	XDE-638 Acute Toxicity to the Mysid, Americamysis bahia DECO HET DR-0357-6203-071 T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/18	Kirk, H.D., Staley, J.L., McMlymo nt, E.L. , McFadden , L.G.	2000	XDE-638: 21 Day Chronic Toxicity Test with the Daphnid, Daphnia magna STRAUS DECO HET DR-0357-6203-075 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/19	Boeri, R.L Ward, T.J.	2002	XDE-638: Chronic Toxicity to the mysid, Americamysis bahia DECO HET DR-0357-6203-082 T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLP Unpublished	N	Dow Agro Science
KCP 10.2/20	Kirk, H.D.,	2000	Effects of XDE-638 on the Growth of the Freshwater Green Alga, Selenastrum Carpricor-	N	Dow Agro-

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**
	Gilles, M.M., McClymo nt, E.L., McFadden , L.G.		nutum Print DECO HET DR-0357-6203-045 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished		Science
KCP 10.2/21	Kirk, H.D., Gilles, M.M., McClymo nt, E.L., McFadden , L.G.	2000	XDE-638: Growth Inhibition Test with the Freshwater Diatom, Navicula Pelliculosa DECO HET DR-0357-6203-065 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/22	Kirk, H.D., Gilles, M.M., McClymo nt, E.L., McFadden , L.G.	2000	XDE-638: Growth Inhibition Test with the Bluegreen Alga, Anabaena Flos-Aquae DECO HET DR-0357-6203-066 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/23	Kirk, H.D., Gilles, M.M., McClymo nt, E.L., McFadden , L.G.	2000	XDE-638:Growth Inhibition Test with the Saltwater Diatom, Skeltonema Costatum DECO HET DR-0357-6203-067 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/24	Hoberg, J.R.	2002	5-Hydroxy-XDE-638 – Toxicity to the Freshwater Green Alga, Pseudkirchneriella subcapitata DR-0370-5235-003 Springborn Laboratories Wareham MA GLP	N	Dow Agro-Sciences

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 10.2/25	Hoberg, J.R.	2002	XDE-638 Metabolite (BSTCA) – Toxicity to the Freshwater Green Alga, Pseudokirchneriella subcapitata DR-0370-5417-001 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/26	Hoberg, J.R.	2002	XDE-638 Metabolite (BST) – Toxicity to the Freshwater Green Alga, Pseudokirchneriella subcapitata DR-0370-5246-003 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/27	Roshon, R.	2002	XDE-638 Metabolite TPSA (TSN 103594): Growth Inhibition Test with the Freshwater Green Alga, Selenastrum Capricornutum Printz DECO HET DR-0374-7566-003 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/28	Roshon, R.	2002	XDE-638 Metabolite BSA (TSN 101980): Growth Inhibition Test with the Freshwater Green Alga, Selenastrum Capricornutum Printz DECO HET DR-0374-7577-003 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/29	Roshon, R.	2002	XDE-638 Metabolite BSA (TSN 101824): Growth Inhibition Test with the Freshwater Green Alga, Selenastrum Capricornutum Printz DECO HET DR-0374-7555-002	N	Dow Agro-Sciences

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
			ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished		
KCP 10.2/30	Roshon, R.	2002	XDE-638 Metabolite 5-OH,2-amino-TP (TSN 101837): Growth Inhibition Test with the Freshwater Green Alga, Selenastrum Capricornutum Printz DECO HET DR-0374-7555-002 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/31	Putt, A.E.	2002	XDE-638 The Full Life-Cycle Toxicity to Midge (Chironomus riparius) Under Static Conditions using Spiked Sediment and Spiked Water DR-0357-6203-084 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agrosciences
KCP 10.2/31	Kirk, H.D., Gilles, M.M., McClymo nt, E.L., McFadden , L.G.	2000	Effect of XDE-638 on the Growth of the Freshwater Aquatic Plant, Duckweed, Lemna Gibba L. G-3 DECO HET DR-0357-6203-056 The Dow Chemical Company Midland Michigan 48674 USA GLP Unpublished	N	Dow Agro-Science
KCP 10.2/32	Hoberg, J.R.	2002	5-Hydroxy-XDE-638 – Toxicity to Duckweed, Lemna gibba DR-0370-5235-001 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agro-Sciences

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 10.2/33	Hoberg, J.R.	2002	XDE-638 Metabolite (BSTCA) Toxicity to Duckweed, Lemna gibba DR-0370-5417-003 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/34	Hoberg, J.R.	2002	XDE-638 Metabolite (BST) – Toxicity to Duckweed, Lemna gibba DR-0370-5246-001 Springborn Laboratories Wareham MA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/35	Roshon, R.	2002	XDE-638 Metabolite TPSA(TSN 103594): Growth Inhibition Test with the Freshwater Aquatic Plant, Lemna Gibba L. G-3 DECO HET DR-0374-7566-002 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/36	Roshon, R.	2002	XDE-638 Metabolite BSA(TSN 101980) Growth Inhibition Test with the Freshwater Aquatic Plant, Lemna Gibba L. G3. DECO HET DR-0374-7577-002 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.2/37	Roshon, R.	2002	XDE-638 Metabolite 2-Amino-TP(TSN 101824) Growth Inhibition Test with the Fresh-water Aquatic Plant, Lemna Gibba L. G-3 DECO HET DR-0374-7555-003 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences

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 10.2/38	Roshon, R.	2002	XDE-638 Metabolite 5-OH-2-Amino-TP (TSN 101837): Growth Inhibition test with the Freshwater Aquatic Plant, Lemna Gibba L. G-3 DECO HET DR-0374-7588-002 ESG International, Inc. 11B Nicholas Beaver Road., Guelph,, Ontario, Canada N1H, 6H9 GLP Unpublished	N	Dow Agro-Sciences
KCP 10.3/01	Kranzfeld er, J.A.	2000	XDE-638: Acute Oral Toxicity Test with the Honeybee (Apis mellifera) DECO HET DR-0357-6203-028 ABC Laboratories, Inc. 7200 E. ABC Lane, Columbia, Missouri 65202 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.3/02	Kranzfeld er, J.A.	2000	XDE-638: Acute contact Toxicity Test with the Honeybee, Apis Mellifera DECO HET DR-0357-6203-027 ABC Laboratories, Inc. 7200 E. ABC Lane, Columbia, Missouri 65202 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.4/01	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999	XDE-638: Acute Toxicity to the Earthworm, Eisenia Foetida DR-0357-6203-029 T R Wilbury Laboratories Inc Marblehead Mass 01945 USA GLP Unpublished	N	Dow Agro-Sciences
KCP 10.5/01	van der Kolk, J.	2002	XDE-638: Determination of Effects on Soil Microflora Activity DR-0357-6203-085 Springborn Laboratories Wareham MA GLP Unbpulished	N	Dow Agro-Sciences
KCP 10.1/06	XXXX	1984	Acute oral toxicity study with M&B 38,544 technical in bobwhite quail	Y	BCS

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**
			R006408 GLP Unpublished		
KCP 10.1/07	XXXXX	1984	The acute oral toxicity (LD50) of M&B38544 to the mallard duck R006406 GLP Unpublished	Y	BCS
KCP 10.1/08	XXXX	1992	Diflufenican Reproduction in the bobwhite quail R015190 GLP Unpublished	Y	BCS
KCP 10.2/39	XXXX	1998	Diflufenican Acute toxicity (96 hours) to rainbow trout (Oncorhynchus mykiss) under static conditions R006584 GLP Unpublished	Y	BCS
KCP 10.2/40	XXXXX	1996	MB38181 Cute toxicity (96 hours) to rainbow trout (Oncorhynchus mykiss) under static conditions R006576 GLP Unpublished	Y	BCS
KCP 10.2/41	XXXXX	1998	Diflufenican Acute toxicity (96 hours) to common carp (Cyprinus carpio) under static conditions R006586 GLP Unpublished	Y	BCS

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 10.2/42	XXXX	1997	Diflufenican Fish, juvenile growth test (28 days) under flow-through conditions R005623 GLP Unpublished	Y	BCS
KCP 10.2/42	XXXX	1998	Diflufenican – Early life-stage toxicity test with feathed minnow (Pimephales promelas) R005752 GLP Unpublished	Y	BCS
KCP 10.2/43	XXXX	1998	(14C)-Diflufenican Bioaccumulation and metabolism in rainbow trout R006596 Yes Unpublished	Y	BCS
KCP 10.2/44	Odin-Feurtet, M.	1999	Diflufenican Acute toxicity (48 hours) to daphnids (Daphnia magna) under static conditions R005989 Rhone-Poulenc; Rhone-Poulenc Agro, Sphia Antipolis; Centre de Recherche, Rhone-Poulenc Agro, Lyon GLP Unpublished	N	BCS
KCP 10.2/45	Suteau, P.	1996	MB38181 Cute toxicity (48 hours) to daphnids (Daphnia magna) under static conditions R006574 Rhone-Poulenc; Rhone-Poulenc Agro, Sphia Antipolis; Centre de Recherche, Rhone-Poulenc Agro, Lyon GLP Unpublished	N	BCS
KCP 10.2/46	Douglas, M.T. Handley, J.W.	1987	Diflufenican: The acute toxicity of diflufenican soil metabolite no.2. M&B 43,625 to Daphnia magna	N	BCS

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
			R008232 Rhone-Poulenc, Huntingdon Research Centre Ltd., Huntingdon, GBR May & Baker Ltd., Dagenham, Exxec, GBR GLP Unpublished		
KCP 10.2/47	Putt, A.E.	2000	The chronic toxicity to Daphnia magna under static-renewal conditions Diflufenican Generated by: Springborn Laboratories, Inc., Wareham, USA; Rhone-Poulenc Agro, Sophia Antipolis, FRA; Document No: C009776 GLP / GEP Yes Unpublished	N	BCS
KCP 10.2/48	Odin-Feurtet M.	1997	Diflufenican Freshwater algal growth inhibition study (72 hours) Selenastrum capricornu-tum Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis; Centre de Recherche Rhone-Poulenc Agro; Document No: R005609 GLP / GEP Yes Unpublished	N	BCS
KCP 10.2/49	Hoberg J.R.	1998	Diflufenican - Toxicity to the freshwater blue-green alga, Microcystis aeruginosa Generated by: Rhone-Poulenc; Springborn Laboratories, Inc., Wareham, USA; Rhone-Poulenc Agro, Sophia Antipolis, FRA; Document No: R008300 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/50	Hoberg J.R.	1998	Diflufenican - Toxicity to the freshwater blue-green alga, Anabaena flos-aquae Generated by: Rhone-Poulenc; Springborn Laboratories, Inc., Wareham, USA; Rhone-Poulenc Agrochimie, Sophia Antipolis, FRA;	N	BCS

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**
			Rhone-Poulenc Secteur Agro; Document No: R008296 GLP / GEP Yes unpublished		
KCP 10.2/50	Hoberg J.R.	1997	Diflufenican technical - Toxicity to the freshwater diatom, Navicula pelliculosa Generated by: Rhone-Poulenc; Springborn Laboratories, Inc., Wareham, USA; Rhone-Poulenc Secteur Agro, FRA; Document No: R008292 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/51	Odin-Feurtet M.	1998	Diflufenican: Freshwater algal growth inhibition study (72 hours) Scenedesmus subspicatus Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis, FRA; Rhone-Poulenc Secteur Agro, Lyon, France; Document No: R015235 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/52	Odin-Feurtet M.	1998	Diflufenican: Freshwater algal growth inhibition study in the sediment water system (Scenedesmus subspicatus) Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis; Centre de Recherche Rhone-Poulenc Agro, Lyon; Document No: R006582 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/53	Odin-Feurtet M.	1998	Diflufenican Freshwater algal growth inhibition study in a sediment water system	N	BCS

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**
			(Scenedesmus subspicatus) Generated by: Rhone-Poulenc; Rhone-Poulenc Agro, Sophia Antipolis; Centre de Recherche Rhone-Poulenc Agro; Document No: R006589 GLP / GEP Yes unpublished		
KCP 10.2/54	Odin-Feurtet M.	1998	Diflufenican Freshwater algal growth inhibition study and recovery phase Scenedesmus subspicatus Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis, FRA; Centre de Recherche Rhone-Poulenc Secteur Agro; Rhone-Poulenc Agro, Lyon, FRA; W.E.Z. Document No: R008294 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/55	Suteau P.	1996	MB38181 Freshwater algal growth inhibition study (72 hours) Scenedesmus subspicatus Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis; Centre de Recherche Rhone-Poulenc Secteur Agro; Document No: R006578 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/56	Mead C., Mullee D.M.	2001	MB 43625: Algal inhibition test Generated by: Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Safepharm Laboratories Limited, Derby GBR; Document No: C021270 GLP / GEP Yes	N	BCS

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 10.2/57	Desjardins D., Kendall T.Z., Krueger H.O.	2002	A 72-hour toxicity test with the freshwater alga (Selenastrum capricornutum) Code: AE 0592370 (MB 44085) Generated by: Wildlife International Ltd.; BCS GmbH, DEU; Document No: C025824 GLP / GEP unpublished	N	BCS
KCP 10.2/58	Desjardins D., Kendall T.Z., Krueger H.O.	2002	A 72-hour toxicity test with the freshwater alga (Selenastrum capricornutum) Code: AE C522392 (MB40401) Generated by: Wildlife International Ltd.; BCS GmbH, DEU; Document No: C028238 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/59	McElligott A.	1996	Diflufenican - Toxicity to the sediment dwelling chironomid larvae (Chironomus riparius) under static conditions - 28 days. Generated by: Rhone-Poulenc; Rhone-Poulenc Agrochimie, Sophia Antipolis, FRA; Centre de Recherche Rhone-Poulenc Agro GmbH, Koeln, Germany; Rhone-Poulenc Secteur Agro; Document No: R008288 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/60	Krueger H.O., Platania S., Kendall T.Z., Jaber M.	2002	AE F088657 (diflufenican): A prolonged sediment toxicity test with Chironomus riparius using spiked sediment Generated by: BCS GmbH, DEU; Ecotoxicology, Frankfurt Wildlife International Ltd., Maryland, USA;	N	BCS

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
			Document No: C026642 GLP / GEP Yes unpublished		
KCP 10.2/61	Krueger H.O., Thomas S., Kendall T.Z.	2003	AE C522392 (MB 40401): A prolonged sediment toxicity test with Chironomus riparius using spiked sediment Generated by: BCS GmbH, DEU; Ecotoxicology, Frankfurt Ecotoxicology, Frankfurt; Ecotoxicology, Frankfurt Wildlife International Ltd., Maryland, USA; Document No: C032889 GLP / GEP Yes unpublished	N	BCS
KCP 10.2/62	Hoberg J.R.	1998	Diflufenican - Toxicity to the duckweed, Lemna gibba Generated by: Rhone-Poulenc; Springborn Laboratories, Inc., Wareham, USA; Rhone-Poulenc Agro, Sophia Antipolis, FRA; Document No: R008298 GLP / GEP Yes unpublished	N	BCS
KCP 10.3/03	Schmitzer S.	1998	Laboratory testing for toxicity (acute contact and oral LD50) of diflufenican on honey bees (Apis mellifera L.), (Hymenoptera, Apidae) Generated by: Rhone-Poulenc; IBACON GmbH, Rossdorf, DEU; Inst. f. Biologische Analytik und Consulting Rhone-Poulenc Agro, Sophia Antipolis, FRA; Ecotoxicology Department Document No: R008302 GLP / GEP Yes unpublished	N	BCS

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.4/02	Odin-Feurtet M.	1997	Diflufenican: Acute toxicity (14 day) to earthworms (Eisenia foetida) Artificial soil method Generated by: Rhone-Poulenc; Rhone-Poulenc Agro, Sophia Antipolis; Centre de Recherche Rhone-Poulenc Agro; Document No: R005596 GLP / GEP Yes unpublished	N	BCS
KCP 10.4/03	Staebler D.	2001	Acute toxicity of MB 38181 on earthworms, Eisenia foetida using an artificial soil test Generated by: Aventis CropScience GmbH, DEU; Arbeitsgemeinsch. GAB GmbH & IFU GmbH, DEU; Document No: C021267 GLP / GEP Yes unpublished	N	BCS
KCP 10.4/04	Wetton P.M.	2001	MB43625: Acute toxicity to earthworms (Eisenia foetida) Generated by: Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Safepharm Laboratories Limited, Derby GBR; Document No: C015390 GLP / GEP Yes	N	BCS
KCP 10.4/05	Lührs U.	1999	Effects of diflufenican on reproduction and growth of earthworms Eisenia fetida (Savigny 1826) in artificial soil. Generated by: Rhone-Poulenc; IBACON GmbH, Rossdorf, DEU; Rhone-Poulenc Secteur Agro; Document No: R005877 GLP / GEP Yes unpublished	N	BCS
KCP 10.5/02	Schaefer E.C., Siddiqui A.I.	2003	AE F088657 (dilfufenican): Soil microorganisms: Nitrogen transformation test Generated by: BCS GmbH, DEU; Ecotoxicology, Frankfurt Wildlife International Ltd.,	N	BCS

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**
			Maryland, USA; Document No: C031491 GLP / GEP Yes unpublished		
KCP 10.5/03	Lamb L.S., Luscombe B.M.	1985	Diflufenican: Effects on soilrespiration and nitrification Generated by: Rhone-Poulenc; May & Baker Ltd., England; Document No: R008108 GLP / GEP unpublished	N	BCS
KCP 10.5/04	Koelzer U.	2002	Assessment of the side effects of MB 38181 on the activity of the soil microflora Generated by: Aventis CropScience GmbH, DEU; Arbeitsgemeinsch. GAB GmbH & IFU GmbH, DEU; Document No: C021268 GLP / GEP Yes unpublished	N	BCS
KCP 10.5/05	Koelzer U.	2002	Assessment of the side effects of MB 43625 on the activity of the soil microflora Generated by: Aventis CropScience GmbH, DEU; Arbeitsgemeinsch. GAB GmbH & IFU GmbH, DEU; Document No: C021269 GLP / GEP Yes unpublished	N	BCS
KCP 10.5/06	Schaefer E.C., Siddiqui A.I.	2003	AE F088657 (dilfufenican): Soil microorganisms: Carbon transformation test Generated by: BCS GmbH, DEU; Ecotoxicology, Frankfurt Wildlife International Ltd., Maryland, USA; Document No: C031490 GLP / GEP Yes Unpublished	N	BCS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1/09	XXXX	1992	Technical FOE 5043: An acute oral LD50 with bobwhite quail. Source: Miles Inc. Bayer AG, Report No. 102642 GLP, Unpublished	Y	Bayer
KCP 10.1/10	XXXX	1993	FOE 5043 technical: A subacute dietary LC50 with mallard duck. Source: Miles Inc. Bayer AG, Report No. 103814 GLP, Unpublished	Y	Bayer
KCP 10.1/11	XXXX	1994	FOE 5043 technical: A subacute dietary LC50 with northern bobwhite. Source: Miles Inc. Bayer AG, Report No. 106583 GLP, Unpublished	Y	Bayer
KCP 10.1/12	XXXX	1994	Effects of a subchronic dietary exposure of FOE 5043 techn. on bobwhite quail including effects on reproduction and health. Bayer AG. Report No. SXR/REP 03 GLP, Unpublished	Y	Bayer
KCP 10.1/13	XXXXX	1994	Effect of technical FOE 5043 on mallard reproduction. Source: Miles Inc. Bayer AG, Report No. 106594 GLP, Unpublished	Y	Bayer

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 10.2/63	XXXXX	1995	Acute toxicity of FOE 5043 technical to the rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions. Source: Mobay Corp. Bayer AG, Report No. 106673 GLP, Unpublished	Y	Bayer
KCP 10.2/64	XXXXX	1995	Acute toxicity of FOE 5043 technical to the rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions. Source: Mobay Corp. Bayer AG, Report No. 106673 GLP, Unpublished	Y	Bayer
KCP 10.2/65	XXXXX	1995	FOE 5043 sulfonic acid - Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) in a static test. Bayer AG, Report No. DOM 95031 GLP, Unpublished	Y	Bayer
KCP 10.2/66	XXXXX	1995	Early life stage toxicity of FOE 5043 technical to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. Source: Miles Inc. Bayer AG, Report No. 106978 GLP, Unpublished	Y	Bayer
KCP 10.2/67	XXXXX	1994	Uptake, depuration and bioconcentration of ^{14}C-FOE 5043 technical by bluegill (*Lepomis macrochirus*) under flow-through conditions. Source: Miles Inc. Bayer AG, Report No. 106760 GLP, Unpublished	Y	Bayer

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 10.2/68	Gagliano G.G Bowers,L.M	1994	Chronic toxicity of FOE 5043 technical to the water flea (*Daphnia magna*) under static renewal conditions. Source: Miles Inc. Bayer AG, Report No. 106762 GLP, Unpublished	N	Bayer
KCP 10.2/69	Anderson, J.P.E.	1995	Range Finding Test: Influence of FOE 5043 T on the Growth of the Green Alga, Selenastrum capricornutum Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: AJO/130095 Date: April 5, 1995 GLP not published	N	Bayer
KCP 10.2/70	Anderson, J.P.E.	1997	Growth of the green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), during and after exposure to high concentrations of FOE 5043 Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: AJO/157097 Date: July 14,1997 GLP not published	N	Bayer
KCP 10.2/71	Dorgerloh, M.	1998	FOE 5043-Methylsulfide -Influence on the Growth of the Green Alga, *Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)* Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: DOM 98011 Date: June 16, 1998	N	Bayer

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 not published		
KCP 10.2/72	Dorgerloh, M.	1998	Toxicity of 14C-FOE 5043 to the Green Alga *Selenastrum capricornutum* Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: DOM 98092 Date: September 9, 1998 originally reported as: Bowers, L.M.: Toxicity of 14C-FOE 5043 to the Green Alga *Selenastrum capricornutum.* Source: Bayer Corp., Kansas, USA; Bayer AG, Report No.: 107114, Date: October 19, 1995 GLP not published	N	Bayer
KCP 10.2/73	Hughes, J.S. Alexander, M.M.	1993	Acute toxicity of FOE 5043 (technical) to *Anabaena flos-aquae* Source: Malcolm Pirnie Inc., Tarrytown, NY 10591, USA Generated by: Miles Inc., Submitted by: Bayer AG, Bayer file No.: 105199 Date: December 17, 1993 GLP not published	N	Bayer
KCP 10.2/74	Dorgerloh, M.	1998	Acute toxicity of FOE 5043 (technical) to *Lemna gibba* G3 Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: DOM 98091 Date: September 1, 1998 GLP not published originally reported as: Hughes, J.S.; Alexander, M. M.: Acute Toxicity of FOE 5043 (technical) to *Lemna gibba* G3; Source: Miles, Kansas; Bayer	N	Bayer

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**
			AG, Report No.: 105198 Date: December 17, 1993		
KCP 10.2/75	Dorgerloh, M.	1995	FOE 5043-sulfonic acid - Toxicity (14 days) to *Lemna gibba* G3. Bayer AG, Report No. DOM 95072 GLP, Unpublished	N	Bayer
KCP 10.2/76	Foekema E.M. and Jak R.G.,	1999	The fate and biological effects of Flufenacet WG 60 in aquatic indoor microcosms Bayer AG, Report No. TNO-MEP – R 99/423 GLP Unpublished	N	Bayer
KCP 10.3/04	Mayer, D.F	1994	FOE 5043 / honey bees acute toxicity. Source: Washington State University Bayer AG, Report No. 106765 GLP, Unpublished	N	Bayer
KCP 10.3/05	Tornier, I.	1995	Results of the screening test on the honey bee *Apis mellifera* L. test substance: FOE 5043 (techn.). Source: GAB Biotechnologie GmbH Bayer AG, Report No. B-958?64 Not GLP, Unpublished	N	Bayer

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 10.3/06	Nengel.S	1995	Assessment of side effects of FOE 5043 (techn.) to the honey bee, *Apis mellifera* L. in the laboratory following the EPPO Guideline No. 170. Source: GAB/IFU, Niedern Bayer AG, Report No. 94137/01-BLEU GLP, Unpublished	N	Bayer
KCP 10.4/06	Nienstedt, K.M.	1999	FOE 5043-Oxalate: A 14-day acute toxicity test with the earthworm (Eisenia fetida) Source: Springborn Laboratories, Horn, Switzerland Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: 1022.006.630 Date: July 19, 1999 GLP not published	N	Bayer
KCP 10.4/07	Nienstedt, K.M.	1999	FOE 5043-Sulfonic acid Na-salt: A 14-day acute toxicity test with the earthworm (Eisenia fetida) Source: Springborn Laboratories, Horn, Switzerland Generated by: Bayer AG, Submitted by: Bayer AG, Bayer file No.: 99-005-1022 Date: July 15, 1999 GLP not published	N	Bayer

Appendix 2 Detailed evaluation of the new studies

Review Comment:

In order to provide sufficient details, where appropriate, the study summaries have been adapted by the zRMS from the full study reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A. 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A.2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.1.2 KCP 10.1.2.1 Acute oral toxicity to mammals

Summarised in Section 6 (Mammalian Toxicology)

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 was conducted according to OECD guideline 202 and principles of GLP. In the definitive test all the validity criteria were met. nNo deviations were observed from the OECD 202 during the study. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. EC_{50}/48 h values is > 100 mg product /L NOEC= > 100 mg product/L and LOEC= > 100 mg product/L

Reference:	KCP 10.2/01
Report	CHR/H/PENDIF 599.5 SC Daphnia magna, Acute Immobilisation Test.; E. Malada, 2021, Study code: W-44-20, 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/PENDIF 599.5 SC; batch no. 042020, content of penoxulam is 38.7 g/L, diflufenican is 259.0 g/L and flufenacet is 318.8 g/L; production date: April 01, 2020, expiry date: April 01, 2022.
Test organism:	*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:	Static test (48 h of exposure); 4 replicates per test item concentration and the control; 5 Daphnia magna in each replicate.
Nominal test item concentrations:	100 mg/L plus the control.
Test conditions:	Temperature: 20.6 – 21.1°C; pH of the control: 7.26 – 7.42; dissolved oxygen concentration in the control: 8.6 – 8.8 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.
Chemical determinations:	The penoxsulam, flufenacet and diflufenican concentration was determined with a validated liquid chromatographic method with DAD detection.
Endpoint values:	EC50/48 h, LOEC/48 h and NOEC/48 h

Results and discussion:

Study conditions

Table 1. pH values and dissolved oxygen concentrations, definitive test

Nominal test item concentration [mg/L]	pH values		Dissolved oxygen concentrations [mg/L]	
	at exposure initiation#	at exposure termination*	at exposure initiation#	at exposure termination*
Control	7.42	7.26	8.8	8.6
100	7.34	7.21	8.5	7.9

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates

Immobilisation of Daphnia magna exposed to the test item, CHR/H/PENDIF 599.5 SC, was investigated during a 48-hour static test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of the test item concentration or the control per replicate. The definitive test was performed with a single test item concentration of 100 mg/L plus the control as a limit test.
The Daphnia magna were observed for immobilisation after 24 h 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 test item concentration of 100 mg/L after 48 h immobilisation of Daphnia magna was 5%. No immobilisation of Daphnia magna was observed during exposure in the control.

Table 2. Immobilisation of Daphnia magna, definitive test

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
100	20	0	0	0	0	0	1	0	0	0	5

Time of exposure: 23.02.2021 – 25.02.2021

Analytical measurements:

The concentrations of penoxsulam, flufenacet and diflufenican were determined using a validated liquid chromatographic method with DAD detection. Samples of the test item concentration and the control were analysed at exposure initiation and at exposure termination.

Table 3. Concentration and stability of penoxulam, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of penoxulam in the test item [mg/L]	Average determined concentration of penoxulam (n=3) in samples collected [mg/L]			
		at exposure initiation	[%] of nominal concentration	at exposure termination	[%] of nominal concentration
Control	---	<LoD	---	<LoD	---
100	3.161	2.744	86.8	3.001	94.9

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
--- no value

Table 4. Concentration and stability of flufenacet, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of flufenacet in the test item [mg/L]	Average determined concentration of flufenacet (n=3) in samples collected [mg/L]			
		at exposure initiation	[%] of nominal concentration	at exposure termination	[%] of nominal concentration
Control	---	<LoD	---	<LoD	---
100	26.039	22.946	88.1	24.406	93.7

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
--- no value

Table 5. Concentration and stability of diflufenican, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of diflufenican in the test item [mg/L]	Average determined concentration of diflufenican (n=3) in samples collected [mg/L]			
		at exposure initiation	[%] of nominal concentration	at exposure termination	[%] of nominal concentration
Control	---	<LoD	---	<LoD	---
100	21.155	19.554	92.4	19.118	90.4

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
--- no value

In samples at exposure initiation the determined concentration of penoxulam was 86.8% and the determined concentration of flufenacet was 88.1%, and the determined concentration of diflufenican was 92.4%. The results confirm that the test item concentration was prepared correctly.
In sample at exposure termination the determined concentration of penoxulam was 94.9% and the determined concentration of flufenacet was 93.7%, and the determined concentration of diflufenican was 90.4%. Therefore, the concentration of penoxsulam, flufenacet and diflufenican was stable under test conditions.
The endpoint values were determined based on nominal test item concentration.

Results:
The endpoint values based on nominal test item concentration are given below:
The EC_{50}/48 h is higher than 100 mg/L.
The LOEC/48 h is higher than 100 mg/L
The NOEC/48 h is higher than 100 mg/L

TEST VALIDITY CRITERIA
In the definitive test the validity criteria were met according to OECD Guideline No. 202 (2004):
– 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 7.9 – 8.8 mg/L (criterion: not less than 3 mg/L).
- pH changes during 48h not more than 1.5 units during the test

A 2.2.1.1.2 Pseudokirchneriella subcapitata

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted during the study. In the definitive test all the validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason end-points are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. Following endpoints are relevant for risk assessment purposes: The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata:* The ErC_{50}/72 h value is 3.267 µg/L (95% confidence interval: 2.634 – 4.095) The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata:* The EyC_{50}/72 h value is 0.636 µg/L (95% confidence interval: 0.493 – 0.820).

Reference:	KCP 10.2/02
Report	CHR/H/PENDIF 599.5 SC *Raphidocelis subcapitata* SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test.; M. Czarnecka,

	2021, Study code: W-45-20, 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. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: CHR/H/PENDIF 599.5 SC; batch no. 042020, content of penoxsulam: 38.7 g/L, diflufenican: 259.0 g/L, flufenacet: 318.8 g/L; production date: April 01, 2020, expiry date: April 01, 2022.

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 104 cells/mL.

Nominal test item concentrations:
15, 5.0, 1.67, 0.56, 0.19, 0.062 µg/L plus the control.

Test conditions: Temperature: 23.0 – 23.6°C; pH of the control: 7.49 – 8.01; mean light intensity: 6402 - 6545 lux; constant illumination and shaking; medium: AAP.

Chemical determinations:
The concentrations of penoxsulam, flufenacet, and diflufenican were determined using the validated high performance liquid chromatographic method with MS/MS detection.

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, Multiple Sequentially-rejective Welsh-t-test after Bonferroni- Holm.

Endpoint values: ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results and discussion:

The growth of the green algae Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) exposed to the test item, CHR/H/PENDIF 599.5 SC 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 algae was 1 x 104 cells/mL. The definitive test was performed with the following test item concentrations: 15, 5.0, 1.67, 0.56, 0.19, 0.062 µg/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 0.062 to 15 µg/L after 72 h of exposure was in the range of 2.4 – 71.8% when compared to the control. Inhibition of yield for the test item concentrations ranging from 0.062 to 15 µg/L after 72 h of exposure was in the range of 10.3 – 97.3% when compared to the control.
In all test item concentrations, no differences in shape, size and colour of algal cells were reported as compared to the algal cells in the control.

Analytical measurements

The concentrations of penoxsulam, flufenacet, and diflufenican were chemically determined using a validated high performance liquid chromatographic method with MS/MS detection (LC- MS/MS). Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of penoxsulam were in the range of 98.8 – 101.1% of nominal concentration, the determined concentrations of flufenacet were in the range of 85.5 – 98.1% of the nominal concentration, the determined concentrations of diflufenican were in the range of 94.9 – 102.0% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of penoxsulam were in the range of 86.2 – 101.3% of nominal concentration, the determined concentrations of flufenacet were in the range of 85.3 – 100.4%, the determined concentrations of diflufenican were in the range of 95.4 – 105.9% of the nominal concentration. Therefore, the concentrations of flufenacet, diflufenican, and penoxsulam were stable under test conditions.
The endpoint values were determined based on the nominal test item concentrations.

Results:
The endpoint values based on nominal test item concentrations:
The ErC50/72 h value is 3.267 µg/L (95% confidence interval: 2.634 – 4.095).
The LOEC/72 h value for growth rate is 0.56 µg/L.
The NOEC/72 h value for growth rate is 0.19 µg/L.
The EyC50/72 h value is 0.636 µg/L (95% confidence interval: 0.493 – 0.820).
The LOEC/72 h value for yield is lower than or equal to 0.062 µg/L.
The NOEC/72 h value for yield is lower than 0.062 µg/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 109.2 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 1.5% (criterion: it must not exceed 7%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 9.8% (criterion: it must not exceed 35%).

A 2.2.1.1.3 Anabaena flos-aquae

Comments of zRMS:	The study was conducted according to OECD guideline 201 and to the principles of GLP. No deviations were noted during the study. In the definitive test all the validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. Following endpoints are relevant for risk assessment purposes: The concentration causing a 50% inhibition of the growth rate of *Anabaena flos-aquae:* ErC_{50}/72 h= 11.07 mg formulation/L nom The concentration causing a 50% inhibition of yield of *Anabaena flos-aquae*:

	EyC_{50}/72 h = 2.01 mg formulation/L nom

Reference:	KCP 10.2/03
Report	CHR/H/PENDIF 599.5 SC Anabaena flos-aquae UTEX B 1444 Growth inhibition test.; M. Czarnecka, 2021, Study code: W-47-20, 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/PENDIF 599.5 SC; batch no. 042020, content of penoxsulam: 38.7 g/L, diflufenican: 259.0 g/L, flufenacet: 318.8 g/L; production date: April 01, 2020, expiry date: April 01, 2022.

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, Eco-toxicology 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 104 cells/mL.

Nominal test item concentrations:
20, 6.25, 1.95, 0.61, and 0.19 mg/L plus the control.
Test conditions: Temperature: 22.8 – 23.5°C; pH of the control: 7.50 – 8.07;
mean light intensity: 3595 - 3698 lux; constant illumination and shaking; medium: AAP.

Chemical determinations:
The concentrations of flufenacet, diflufenican and penoxsulam were determined using the validated high performance liquid chromatographic method with MS/MS detection.
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-Terpsta Test Procedure.

Endpoint values: ErC50/72 h, EyC50/72 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/PENDIF 599.5 SC 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: 20, 6.25, 1.95, 0.61, and 0.19 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 all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control. The concentrations of flufenacet, diflufenican, and penoxsulam were chemically determined using a validated high performance liquid chromatographic method with MS/MS detection. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of flufenacet were in the range of 94.9 – 101.3% of the nominal concentration, the determined concentrations of diflufenican were in the range of 101.3 – 108.5% of the nominal concentration, the determined concentrations of penoxsulam were in the range of 98.0 – 101.6% of nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of flufenacet were in the range of 88.2 – 96.0%, the determined concentrations of diflufenican were in the range of 90.7 – 95.6% of the nominal concentration, the determined concentrations of penoxsulam were in the range of 91.1 – 102.1% of nominal concentration. Therefore, the concentrations of flufenacet, diflufenican, and penoxsulam were stable under testconditions. The endpoint values were determined based on nominal test item concentrations.

The endpoint values based on the nominal test item concentrations are given below:
The ErC50/72 h value is 11.07 mg/L (95% confidence interval: 8.42 – 15.57).
The LOEC/72 h value for growth rate is 1.95 mg/L.
The NOEC/72 h value for growth rate is 0.61 mg/L.
The EyC50/72 h value is 2.01 mg/L (95% confidence interval: 1.37 – 2.94).
The LOEC/72 h value for yield is 1.95 mg/L.
The NOEC/72 h value for yield is 0.61 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 56.2 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 4.0% (criterion: it must not exceed 10%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 32.1% (criterion: it must not exceed 35%).

A 2.2.1.1.4 Lemna Gibba

Comments of zRMS:	Growth inhibition test was conducted according to OECD guideline 221 and to the principles of GLP. No deviations were noted during the study. In the definitive test all the validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. Following endpoints are relevant for risk assessment purposes: based on the <u>frond number</u> of *Lemna gibba* ErC_{50}/7 d value is 0.056 mg/L (95% confidence interval 0.045 – 0.069) EyC_{50}/7 d value is 0.019 mg/L (95% confidence interval 0.015 – 0.024) based on the <u>dry weight</u> of Lemna gibba ErC_{50}/7 d = 0.162 mg/L (95% confidence interval 0.119 – 0.235) EyC50/7 d value is 0.024 mg/L (95% confidence interval 0.018 – 0.031).

Reference:	KCP 10.2/04
Report	CHR/H/PENDIF 599.5 SC *Lemna gibba* CPCC 310, Growth inhibition test.; M. Czarnecka, 2021, Study code: W-46-20, 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/PENDIF 599.5 SC; batch no. 042020, content of penoxsulam: 38.7 g/L, diflufenican: 259.0 g/L, flufenacet: 318.8 g/L; production date: April 01, 2020, expiry date: April 01, 2022.

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 with daily renewals (7 days of exposure); three replicates for each test item concentration and six replicates for the control.

Nominal test item concentrations:
0.4, 0.13, 0.04, 0.012, 0.004, 0.001 mg/L plus control

Test conditions: Temperature: 22.9 – 23.2ºC; pH of the control: 7.45 – 8.90; light intensity: 6894 – 7513 lux; constant illumination; test vessels: glass crystallizers containing 150 mL of each treatment; initial frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP.

Chemical determinations:
The concentrations of flufenacet, diflufenican and penoxsulam were determined using the validated high performance liquid chromatographic method with MS/MS detection.

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

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/PENDIF 599.5 SC, was investigated in a 7 day semi-static test with daily renewals. The test was performed in glass crystallizers containing 150 mL of either the 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: 0.4, 0.13, 0.04, 0.012, 0.004, and 0.001 mg/L plus the control. The total number of fronds in each test vessel was counted twice during exposure (day 3 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 of 0.001, 0.004, and 0.012 mg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of 0.04 mg/L overlapping colonies, spots of chlorosis and discoloration of fronds were observed. In the test item concentrations of 0.13 and 0.4 mg/L bending down of colonies and spots of chlorosis were observed. The concentrations of flufenacet, diflufenican, and penoxsulam were

chemically determined using a validated high performance liquid chromatographic method with MS/MS detection. Samples of all fresh test item concentrations and the control collected at exposure initiation and all spent test item concentrations and the control collected at the first renewal were chemically determined. Moreover, fresh and spent samples of the highest (0.4 mg/L) and the lowest test item concentration (0.001 mg/L) and the control at each renewal and at exposure termination were chemically analyzed. In fresh samples at exposure initiation and at renewals, the determined concentrations of flufenacet were in the range of 97.6 – 107.5% of the nominal concentration, the determined concentrations of diflufenican were in the range of 92.3 – 107.8% of the nominal concentration, and the determined concentrations of penoxsulam were in the range of 93.4 – 109.8% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. In spent samples at renewals and at exposure termination, the determined concentrations of flufenacet were in the range of 98.5 – 108.7% of the nominal concentration, the determined concentrations of diflufenican were in the range of 93.8 – 109.2% of the nominal concentration, the determined concentrations of penoxsulam were in the range of 91.9 – 106.3% of the nominal concentration. Therefore, the concentrations of flufenacet, diflufenican, and penoxsulam were stable under test conditions. The endpoint values were determined based on the nominal test item concentrations.

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:
The ErC50/7 d value is 0.056 mg/L (95% confidence interval 0.045 – 0.069).
The ErC20/7 d value is 0.013 mg/L (95% confidence interval 0.009 – 0.017).
The ErC10/7 d value is 0.006 mg/L (95% confidence interval 0.004 – 0.009).
For growth rate, the NOEC/7 d value is 0.004 mg/L, whereas LOEC/7 d value is 0.012 mg/L.

The EyC50/7 d value is 0.019 mg/L (95% confidence interval 0.015 – 0.024).
The EyC20/7 d value is 0.004 mg/L (95% confidence interval 0.002 – 0.005).
The EyC10/7 d value is 0.002 mg/L (95% confidence interval 0.001 – 0.003).
For yield, the NOEC/7 d value is 0.004 mg/L, whereas LOEC/7 d value is 0.012 mg/L.

Endpoints based on the dry weight:
The ErC50/7 d value is 0.162 mg/L (95% confidence interval 0.119 – 0.235).
The ErC20/7 d value is 0.010 mg/L (95% confidence interval 0.006 – 0.015).
The ErC10/7 d value is 0.002 mg/L (95% confidence interval 0.001 – 0.004).

For growth rate NOEC/7 d value is lower than 0.001 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.001 mg/L.
The EyC50/7 d value is 0.024 mg/L (95% confidence interval 0.018 – 0.031).
The EyC20/7 d value is 0.002 mg/L (95% confidence interval 0.001 – 0.003).
EyC10/7 d value is lower than 0.001 mg/L.
For yield, the NOEC/7 d value is lower than 0.001 mg//L, whereas the LOEC/7 d value is lower than or equal to 0.001 mg/L.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1.1 KCP 10.3.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment.

	Overall, the study is considered acceptable with following endpoints: 48 h LD_{50} > 200.0 µg/honeybee

Reference:	KCP 10.3.1/01
Report	CHR/H/PENDIF 599.5 SC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test.; P. Holewik, 2021, Study code: B-63-20, 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/PENDIF 599,5 SC content: 318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam batch no.: 042020 production date: 01.04.2020 expiry date: 01.04.2022
Biological test system:	the honeybee, *Apis mellifera* L., strain: carnica
– age:	approximately 3 weeks
– source:	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:	24 - 26°C
– relative air humidity:	63 - 65%
Place:	Dark room
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 and LD_{50}/48 h of the test item,

– the oral LD_{50}/24 h of the reference item (dimethoate).

Results and discussion

The acute oral toxicity study of CHR/H/PENDIF 599,5 SC was conducted in order to determine the LD_{50}. 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 a 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 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 oral toxicity test finished after the 48-hour observation. The acute oral toxicity study of the test item, CHR/H/PENDIF 599,5 SC 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.]	[%]	[%][c]	
0.0 (Control)	30	1	3.3	-	> 200.0*
12.50	30	0	0.0	-3.5**	
25.00	30	0	0.0	-3.5**	
50.00	30	2	6.7	3.5	
100.0	30	2	6.7	3.5	
200.0	30	2	6.7	3.5	

[c]: mortality corrected according formula of Abbott's [7]

*: oral LD_{50} value (with 95% confidence limits) was estimated with the probit method (ToxRat Proffesional 3.3.0 computer software), [9], [SOP/B/67]

**: the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item

Conclusions:
The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than 200.0 µg/honeybee.

TEST VALIDITY CRITERIA
The following validity criteria were met during the test:
- the mortality for the control was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- the LD_{50}/24 h of the reference item (dimethoate) was 0.16 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilisation. The mentioned deviation had not effect on the results of the stud In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment.

	Overall, the study is considered acceptable with following endpoints: 48 h LD_{50} > 200.0 µg/honeybee

Reference:	KCP 10.3.1/02
Report	CHR/H/PENDIF 599.5 SC Honeybees (Apis mellifera L.), Acute Contact Tox-icity Test.; P. Holewik, 2021, Study code: B-64-20, 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/PENDIF 599,5 SC content: 318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam batch no.: 042020 production date: 01.04.2020 expiry date: 01.04.2022
Biological test system:	the honeybee, *Apis mellifera* L., strain: carnica
– age:	approximately 3 weeks
– source:	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 one 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
Test conditions:	
– temperature:	24.0 - 25.0°C
– relative air humidity:	63 - 65%
16 hours light : 8 hours dark	
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 LD_{50}/24 h of the reference item (dimethoate).

Results and discussion

Mortality of honeybees, Apis mellifera, exposed to CHR/H/PENDIF 599,5 SC 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/PENDIF 599,5 SC 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.]	[%]	[%] Corr[a]	
0.0 (control)	30	1	3.3	–	> 200.0
12.5	30	0	0.0	-3.5*	
25.0	30	0	0.0	-3.5*	
50.0	30	2	6.7	3.5	
100.0	30	0	0.0	-3.5*	
200.0	30	1	3.3	0.0	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

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 3.3% after 48 h (criterion: it must not exceed 10.0%),
- the LD_{50}/24 h of the reference item (dimethoate) was 0.28 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 was conducted to OECD guideline 245 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: 48 h LD_{50} > 666.7 µg/honeybee LDD_{50} >17.7 µg/honeybee/day

Reference:	KCP 10.3.1/03
Report	CHR/H/PENDIF 599.5 SC Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test.; P. Holewik, 2021, Study code: B-62-20, 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. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	CHR/H/PENDIF 599,5 SC batch number: 042020 content: 318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam production date: 01.04.2020 expiry date: 01.04.2022
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:	666.7 mg/kg
Nominal dose of the test item:	20.0 µg/bee/day
Test item dietary dose:	17.7 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:	0.013 µg/bee/day
Test conditions:	temperature: 31.9 – 34.8ºC; relative humidity: 51.0 – 69.5%;
Statistical analysis:	Fisher's Exact Binomial Test

Endpoints: honeybee mortality after 10 days of exposure

Aim of the study

The aims of the study were to determine the chronic oral toxicity of the test item, CHR/H/PENDIF 599,5 SC to honeybees (*Apis mellifera* L.) and to demonstrate that the median lethal concentration, i.e. the LC50 and median lethal dietary dose, i.e. LDD_{50} are higher than the test item concentration used for exposure (limit test)

Summary

The mortality of honeybees exposed to CHR/H/PENDIF 599,5 SC 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 17.7 µg/bee/day (dietary dose). Daily dose was calculated on the basis of average consumption of a treated 50% sucrose solution 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

The insects were observed for mortality and behavioural abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure.

Analytical determination

The concentration of penoxulam, flufenacet and diflufenican were chemically determined using the validated high performance liquid chromatographic method with DAD detection. 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 penoxulam was 81.8% of nominal concentration, the determined concentration of flufenacet was 84.3% of nominal concentration and the determined concentration of diflufenican was 84.0% of nominal concentration. The results 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 penoxulam was 83.7% of nominal concentration, the determined concentration of flufenacet was 88.9% of nominal concentration, the determined concentration of diflufenican was 87.3% of nominal concentration. Based on the results of chemical analyses, the concentrations of penoxulam, flufenacet and diflufenican were stable under storage conditions

Results and discussion

The validity criterion concerning mortality was met, because mortality in the control was 2.0% 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 17.7 µg/bee/day) at exposure termination (after 10 days), corrected according to the Abbott formula [8], was 2.0%.

On the basis of the obtained mortality results the LC_{50} is higher than 666.7 mg/kg, and the LDD_{50} value is higher than 17.7 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/PENDIF 599,5 SC on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested[a] dose [µg/bee/day]	Number of tested bees [no]	Total mortality			LC_{50} [mg/kg]	LDD_{50} [µg/bee/day]
[µg/30 mg/day] [µg/bee/day]	[mg/kg]			No.	[%]	[%][c]		
CHR/H/PENDIF 599,5 SC								
0.0 (Control)			50	1	**2.0**	-	**> 666.7**	**> 17.7**
20.0	**666.7**	**17.7**	50	2	**4.0**	**2.0**		
Dimethoate (reference item)								
0.024	**0.8**	**0.013**	30	19	**100.0**	-	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and average sucrose solution consumption
[c]: mortality corrected according formula of Abbott [8]

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 2.0% (criterion: it must not exceed 15%) [1].

- After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.013 µg/bee/day) was 100.0% (criterion: it must be ≥ 50% on day 10 of exposure).

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

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 follows the guideline specified by Blümel et al. (2000) and according to the principles of GLP. 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. Since n the definitive test all the validity criteria were met it didn't impact the results of the study. Considering the current test guideline (Blümel et al., 2000) the study is considered valid. LR_{50} = 0.128 L formulation//ha $ER_{50=}$ 0.067 L formulation/ha

Reference:	KCP 10.3.1/04
Report	An extended laboratory test for evaluating the effects of CHR/H/PENDIF 599,5 SC on the predatory mite, Typhlodromus pyri (Sch.)..; P. Holewik, 2021, Study code: B-59-20, 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/PENDIF 599.5 SC
Active substance:		318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam
Batch number:		042020
Manufacture date:		01.04.2020
Expiry date:		01.04.2022
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:		6 study groups: - a control group (0.0 L/ha) - 0.007 L/ha - 0.022 L/ha - 0.067 L/ha - 0.200 L/ha - reference item: Bi 58 Top 400 EC at the rate of 9.0 mL/ha - number of replicates: 3 number of mites in each replicate: 20
Test conditions:		
– temperature:		23 – 26°C
– relative air humidity:		60 – 80%
– photoperiod:		16 h light : 8 h dark
– light intensity:		646 lux
Statistical analysis:		Logit analysis using max. likelihood regression, Probit analysis using linear weighted regression Step-down Cochran-Armitage Test Procedure, Shapiro Wilk's Test on Normal Distribution, Levene's Test on Variance Homogenity (with Residuals), Williams Multiple Sequential t-test Procedure, Chi2 2x2 Table Test with Bonferroni Correction

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 $NOER_{reproduction}$

Summary

The aim of the laboratory test was to evaluate the effects of the test item, CHR/H/PENDIF 599,5 SC on mortality and reproduction of the predatory mite, T. pyri (Sch.). On the basis of the preliminary tests results, it was decided to use four rates of the test item in the definitive test. These were 0.007, 0.022, 0.067 and 0.200 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 0.007, 0.022 and 0.067 L/ha were made after 8, 11, and 14 days of the treatment. Mortality of T. pyri after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints. To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Bi 58 Top 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Plant material

An extended laboratory test is conducted on the natural substrate. As the plant material the discs of a 45 mm diameter, cut off from rose (Rosa Sp.), leaves were used. The leaf discs were prepared for each study group on the day of application and kept individually on pads of damp tissue-paper in Petri dishes for applications.

Results and discussion

In the definitive test, mortality of the control group after 7 days of exposure was 1.7%. After 7 days of exposure to CHR/H/PENDIF 599,5 SC at rates of 0.007, 0.022, 0.067 and 0.200 L/ha, the percentages of *T. pyri*, mortality, corrected according to the Abbott formula, were (-1.7), 18.6, 33.9 and 59.3%, respectively.
There were no statistically significant differences in mortality between group treated with the test item at the rate of 0.007 L/ha and the control group. There were statistically significant differences in mortality between group treated with the test item at the rates of 0.022, 0.067 and 0.200 L/ha and the control group (Step-down Cochran-Armitage Test Procedure, $p(trend)>\alpha$).
The LR50 value is equal to 0.128 L/ha of CHR/H/PENDIF 599,5 SC (95% confidence limits: 0.094 – 0.195). NOERmortality is 0.007 L/ha of CHR/H/PENDIF 599,5 SC.
After 7 days of exposure to Bi 58 Top 400 EC at the rate of 9.0 mL/ha mortality, corrected according to the Abbott formula, was 76.3%. 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 the groups treated with CHR/H/PENDIF 599,5 SC at the rates of 0.007, 0.022 and 0.067 L/ha, was assessed since mortality of these groups was < 50.0%.

The mean reproduction rate (Rr) in the control group was 7.3 eggs/female. The mean Rr after 14 days of exposure to CHR/H/PENDIF 599,5 SC at the rates of 0.007, 0.022 and 0.067 L/ha were 5.5, 4.6 and 4.2 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.007, 0.022 and 0.067 L/ha were 25.8, 37.1 and 42.3%, respectively.
There were statistically significant differences in reproduction between group treated with the test item at the rates of 0.007, 0.022 and 0.067 L/ha and the control group (Williams Multiple Sequential t-test Procedure,|t|>|t*|).
On the basis of the obtained reproduction results, it could be assumend that the ER50 value is higher than 0.067 L/ha of CHR/H/PENDIF 599,5 SC. NOERreproduction is lower than 0.007 L/ha of CHR/H/PENDIF 599,5 SC.

The effects of CHR/H/PENDIF 599,5 SC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized in the table.

Test item rate [L/ha]	Parameter (endpoint)						
	Mortality			Reproduction			
	Total [%]	Total [%]*	LR_{50} [L/ha]	Test item rate [L/ha]	Mean number of eggs/ female (Rr) [no.]	Repro-duction reduction Pr [%]	ER_{50} [L/ha]
Control (0.0)	1.7	-	0.128 *(0.094 – 0.195)**	Control (0.0)	7.3	–	> 0.067
0.007	0.0	-1.7**		0.007*	5.5	25.8	
0.022*	20.0	18.6		0.022*	4.6	37.1	
0.067*	35.0	33.9		0.067*	4.2	42.3	
0.200*	60.0	59.3					
$NOER_{mortality}$ 0.007 [L/ha]				$NOER_{reproduction}$ < 0.007 [L/ha]			
Reference item: Bi 58 Top 400 EC							
Reference item [mL/ha]				9.0			
Mortality							
Total [%]*				76.3			

*: mortality corrected according formula of Abbott [1]
*: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]
*: 95%-confidence limits
**: the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item

Conclusions:
Based on the results it can be stated that CHR/H/PENDIF 599,5 SC, at the rates of 0.007 L/ha has no significant adverse effect on mortality of the mites. Based on the results it can be stated that CHR/H/PENDIF 599,5 SC, at the rates of 0.022, 0.067 and 0.200 L/ha has significant adverse effect on mortality of the mites. Based on the results it can be stated that CHR/H/PENDIF 599,5 SC, at the rates of 0.007, 0.022 and 0.067 L/ha has significant adverse effect on reproduction of the mites.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [3]:

- mortality of the control group was 1.7% on day 7 of exposure (criterion: a maximum of 20%),

- mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha, after correction, was 76.3% on day 7 of exposure (criterion: from 50 to 100%),

- the mean number of eggs per female in the control group was 7.3 (required: ≥ 4 eggs per female).

A 2.3.1.4.2 Aphidius rhopalosiphi

Comments of zRMS:	The study follows the guideline specified by Mead Briggs M.A. et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met Considering the current test guideline (Mead Briggs M.A. et al, 2000) the study is considered valid. LR_{50} > 0.4 L formulation /ha which is equicalent to > 489.72 g formulation/ha

Reference:	KCP 10.3.1/05
Report	An extended laboratory test for evaluating the effects of CHR/H/PENDIF 599,5 SC on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez)P. Holewik, 2021, Study code: B-60-20, Łukasiewicz Re-search Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Ecotoxico-logical Studies, Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	Mead-Briggs et al. 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Name:	CHR/H/ PENDIF 599,5 SC
Active substance:		318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam
Batch number:		042020
Manufacture date:		01.04.2020
Expiry date:		01.04.2022
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:	5 study groups: □ a control group (0.0 L/ha) – 0.04 L/ha – 0.13 L/ha – 0.4 L/ha □ Reference item: Bi 58 Top 400 EC at the rate of 5.0 mL/ha mortality assessment: 6 replicates/group; 5 females/replicate fecundity assessment: 15 replicates/group; 1 females/replicate
Test conditions:	
– temperature:	18 – 21°C
– relative air humidity:	63 –72%
– photoperiod:	16 hours light : 8 hours dark
– light intensity:	mortality and oviposition assessment: 2203 lx fecundity phase: 5033 lx
Statistical analyses:	– 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 Homogeneity, – Williams Multiple Sequential t-test Procedure.
Endpoints:	– wasp mortality after 48 hours of exposure, – determination of the LR_{50} and the $NOER_{mortality}$, – determination of the ER_{50} and the $NOER_{fecundity}$. – reduction in fecundity (Pr) of the surviving female wasps exposed to CHR/H/PENDIF 599,5 SC, 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/PENDIF 599,5 SC on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi*. On the basis of the results of the preliminary test, it was decided to use three rates of the test item in the definitive test. These were 0.04, 0.13 and 0.4 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/PENDIF 599,5 SC 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 CHR/H/PENDIF 599,5 SC and the ones from the control group were subjected to fecundity assessments. Fifteen female wasps from the four 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, Bi 58 Top 400 EC (400 g dimethoate/L), which is an insecticide, was used as a reference item. The rate of the reference item was 5.0 mL/ha (2.0 g dimethoate/ha). The control group was treated with distilled water.

In the definitive test, after 48 hours mortality of the control wasps was 3.3%. The corrected mortality, in the groups treated with CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 L/ha were -3.5, -3.5 (the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item) and 0.0%, respectively.
At the significance level of 0.05, there were no statistically significant differences in mortality between the wasps exposed to the test item at the rates of 0.04, 0.13 and 0.4 L/ha and the control group (Chi2 2x2 Table Test with Bonferroni Correction, $p>0.05$).
Based on the obtained results the LR50 value could not be estimated. It could be assumend that LR50 is higher than 0.4 L/ha. The NOERmortality is higher than or equal to 0.4 L/ha.
The corrected mortality of the wasps exposed to Bi 58 Top 400 EC at the rate of 5.0 mL/ha was 75.9% 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 16.4 (after 12 days after oviposition). As for the wasps treated with CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 L/ha the mean number of mummies per female were 14.9, 13.7 and 10.9, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.04, 0.13 and 0.4 L/ha were 8.9, 16.3 and 33.7%, respectively.
At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the rate of 0.4 L/ha and the control group (Williams Multiple Sequential t-test Procedure, $p>0.05$).
Based on the obtained fecundity results it could be assumed that the ER50 value is higher than 0.4 L/ha and the NOERfecundity is equal to 0.13 L/ha of the test item.

The effects of the test item, CHR/H/PENDIF 599,5 SC on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Parametr (endpoint)							
Mortality				**Fecundity**			
Test item [L/ha]	**Total [%]**	**Total [%][a]**	**LR_{50} [L/ha]**	**Test item [L/ha]**	**Mean no. of mummies/ female**	**Fecundity reduction Pr [%]**	**ER_{50} [L/ha]**
Control	3.3	-	**>0.4**	**Control**	16.4	–	**>0.4**
0.04	0.0	-3.5		**0.04**	14.9	8.9	
0.13	0.0	-3.5		**0.13**	13.7	16.3	
0.4	3.3	0.0		**0.4[+]**	10.9	33.7	
$NOER_{mortality}$ ≥ 0.4 [L/ha]				**$NOER_{fecundity}$ = 0.13 [L/ha]**			
Reference item: Bi 58 Top 400 EC							
Reference item [mL/ha]	**5.0**						
Mortality (after 48 h)							
Total [%]	**75.9**						

[a]: mortality corrected according formula of Abbott [1]
[+]: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3],
**: the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item

Conclusion:
On the basis of the obtained mortality results it can be concluded that CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 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/PENDIF 599,5 SC at the rates of 0.04 and 0.13 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/PENDIF 599,5 SC at the rate of 0.4 L/ha has an adverse effect on the fecundity of the wasps.

TEST VALIDITY CRITERIA
The following validity criteria were met during the study [5]:
– after 48 hours, mortality of the control group was 3.3% (criterion: a maximum of 10.0%),
– after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 75.9% (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 16.4 (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 follows the guideline specified by Vogt et al. and according to the principles of GLP. 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. Since the definitive test all the validity criteria were met. The study is considered valid. $NOER_{mortality}$ is higher than or equal to 0.4 L/ha not 0.04 as is mentioned in the table with the results. Please, update the table with correct values.

Reference:	KCP 10.3.1/06
Report	An extended laboratory test for evaluating effects of CHR/H/PENDIF 599,5 SC on the green lacewing, Chrysoperla carnea (Steph.), P. Holewik, 2021, Study code: B-02-21, 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 developed 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/PENDIF 599.5 SC content: 318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam batch no.: 042020 production date: 01.04.2020 expiry date: 01.04.2022
Biological test system:	the green lacewing, *Chrysoperla carnea* (Steph.), Neuroptera: *Chrysopidae*
– age:	first instars' larvae (3 days old)
– source:	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/PENDIF 599,5 SC at the rates of - 0.04 L/ha - 0.13 L/ha - 0.4 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:	23.0 - 25.9°C
– relative air humidity:	60.1 - 89.8%
– photoperiod:	16 hours light : 8 hours dark
– light intensity	3011 lux
Statistical analysis:	Logit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure
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/PENDIF 599,5 SC on mortality and reproductive capacity of the green lacewing, Chrysoperla carnea. In a definitive test, three test item application rates of 0.04, 0.13, and 0.4 L/ha were used. 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 during 6 days. 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. The effects of the test item, CHR/H/PENDIF 599,5 SC on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Study group [application rate]	Parameter (endpoints)				
	Mortality			Reproduction	
Test item [L/ha]	[%]	[%][a]	LR_{50} [L/ha]	Mean number of eggs/female /day [no.]	Mean hatching rate [%]
Control (0.0)	6.7	-	> 0.4	18.7	89.0
0.04	6.7	0.0		17.6	77.6
0.13	10.0	3.6		26.1	78.2
0.4	3.3	-3.6*		15.0	82.2
$NOER_{mortality}$	≥ 0.04 [L/ha]				
Reference item [g/ha]	Dimethoate				
15.0	70.0	67.9	-		

*: mortality was corrected according Abbott's equation [1]
*: the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item

Conclusion:
The validity criterion concerning mortality was met, because mortality of the green lacewings, Chrysoperla carnea (Steph.) in the control group was 6.7%. The corrected mortality of the green lacewings exposed to the test item at the rates of 0.04, 0.13 and 0.4 L/ha of CHR/H/PENDIF 599,5 SC was 0.0, 3.6 and (-3.6)%, (the negative value indicates that the mortality in the control group was higher than in the group exposed to the test item), respectively.
There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.04, 0.13 and 0.4 L/ha in comparison to the control group (Step-down Cochran-Armitage Test Procedure, p(trend)> α, (α=0.05)).
The LR_{50} value is higher than 0.4 L/ha. The $NOER_{mortality}$ value is higher than or equal to 0. 4 L/ha.
The percentage of mortality of Ch. carnea (Steph.) exposed to dimethoate at rate of 15.0 g/ha, after Abbott's corrections, was 67.9%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.
The mean number of fertile eggs/female/day in the control group was equal to 18.7 (criterion: ≥ 15.0). The mean numbers of fertile eggs/female/day in the groups treated with CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0. 4 L/ha were equal to 17.6, 26.1 and 15.0, respectively.
The mean hatching rate in the control group was 89.0% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of 0.04, 0.13 and 0.4 L/ha were 77.6, 78.2 and 82.2%, respectively.
Fecundity reduction (Pr) in the group treated with the test item at the rates 0.04, 0.13 and 0.4 L/ha were 12.8, 12.1 and 7.6%, respectively.
Based on the results it can be stated that CHR/H/PENDIF 599,5 SC has no an adverse effect on mortality of the tested organisms at the rates of 0.04, 0.13 and 0.4 L/ha. Based on the results, it can be presumed that CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 L/ha had no adverse effect on the reproductive performance of the lacewings..

TEST VALIDITY CRITERIA
The following validity criteria were met during the study [5]:
– pre-imaginal mortality of the control group was 6.7% (criterion: a maximum of 20.0%),
– mean mortality of the reference item group was 67.9% (criterion: a minimum of 50%),
– the mean number of eggs per female per day in the control group (fecundity) was 18.7 (criteri-

on: ≥ 15.0),
– the mean hatching rate in the control group (fertility) was 89.0 (criterion: ≥ 70%).

A 2.3.1.4.4 Coccinella Septempunctata

Comments of zRMS:	The study follows the guideline specified by. Schmuck et al. (2000) in Candolfi (2000) guidelines according to the principles of GLP. 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. Since the definitive test all the validity criteria were met. The study is considered valid. In the definitive test all the validity criteria were met. The study is considered valid.

Reference:	KCP 10.3.1/07
Report	An extended laboratory test for evaluating effects of CHR/H/PENDIF 599,5 SC on the ladybird beetle, Coccinella septempunctata (L.), P. Holewik, 2021, Study code: B-01-21, 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 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/PENDIF 599.5 SC content: 318.8 g/L of flufenacet 259.0 g/L of diflufenican 38.7 g/L of penoxulam batch no.: 042020 production date: 01.04.2020 expiry date: 01.04.2022
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 L/ha) □ CHR/H/PENDIF 599,5 SC at the rates of: - 0.04 L/ha - 0.13 L/ha - 0.4 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:	23.0 – 26.9°C
– relative air humidity:	60.9 □ 74.8%
– photoperiod:	16 hours light : 8 hours dark
– light intensity	3052 lx
Statistical analysis:	probit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure
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/PENDIF 599,5 SC on mortality and reproductive capacity of the ladybird beetle, Coccinella septempunctata. In a definitive test, three test item application rates of 0.04, 0.13 and 0.4 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, pupae, and adults which died during emergence. After completion of mortality assessment, healthy hatched beetles from the control group and from group treated with the test item at the rates of application rates of 0.04, 0.13 and 0.4 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 over a period of 14 days. 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. The effects of the test item, CHR/H/PENDIF 599,5 SC on mortality and reproductive capacity of the ladybird beetle, Coccinella septempunctata L. in the laboratory test are summarized below.

Study group	Parameters (endpoints)					
	Mortality			Reproduction		
Test item [L/ha]	[%]	[%][a]	LR_{50} [L/ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]***
Control (0.0)	5.0	–	> 0.4	7.7	5.7	–
0.04	2.5	-2.6**		9.5	7.5	-31.6
0.13	0.0	-5.3**		9.7	8.1	-42.1
0.4	0.0	-5.3**		9.8	7.1	-24.6
$NOER_{mortality}$	≥ 0.4 [L/ha]					
dimethoate						
Reference item [g/ha]	100.0	100.0	–			
3.2						

*: mortality was corrected according Abbott's equation [1]
* - confidence limits
** - The negative values means that in the tested rates there were lower mortality than in the control group
*** - The negative values means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group

Conclusion:
The validity criterion concerning mortality was met, because mortality of the ladybird beetle, Coccinella septempunctata L. in the control group was equal to 5.0% (≤ 30.0%). The mortality of the ladybird beetles exposed to the test item at the rates of 0.04, 0.13 and 0.4 L/ha, after Abbott's correction, were -2.6, -5.3 and -5.3%, respectively. The negative values means that in the tested rates there were lower mortality than in the control group 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 0.04, 0.13 and 0.4 L/ha of CHR/H/PENDIF 599,5 SC and the control group (Step-down Cochran-Armitage Test Procedure, (α=0.05)). The LR50 value is above 0.4 L/ha of CHR/H/ PENDIF 599,5 SC. The NOERmortality is higher or equal to 0.4 L/ha of CHR/H/ PENDIF 599,5 SC. The mortality of the ladybird beetles exposed to the reference item at the rate of 3.2 g of dimethoate/ha, after Abbott's correction, 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 5.7 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 L/ha were equal to 7.5, 8.1 and 7.1 it refers to -31.6, -42.1 and -24.6% reproduction reduction. The negative values means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group. It can be concluded that CHR/H/PENDIF 599,5 SC at the rates of 0.04, 0.13 and 0.4 L/ha had no adverse effect on the reproduction capacity of the ladybird beetle.

TEST VALIDITY CRITERIA
The following validity criteria were met during the study [6]:
- pre-imaginal mortality of the control group was 5.0% (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 5.7 (criterion: ≥ 2 fertile eggs/female).%).

A 2.3.1.4.5 Aged Residue study

Comments of zRMS:	The study follows the guideline specified by Blümel S. et al., 2000 and according to the principles of GLP. No deviations to the guideline were noted. All the validity criteria were met.

Reference:	KCP 10.3.1/08
Report	CHR/H/PENDIF 599.5 SC – Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae), L. Fallowfield, 2021, Study code: CHR-21-07, Mambo-Tox A Division of Cawood Scientific Ltd., 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/PENDIF 599.5 SC
Formulation type = suspension concentrate (SC)
Sample identification = 220111704704
Batch number = 042020
Active substances = a) flufenacet b) diflufenican c) penoxulam
Nominal content of a.s. = a) 312.0 g/L b) 250 g/L c) 37.5 g/L
Measured content of a.s. = a) 318.8 g/L b) 259.0 g/L c) 38.7 g/L
Measured density = 1.2243 g/cm3
Appearance = white opaque liquid
Storage at Test Facility = ambient laboratory conditions
Sample expiry date = 01 April 2022

CHR/H/PENDIF 599.5 SC was evaluated at a single application rate, equivalent to 0.4 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 60 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 ring 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.
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 and discussion

The test item CHR/H/PENDIF 599.5 SC is a suspension concentrate formulation containing flufenacet (nominally 312.0 g/L), diflufenican (nominally 250.0 g/L) and penoxulam (nominally 37.5 g/L). The aim of this study was to determine the effects of both freshly-dried and field-aged residues of CHR/H/PENDIF 599.5 SC on the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae), in a series of extended laboratory tests. 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	-	13	-	10.4	-
	CHR/H/PENDIF 599.5 SC	0.4	49 *	41.4	5.8 *	44.0
	Toxic reference	-	100 *	100	~	-
14 DAT	Control	-	5	-	9.9	-
	CHR/H/PENDIF 599.5 SC	0.4	16 *	11.6	10.1	-2.2

a) For each bioassay, treatment mortalities were compared to the control using chi² 2x2 table test (α = 0.05, one-sided, > 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 by Student's t-test for homogenous variances (α = 0.05, one-sided, < control), a statistically significant effect is denoted by an asterisk (*).
d) Percentage change in numbers of eggs per female, relative to the respective control. A positive value indicates a decrease and a negative value indicates an increase.
~ indicates no assessments were made for this treatment.

Conclusions
The effects of freshly-dried and field-aged foliar residues of CHR/H/PENDIF 599.5 SC 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 0.4 L test item/ha, fresh-dried 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 control)..

TEST 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 was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD Guideline No. 222: The study is reliable and suitable for the risk assessment.

~~Reference:~~	~~KCP 10.4/01~~
~~Report~~	~~CHR/H/PENDIF 599.5 SC Earthworm reproduction test (Eisenia andrei); P. Pieczka, 2021, Study code: G-39-20, 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. 222 (2016)~~
~~Deviations:~~	~~No~~
~~GLP:~~	~~Yes~~
~~Acceptability:~~	~~Yes~~
~~Duplication (if vertebrate study)~~	~~No~~

~~**Materials and methods**~~

~~Test item:~~	~~**CHR/H/PENDIF 599.5 SC**~~ ~~batch no.: 042020~~
~~Active substances:~~	~~flufenacet – 318.8 g/L~~ ~~diflufenican – 259.0 g/L~~ ~~penoxulam – 38.7 g/L~~
~~Artificial soil:~~	~~10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand~~
~~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, 0.31, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0 mg/kg dry weight of the artificial soil~~
~~Test conditions:~~	~~temperature: 19.8 – 22.0°C;~~ ~~pH at the beginning of the experiment: 5.68 – 5.89;~~ ~~pH at the end of the experiment: 5.56 – 6.18;~~

	soil moisture content at the beginning of the experiment: 24.8 – 29.1% (47.6 – 55.8% of the maximum water holding capacity); soil moisture content at the end of the experiment: 25.4 – 29.6% (48.7 – 56.8% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 427 – 617 lux light intensity at the end of the experiment: 524 – 679 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/PENDIF 599,5 SC** on reproduction of the earthworm, *Eisenia andrei* and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. The concentrations of the test item were: 0.31, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0 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.

At concentrations ranging from 0.31 to 180.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 25.0%.
As for the control group, mortality of the adult earthworms was equal to 7.5%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 180.0 mg/kg dry weight of the artificial soil (above 46.87 mg of flufenacet + 38.08 mg of diflufenican + 5.69 mg of penoxulam/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 0.31 to 180.0 mg/kg dry weight of artificial soil, the body weight increase was between 3.3 and 12.4%. As for the control group, the body weight increase was equal to 5.1%.

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 0.31 to 180.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 95.3 and 285.8 per replicate. The mean number of juveniles in the control group was equal to 257.9 per replicate.

~~After 8 weeks of the experiment, it was concluded that **CHR/H/PENDIF 599,5 SC** had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 18.0 to 180.0 mg/kg dry weight of the artificial soil.~~
~~The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.~~

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of flufenacet/kg dry weight of artificial soil]	Value [mg of diflufenican/kg dry weight of artificial soil]	Value [mg of penoxulam/kg dry weight of artificial soil]
EC_{10}	10.77 (7.56 – 14.08)	2.80 (1.97 – 3.67)	2.28 (1.60 – 2.98)	0.34 (0.24 – 0.45)
EC_{20}	23.10 (18.28 – 27.81)	6.02 (4.76 – 7.24)	4.89 (3.87 – 5.88)	0.73 (0.58 – 0.88)
EC_{50}	99.37 (85.44 – 118.50)	25.88 (22.25 – 30.86)	21.02 (18.07 – 25.07)	3.14 (2.70 – 3.75)
NOEC (reproduction)	10.00	2.60	2.12	0.32
LOEC (reproduction)	18.00	4.69	3.81	0.57
LC_{50}	>180.00	>46.87	>38.08	>5.69
NOEC (survival)	≥180.00	≥46.87	≥38.08	≥5.69
LOEC (survival)	>180.00	>46.87	>38.08	>5.69

~~VALIDITY CRITERIA~~
~~The results are considered valid because the following criteria were satisfied in the controls:~~
~~- each replicate produced from 210 to 301 juveniles (257.9 mean) at the end of the experiment (criterion: ≥ 30 juveniles by the end of the experiment),~~
~~- the coefficient of variation of reproduction was 12.1% (criterion: ≤ 30%),~~
~~- adult mortality over the initial 4 weeks of the experiment was 7.5% (criterion: ≤ 10%).~~

Reference:	KCP 10.4/01
Report	CHR/H/PENDIF 599,5 SC Earthworm reproduction test (Eisenia andrei); P. Pieczka, 2021, Study code: G-39-20, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Department of Eco-toxico-logical 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/PENDIF 599.5 SC** **batch no.: 042020**
Active substances:	flufenacet – 318.8 g/L diflufenican – 259.0 g/L penoxulam – 38.7 g/L
Artificial soil:	10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
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, 0.31, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0 mg/kg dry weight of the artificial soil
Test conditions:	temperature: 19.8 – 22.0°C; pH at the beginning of the experiment: 5.68 – 5.89; pH at the end of the experiment: 5.56 – 6.18; soil moisture content at the beginning of the experiment: 24.8 – 29.1% (47.6 – 55.8% of the maximum water holding capacity); soil moisture content at the end of the experiment: 25.4 – 29.6% (48.7 – 56.8% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 427 – 617 lux light intensity at the end of the experiment: 524 – 679 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/PENDIF 599,5 SC on reproduction of the earthworm, Eisenia andrei and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. The concentrations of the test item were: 0.31, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0 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.

At concentrations ranging from 0.31 to 180.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 25.0%.
As for the control group, mortality of the adult earthworms was equal to 7.5%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 180.0 mg/kg dry weight of the artificial soil (above 46.87 mg of flufenacet + 38.08 mg of diflufenican + 5.69 mg of penoxulam/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 0.31 to 180.0 mg/kg dry weight of artificial soil, the body weight increase was between 3.3 and 12.4%. As for the control group, the body weight increase was equal to 5.1%. 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 0.31 to 180.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 95.3 and 285.8 per replicate. The mean number of juveniles in the control group was equal to 257.9 per replicate.
After 8 weeks of the experiment, it was concluded that CHR/H/PENDIF 599,5 SC had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 18.0 to 180.0 mg/kg dry weight of the artificial soil.
The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below..

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of flufenacet/kg dry weight of artificial soil]	Value [mg of diflufenican/kg dry weight of artificial soil]	Value [mg of penoxulam/kg dry weight of artificial soil]
EC_{10}	10.77 (7.56 – 14.08)	2.80 (1.97 – 3.67)	2.28 (1.60 – 2.98)	0.34 (0.24 – 0.45)
EC_{20}	23.10 (18.28 – 27.81)	6.02 (4.76 – 7.24)	4.89 (3.87 – 5.88)	0.73 (0.58 – 0.88)
EC_{50}	99.37 (85.44 – 118.50)	25.88 (22.25 – 30.86)	21.02 (18.07 – 25.07)	3.14 (2.70 – 3.75)
NOEC (reproduction)	10.00	2.60	2.12	0.32
LOEC (reproduction)	18.00	4.69	3.81	0.57
LC_{50}	>180.00	>46.87	>38.08	>5.69
NOEC (survival)	≥180.00	≥46.87	≥38.08	≥5.69
LOEC (survival)	>180.00	>46.87	>38.08	>5.69

VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:
- each replicate produced from 210 to 301 juveniles (257.9 mean) at the end of the experiment (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 12.1% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 7.5% (criterion: ≤ 10%).

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 was conducted to OECD guideline 232 and according to the principles of GLP. Following deviations from the guideline 232 were noted: - 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 - 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) Since all validity criteria were met these deviations did not affect the results of the study. The study is reliable and suitable for the risk assessment.

~~Reference:~~ ~~KCP 10.4/02~~

~~Report~~ ~~CHR/H/PENDIF 599.5 SC Collembolan (Folsomia candida) Reproduction Test, A. Gierbuszewska, 2021, Study code: G-40-20, 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. 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: CHR/H/PENDIF 599,5 SC
batch no.: 042020
Active substances: flufenacet – 318.8 g/L
diflufenican – 259.0 g/L
penoxulam – 38.7 g/L (Appendix No. 1)
Artificial soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

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 11 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, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, and 320.0 mg of the test item/kg of dry weight of the artificial soil

Test conditions: temperature: 19.8 – 22.0°C;
pH at the beginning of the test: 5.52 – 5.57;
pH at the end of the test: 5.67 – 5.84;
soil moisture content at the beginning of the test: 15.2 – 16.4% (47.43 – 51.04% of the maximum water holding capacity);
soil moisture content at the end of the test: 14.6 – 15.5% (45.59 – 48.37% of the maximum water holding capacity);
lighting: 16 h light and 8h dark;
light intensity at the beginning of the experiment: 495 – 510 lux;
light intensity at the end of the experiment: 515 – 531 lux
Statistical analysis: EC10, EC20, EC50 – logit analysis using linear max. likelihood regression
LC10, LC20 and LC50 – probit analysis using linear weighted regression
NOEC (number of juveniles):
- Shapiro-Wilk's Test on Normal Distribution,
- Bartlett's Test Procedure on Variance Homogeneity,
- Williams Multiple Sequential t-test Procedure
NOEC (survival):

~~- Fisher's Exact Binomial 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/PENDIF 599,5 SC 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 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, and 320.0 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 suspension 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. At the concentrations ranging from 1.8 to 320.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 2.5 to 25.0%. As for the control group, it was equal to 6.3%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 320 mg/kg dry weight of the artificial soil (83.33 mg of flufenacet + 67.70 mg of diflufenican + 10.12 mg of penoxulam/kg dry weight of the artificial soil). The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below.~~

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/kg dry weight of the artificial soil]	Value [mg of penoxulam/kg dry weight of the artificial soil]
LC_{10}	100.91 (42.91 – 368.17)	26.28 (11.17 – 95.87)	21.35 (9.08 – 77.89)	3.19 (1.36 – 11.64)
LC_{20}	431.83 (162.50 – 7516.00)	112.45 (42.31 – 1957.12)	91.35 (34.38 – 1590.01)	13.65 (5.14 – 237.58)
LC_{50}	320.00	83.33	67.70	10.12
NOEC	320.00	83.33	67.70	10.12

~~After the exposure of collembolans to the test item at the concentrations ranging from 1.8 to 320.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 361.5 – 967.5 per replicate. As for the control group, the number of juveniles was equal to 899.4 per replicate. The endpoint values showing the impact of the test item on reproduction of Folsomia candida are presented in the table given below.~~

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/kg dry weight of the artificial soil]	Value [mg of penoxulam/kg dry weight of the artificial soil]
EC_{10}	249.23	64.90	52.72	7.88
EC_{20}	269.48	70.17	57.01	8.52
EC_{50}	307.98	80.20	65.15	9.74
NOEC	180.00	46.87	38.08	5.69

Report	CHR/H/PENDIF 599.5 SC Collembolan (Folsomia candida) Reproduction Test, A. Gierbuszewska, 2021, Study code: G-4020, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna De-part-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/PENDIF 599,5 SC
batch no.: 042020
Active substances: flufenacet – 318.8 g/L
diflufenican – 259.0 g/L
penoxulam – 38.7 g/L

Artificial soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

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 11 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, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, and 320.0 mg of the test item/kg of dry weight of the artificial soil

Test conditions: temperature: 19.8 – 22.0°C;
pH at the beginning of the test: 5.52 – 5.57;
pH at the end of the test: 5.67 – 5.84;
soil moisture content at the beginning of the test: 15.2 – 16.4% (47.43 – 51.04% of the maximum water holding capacity);
soil moisture content at the end of the test: 14.6 – 15.5% (45.59 – 48.37% of the maximum water holding capacity);
lighting: 16 h light and 8h dark;
light intensity at the beginning of the experiment: 495 – 510 lux;
light intensity at the end of the experiment: 515 – 531 lux

Statistical analysis: EC10, EC20, EC50 – logit analysis using linear max. likelihood regression
LC10, LC20 and LC50 – probit analysis using linear weighted regression
NOEC (number of juveniles):

- Shapiro-Wilk's Test on Normal Distribution,
- Bartlett's Test Procedure on Variance Homogeneity,
- Williams Multiple Sequential t-test Procedure
NOEC (survival):
- Fisher's Exact Binomial 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/PENDIF 599,5 SC 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 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, and 320.0 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 suspension 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. At the concentrations ranging from 1.8 to 320.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 2.5 to 25.0%. As for the control group, it was equal to 6.3%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 320 mg/kg dry weight of the artificial soil (83.33 mg of flufenacet + 67.70 mg of diflufenican + 10.12 mg of penoxulam/kg dry weight of the artificial soil). The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/kg dry weight of the artificial soil]	Value [mg of penoxulam/kg dry weight of the artificial soil]
LC_{10}	100.91 (42.91 – 368.17)	26.28 (11.17 – 95.87)	21.35 (9.08 – 77.89)	3.19 (1.36 – 11.64)
LC_{20}	431.83 (162.50 – 7516.00)	112.45 (42.31 – 1957.12)	91.35 (34.38 – 1590.01)	13.65 (5.14 – 237.58)
LC_{50}	320.00	83.33	67.70	10.12
NOEC	320.00	83.33	67.70	10.12

After the exposure of collembolans to the test item at the concentrations ranging from 1.8 to 320.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 361.5 – 967.5 per replicate. As for the control group, the number of juveniles was equal to 899.4 per replicate.
The endpoint values showing the impact of the test item on reproduction of Folsomia candida are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/kg dry weight of the artificial soil]	Value [mg of penoxulam/kg dry weight of the artificial soil]
EC_{10}	249.23	64.90	52.72	7.88
EC_{20}	269.48	70.17	57.01	8.52
EC_{50}	307.98	80.20	65.15	9.74
NOEC	180.00	46.87	38.08	5.69

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 6.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 899.4 (criterion: ≥100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 9.2 (criterion: ≤ 30%).

A 2.4.2.1.2 Hypoaspis aculeifer

Comments of zRMS:	The study was conducted to OECD guideline 226 and according to the principles of GLP. Following deviations from the guideline 232 were noted, however they did not affect the results since all the validity criteria of the method were met: 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate 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 mites. The study is reliable and suitable for the risk assessment.

~~Reference:~~ ~~KCP 10.4/03~~

~~Report~~ ~~CHR/H/PENDIF 599.5 SC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, A. Arendarczyk, 2021, Study code: G 41 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~~ ~~No~~

(if vertebrate study)

Materials and methods

Test item:
CHR/H/PENDIF 599,5 SC
batch number: 042020

Active substance:
flufenacet: 318.8 g/L
diflufenican: 259.0 g/L
penoxsulam: 38.7 g/L

Artificial soil:
5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

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, 5.60, 10.00, 18.00, 32.00, 56.00, 100.00, 180.00 and 320.00 mg test item/kg dry weight of the artificial soil.

Test conditions:
temperature: 20.1 – 22.0°C
pH at the beginning of the test: 5.34 – 5.51
pH at the end of the test: 5.52 – 5.59
soil moisture content at the beginning of the test: 13.4 – 15.2% (42.8 – 48.5% of the maximum water holding capacity)
soil moisture content in the middle of the test: 13.4 – 15.3% (42.7 – 49.1% of the maximum water holding capacity)
soil moisture content at the end of the test: 12.7 – 14.7% (40.7 – 46.9% of the maximum water holding capacity)
light-dark cycle: 16 h light and 8 h dark
light intensity at the beginning of the test: 503 – 517 lux
light intensity at end of the test: 504 – 521 lux

Statistical analysis:
EC10, EC20, EC50 – a probit analysis using linear max. likelihood regression
LC10, LC20, LC50 – a probit analysis using linear max. likelihood regression
NOEC:
- offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrast (Monotonicity of Response), Dunnett's Multiple Multiple t-test Procedure
- survival – Qualitative Trend Analysis by Contrast (Monotonicity of Response), Chi2×2 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 CHR/H/PENDIF 599,5 SC on reproduction of the predatory mite, Hypoaspis (Geolaelaps) aculeifer and to determine the EC10, EC20, EC50, and NOEC.~~
~~Eight concentrations of the test item were used. These included: 5.60, 10.00, 18.00, 32.00, 56.00, 100.00, 180.00 and 320.00 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 suspension 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 320.00 mg/kg dry weight of the artificial soil was between 2.5% and 12.5%. Mortality of the control group was equal to 6.3%.~~
~~After the application of the test item at the concentrations ranging from 5.6 to 320.00 mg/kg dry weight of the artificial soil the mean number of juveniles was between 90.3 - 104.3 per replicate. The mean number of juveniles in the control group was equal to 96.3 per replicate. The results are summarized in the table given below.~~

Concentration [mg/kg dry weight of the artificial soil]	Adult mites		Number of juveniles (mean)
	Number of tested mites	Number of dead mites after 14 days	
Control	80	5	96.3
5.60	40	3	91.5
10.00	40	3	104.3
18.00	40	4	98.3
32.00	40	5	99.0
56.00	40	2	90.3
100.00	40	1	96.5
180.00	40	4	93.3
320.00	40	3	92.8

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/ kg dry weight of the artificial soil]	Value [mg of penoxsulam/kg dry weight of the artificial soil]
EC_{10}	> 320.0	> 83.3	> 67.7	> 10.1
EC_{20}	> 320.0	> 83.3	> 67.7	> 10.1
EC_{50}	> 320.0	> 83.3	> 67.7	> 10.1
NOEC (reproduction)	≥ 320.0	≥ 83.3	≥ 67.7	≥ 10.1
LC_{10}	> 320.0	> 83.3	> 67.7	> 10.1
LC_{20}	> 320.0	> 83.3	> 67.7	> 10.1
LC_{50}	> 320.0	> 83.3	> 67.7	> 10.1
NOEC (survival)	≥ 320.0	≥ 83.3	≥ 67.7	≥ 10.1

Reference:	KCP 10.4/03
Report	CHR/H/PENDIF 599,5 SC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, A. Arendarczyk, 2021, Study code: G-41-20, 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:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
CHR/H/PENDIF 599,5 SC
batch number: 042020

Active substance:
flufenacet: 318.8 g/L
diflufenican: 259.0 g/L
penoxsulam: 38.7 g/LL

Artificial soil:
5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

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, 5.60, 10.00, 18.00, 32.00, 56.00, 100.00, 180.00 and 320.00 mg test item/kg dry weight of the artificial soil.

Test conditions:
temperature: 20.1 – 22.0°C
pH at the beginning of the test: 5.34 – 5.51
pH at the end of the test: 5.52 – 5.59
soil moisture content at the beginning of the test: 13.4 – 15.2% (42.8 – 48.5% of the maximum water holding capacity)
soil moisture content in the middle of the test: 13.4 – 15.3% (42.7 – 49.1% of the maximum water holding capacity)
soil moisture content at the end of the test: 12.7 – 14.7% (40.7 – 46.9% of the maximum water holding capacity)
light-dark cycle: 16 h light and 8 h dark
light intensity at the beginning of the test: 503 – 517 lux
light intensity at end of the test: 504 – 521 lux

Statistical analysis:
EC10, EC20, EC50 – a probit analysis using linear max. likelihood regression
LC10, LC20, LC50 – a probit analysis using linear max. likelihood regression
NOEC:
- offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrast (Monotonicity of Response), Dunnett's Multiple Multiple t-test Procedure
- survival – Qualitative Trend Analysis by Contrast (Monotonicity of Response), Chi2×2 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 CHR/H/PENDIF 599,5 SC on reproduction of the predatory mite, Hypoaspis (Geolaelaps) aculeifer and to determine the EC10, EC20, EC50, and NOEC.
Eight concentrations of the test item were used. These included: 5.60, 10.00, 18.00, 32.00, 56.00, 100.00, 180.00 and 320.00 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 suspension 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 320.00 mg/kg dry

weight of the artificial soil was between 2.5% and 12.5%. Mortality of the control group was equal to 6.3%.
After the application of the test item at the concentrations ranging from 5.6 to 320.00 mg/kg dry weight of the artificial soil the mean number of juveniles was between 90.3 – 104.3 per replicate. The mean number of juveniles in the control group was equal to 96.3 per replicate.
The results are summarized in the table given below..

Concentration [mg/kg dry weight of the artificial soil]	Adult mites		Number of juveniles (mean)
	Number of tested mites	Number of dead mites after 14 days	
Control	80	5	96.3
5.60	40	3	91.5
10.00	40	3	104.3
18.00	40	4	98.3
32.00	40	5	99.0
56.00	40	2	90.3
100.00	40	1	96.5
180.00	40	4	93.3
320.00	40	3	92.8

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of flufenacet/kg dry weight of the artificial soil]	Value [mg of diflufenican/ kg dry weight of the artificial soil]	Value [mg of penoxsulam/kg dry weight of the artificial soil]
EC_{10}	> 320.0	> 83.3	> 67.7	> 10.1
EC_{20}	> 320.0	> 83.3	> 67.7	> 10.1
EC_{50}	> 320.0	> 83.3	> 67.7	> 10.1
NOEC (reproduction)	≥ 320.0	≥ 83.3	≥ 67.7	≥ 10.1
LC_{10}	> 320.0	> 83.3	> 67.7	> 10.1
LC_{20}	> 320.0	> 83.3	> 67.7	> 10.1
LC_{50}	> 320.0	> 83.3	> 67.7	> 10.1
NOEC (survival)	≥ 320.0	≥ 83.3	≥ 67.7	≥ 10.1

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the control:

- mean adult mortality: 6.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 96.3 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 9.1% (criterion: ≤ 30%).

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 was conducted to OECD guideline 216 and according to the prin-ciples of GLP. Following deviations from the OECD Guideline No. 216 (2000), the EU Method C.21were noted: - the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 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 - The predicted environmental concentration (PEC) was calculated assuming 1 cm of the soil depth according to the German conditions for the active substances with the mobility in soil KFoc > 500 mL/g. Thus, the applied soil depth is a deviation from the OECD Guideline No. 216 (2000) and EU Method C.21 where the PEC is calculated by using 5 cm of the soil depth In the definitive test all the validity criteria were met as follows: - The coefficients of variation (CV) in the control were 5.2; 10.7; 0.7; 2.2; 1.3 % after 0, 7, 14, 28 and 42 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

~~Reference:~~ ~~KCP 10.5/01~~

~~Report~~ ~~CHR/H/PENDIF 599.5 SC Soil Microorganisms: Nitrogen Transformation Test, A. Gierbuszewska, 2021, Study code: G-42-20, 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~~ ~~No~~
~~(if vertebrate study)~~

~~**Materials and methods**~~

~~Test material: CHR/H/PENDIF 599,5 SC~~
~~batch no.: 042020~~
~~Active substance: flufenacet – 318.8 g/L~~
~~diflufenican – 259.0 g/L~~

penoxulam – 38.7 g/L
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. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil.

Test duration: 42 days.

Concentrations of the test item:
control; PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil);
5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil)

Test conditions:
temperature: 18.8 – 21.5°C,
soil moisture: 42.2 – 50.5% of the maximum water holding
capacity, incubation in darkness

Endpoints: The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28 and 42 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, 0 – 42 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42 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/PENDIF 599,5 SC 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 in the experiment:
- PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil)
- 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil)
The treated and the control soils were divided into three replicates.
On days 0, 7, 14, 28 and 42 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.

On 28 day of analysis the percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at the concentrations corresponding to the PEC and 5 x PEC exceeded 25%, therefore, according to the OECD No. 216, EU Method C.21 and the study plan, the experiment was continued. 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: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet +

~~3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil) did not exceed 25% on 42 day of analysis.~~

~~**Conclusions:**~~
~~On the basis of the results, it was concluded that CHR/H/PENDIF 599,5 SC at the concentrations corresponding to the PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.~~

Reference:	KCP 10.5/01
Report	CHR/H/PENDIF 599,5 SC Soil Microorganisms: Nitrogen Transformation Test, A. Gierbuszewska, 2021, Study code: G-42-20, 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. 216 (2000)/EU Method C.21
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material: CHR/H/PENDIF 599,5 SC
batch no.: 042020

Active substance: flufenacet – 318.8 g/L
diflufenican – 259.0 g/L
penoxulam – 38.7 g/L

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. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil.

Test duration: 42 days.

Concentrations of the test item:
control;
PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil);
5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil)

Test conditions:
temperature: 18.8 – 21.5°C,
soil moisture: 42.2 – 50.5% of the maximum water holding capacity, incubation in darkness

Endpoints: The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28 and 42 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, 0 – 42 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42 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/PENDIF 599,5 SC 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 in the experiment:
- PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil)
- 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil)
The treated and the control soils were divided into three replicates.
On days 0, 7, 14, 28 and 42 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.

On 28 day of analysis the percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at the concentrations corresponding to the PEC and 5 x PEC exceeded 25%, therefore, according to the OECD No. 216, EU Method C.21 and the study plan, the experiment was continued.
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: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/kg dry weight of soil) did not exceed 25% on 42 day of analysis.

Conclusions:
On the basis of the results, it was concluded that CHR/H/PENDIF 599,5 SC at the concentrations corresponding to the PEC: 3.26 mg of the test item/kg dry weight of soil (i.e. 0.85 mg of flufenacet + 0.69 mg of diflufenican + 0.10 mg of penoxulam/kg dry weight of soil) and 5 x PEC: 16.30 mg of the test item/kg dry weight of soil (i.e. 4.24 mg of flufenacet + 3.45 mg of diflufenican + 0.52 mg of penoxulam/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 5.2, 10.7, 0.7, 2.2 and 1.4%, after 0, 7, 14, 28 and 42 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 seedling emergence study was conducted to OECD guideline 208 and according to the principles of GLP. In the definitive test all the validity criteria were met. Following deviation from OECD Guideline No. 208 was noted: 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 92.37 and 172.7 µE/m2 /s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. This deviation did not affect results of the experiment The study is acceptable and reliable for risk assessment purposes.

~~Reference:~~	~~KCP 10.6.1/01~~
~~Report~~	~~CHR/H/PENDIF 599.5 SC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, A. Arendarczyk, 2021, Study code: G 44 20, Łukasiewicz Research Network – Institute of Industrial Organic Chemis try, Branch Pszczyna Depart ment of Ecotoxicological Studies, Doświ adczalna 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/PENDIF 599,5 SC**~~ ~~batch number: 042020~~ ~~active substances: flufenacet – 318.8 g/L~~ ~~diflufenican – 259.0 g/L~~ ~~penoxsulam – 38.7 g/L~~ ~~(Appendix No. 1)~~
~~Test species:~~	~~sunflower (*Helianthus annuus*), flax (*Linum usitatissimum*), pea (*Pisum sativum*), carrot (*Daucus carota*), onion (*Allium cepa*), corn (*Zea mays*)~~
~~Soil:~~	~~Sandy loam~~
~~Study design:~~	~~number of rates:~~ ~~- 6 + control for corn (range from 12.5 to 400 mL/ha),~~ ~~- 8 + control for flax and onion (range from 1.6 to 200.0 mL/ha),~~ ~~- 8 + control for sunflower and carot (range from 3.2 to 400.0 mL/ha),~~ ~~- 9 + control for pea (range from 1.6 to 400.0 mL/ha);~~ ~~number of replicates/rate:~~ ~~- 4 for carrot, flax, onion,~~

	- 7 for pea, sunflower, - 10 for corn; The total number of seeds per application rate: - 20 for carrot, flax, onion and corn, - 21 for pea and sunflower; test termination: 14 days after the emergence of 50% of the control seedlings.
Application rates:	- a control, - 1.6 mL of the test item /ha (0.5 g of flufenacet + 0.4 g of diflufenican/ha + 0.06 g of penoxsulam/ha), - 3.2 mL of the test item /ha (1.0 g of flufenacet + 0.8 g of diflufenican/ha + 0.12 g of penoxsulam/ha), - 6.3 mL of the test item /ha (2.0 g of flufenacet + 1.6 g of diflufenican/ha + 0.24 g of penoxsulam/ha), - 12.5 mL of the test item /ha (4.0 g of flufenacet + 3.2 g of diflufenican/ha + 0.48 g of penoxsulam/ha), - 25.0 mL of the test item /ha (8.0 g of flufenacet + 6.5 g of diflufenican/ha + 0.97 g of penoxsulam/ha), - 50.0 mL of the test item /ha (15.9 g of flufenacet + 13.0 g of diflufenican/ha + 1.94 g of penoxsulam/ha), - 100.0 mL of the test item /ha (31.9 g of flufenacet + 25.9 g of diflufenican/ha + 3.87 g of penoxsulam/ha), - 200.0 mL of the test item /ha (63.8 g of flufenacet + 51.8 g of diflufenican/ha + 7.74 g of penoxsulam/ha), - 400.0 mL of the test item /ha (127.5 g of flufenacet + 103.6 g of diflufenican/ha + 15.48 g of penoxsulam/ha).
Volume of deionized water:	volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha.
Test conditions:	temperature: 17.4 – 26.5°C, humidity: 48.2 – 82.7%, lighting: 16 h light : 8 h dark; light intensity: 61.7 – 163.2 µE/m^2/s; carbon dioxide concentration: 335 – 372 ppm
Statistical analysis:	ER_{25}, ER_{50} - probit analysis with the linear max. likelihood regression (final number of plants), non linear regression – 3 parametric normal Distribution Function (CDF) (plant shoot length and plant shoot weight). NOER (plant emergence): - Multiple Sequentially rejective Fisher Test After Bonferroni – Holm, - Fisher's Exact Binomial Test with Bonferroni Correction, - Tarone's Test Procedure, - Williams Multiple Sequential t test Procedure were used. NOER (plant shoot length and plant shoot weight): - Shapiro Wilk's Test on Normal Distribution, - Levene's Test on Variance Homogeneity (with Residuals), - Multiple Sequentially rejective Welsch t test After Bonferroni – Holm, - Step down Jonckheere Terpstra Test Procedure, - Williams Multiple Sequential t test Procedure.
Endpoints:	ER25, ER50, NOER

Results and discuss:

The study, aimed at evaluating the effect CHR/H/PENDIF 599,5 SC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 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 3 (pea, sunflower) or 5 (carrot, flax, onion) or 2 (corn) 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 1 to 2 days to the emergence of 50% of the control seedlings and after then every 2 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, 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 ER25, ER50, and NOER.

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>400.0*	168.5 (99.6 – 421.5**)	280.7 (211.2 – 433.8**)	>400.0*	182.2 (90.2 – 2541.1**)	>400.0*
NOER	≥400.0	50.0	200.0	≥400.0	50.0	≥400.0
Shoot length (plants without roots)						
ER_{50}	337.4 (139.3 – 794.7**)	110.1 (68.2 – 177.4)	213.1 (132.7 – 338.0)	94.0 (49.5 – 184.1)	117.6 (39.8 – 352.6)	>400.0*
NOER	12.5	25.0	25.0	6.3	6.3	100.0
Plant dry weight (plants without roots)						
ER_{50}	714.6** (280.8 – 1745.9**)	121.1 (48.1 – 298.4**)	251.4 (152.9 – 408.2**)	35.8 (16.1 – 82.8)	88.2 (52.7 – 150.5)	>400.0*
NOER	50.0	12.5	50.0	12.5	25.0	≥400.0

The ER_{10}, ER_{25}. ER_{50} and NOER values were calculated using the ToxRat Professional 3.3.0 computer software.

*the value could not be derermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 200.0 or 400.0 mL/ha

**the value determined as higher than the highest application rate, i.e. 200.0 or 400.0 mL/ha

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>127.5*	53.7 (31.8 – 134.4**)	89.5 (67.3 – 138.3**)	>127.5*	58.1 (28.8 – 810.1**)	>127.5*
NOER	≥127.5	15.9	63.8	≥127.5	15.9	≥127.5
Shoot length (plants without roots)						
ER_{50}	107.6 (44.4 – 253.4**)	35.1 (21.7 – 56.6)	67.9 (42.3 – 107.8)	30.0 (15.8 – 58.7)	37.5 (12.7 – 112.4**)	>127.5*
NOER	3.99	8.0	8.0	2.0	2.0	31.9
Plant dry weight (plants without roots)						
ER_{50}	227.8** (89.5 – 556.6**)	38.6 (15.3 – 95.1**)	80.2 (48.7 – 130.1**)	11.4 (5.1 – 26.4)	28.1 (16.8 – 48.0)	>127.5*
NOER	15.9	4.0	15.9	4.0	8.0	≥127.5

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 63.8 or 127.5 g of flufenacet / ha

**the value determined as higher than the highest application rate, i.e. 63.8 or 127.5 g of flufenacet / ha

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>103.6*	43.6 (25.8 – 109.2**)	72.7 (54.7 – 112.4**)	>103.6*	47.2 (23.4 – 658.1**)	>103.6*
NOER	≥103.6	13.0	51.8	≥103.6	13.0	≥103.6
Shoot length (plants without roots)						
ER_{50}	87.4 (36.1 – 205.8**)	28.5 (17.7 – 46.0)	55.2 (34.4 – 87.5)	24.3 (12.8 – 47.8)	30.5 (10.3 – 91.3)	>103.6*
NOER	3.2	6.5	6.5	1.6	1.6	25.9
Plant dry weight (plants without roots)						
ER_{50}	185.1** (72.7 – 452.2**)	31.4 (12.5 – 77.3**)	65.1 (39.6 – 105.7**)	9.3 (4.2 – 21.4)	22.8 (13.7 – 39.0)	>103.6*
NOER	13.0	3.2	13.0	3.2	6.5	≥103.6

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 51.8 or 103.6 g of diflufenican / ha

**the value determined as higher than the highest application rate, i.e. 51.8 or 103.6 g of diflufenican / ha

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

	Sunflower *Helianthus annuus*	Flax *Linum usitatissimum*	Pea *Pisum sativum*	Carrot *Daucus carota*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>15.48*	6.52 (3.85 – 16.31**)	10.86 (8.17 – 16.79**)	>15.48*	2.93 (0.98 – 7.69**)	>15.48*
NOER	≥15.48	1.94	7.74	≥15.48	1.94	≥15.48
Shoot length (plants without roots)						
ER_{50}	13.06 (5.39 – 30.75**)	4.26 (2.64 –6.87)	8.25 (5.14 – 13.08)	3.64 (1.92 – 7.12)	4.55 (1.54 – 13.7)	>15.48*
NOER	0.48	0.97	0.97	0.24	0.24	3.87
Plant dry weight (plants without roots)						
ER_{50}	27.66** (10.87 – 67.57**)	4.69 (1.86 – 11.55)	9.73 (5.92 – 15.80**)	1.39 (0.62 – 3.20)	3.41 (2.04 – 5.82)	>15.48*
NOER	1.94	0.48	1.94	0.48	0.97	≥15.48

*the value could not be derterminded, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 7.74 or 15.48 g of penoxsulam / ha

**the value determined as higher than the highest application rate, i.e. 7.74 or 15.48 g of penoxsulam / ha

~~On the basis of the obtained results it was proved that the test item i.e. CHR/H/PENDIF 599,5 SC had varied impact on seedling emergence and seedling growth of the test plant species.~~
~~1. For the selected application rates, seedling emergence of sunflower, pea and onion was delayed by one day when compared with the control. The death of tested plants was not observed during the experiment for any of the tested plant species.~~
~~2. The lowest ER50 value determined on the basis of the plant shoot length at the end of the experiment, was observed for carrot and it was equal to 94.0 mL of the test item/ha.~~
~~3. The lowest ER50 value determined on the basis of the plant shoot weight at the end of the experiment, was observed for carrot and it was equal to 35.8 mL of the test item/ha.~~
~~4. Significant and moderate inhibition of plant shoot length was observed for carrot, onion, flax, pea and sunflower. Slightly inhibition of plant shoot length could be noticed only for corn.~~
~~5. Significant and moderate inhibition of plant shoot weight was observed for carrot, onion, flax, pea and sunflower. No inhibition of plant shoot weight could be noticed only for corn.~~
~~6. **Phytotoxic symptoms of plants,** at selected application rates, were observed during the experiment. It was stunted growth for sunflower and flax, stunted growth and chlorosis for pea, carrot, corn and stunted growth, chlorosis and wiliting for onion plants.~~
~~7. The following order of the test plant sensitivity was noticed:~~
~~carrot > onion, flax > sunflower, pea > corn.~~

Reference: KCP 10.6.1/01

Report: CHR/H/PENDIF 599,5 SC: Seedling Emergence and Seedling Growth Test, A. Arendarczyk, 2021, Study code: G-44-20, Łukasiewicz Research Net-

	work – Institute of Industrial Organic Chemis-try, Branch Pszczyna Depart-ment of Ecotoxicological Studies, Doświ-adczalna 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/PENDIF 599,5 SC batch number: 042020 active substances: flufenacet – 318.8 g/L diflufenican – 259.0 g/L penoxsulam – 38.7 g/L
Test species:	sunflower (*Helianthus annuus),* flax (*Linum usitatis-simum*), pea *(Pisum sativum),* carrot *(Daucus carota),* onion *(Allium cepa),* corn *(Zea mays)*
Soil: Study design:	Sandy loam number of rates: - 6 + control for corn (range from 12.5 to 400 mL/ha), - 8 + control for flax and onion (range from 1.6 to 200.0 mL/ha), - 8 + control for sunflower and carot (range from 3.2 to 400.0 mL/ha), - 9 + control for pea (range from 1.6 to 400.0 mL/ha); number of replicates/rate: - 4 for carrot, flax, onion, - 7 for pea, sunflower, - 10 for corn; The total number of seeds per application rate: - 20 for carrot, flax, onion and corn, - 21 for pea and sunflower; test termination: 14 days after the emergence of 50% of the control seedlings.

Application rates:	- a control, - 1.6 mL of the test item /ha (0.5 g of flufenacet + 0.4 g of diflufenican/ha + 0.06 g of penoxsulam/ha), - 3.2 mL of the test item /ha (1.0 g of flufenacet + 0.8 g of diflufenican/ha + 0.12 g of penoxsulam/ha), - 6.3 mL of the test item /ha (2.0 g of flufenacet + 1.6 g of diflufenican/ha + 0.24 g of penoxsulam/ha), - 12.5 mL of the test item /ha (4.0 g of flufenacet + 3.2 g of diflufenican/ha + 0.48 g of penoxsulam/ha), - 25.0 mL of the test item /ha (8.0 g of flufenacet + 6.5 g of diflufenican/ha + 0.97 g of penoxsulam/ha), - 50.0 mL of the test item /ha (15.9 g of flufenacet + 13.0 g of diflufenican/ha + 1.94 g of penoxsulam/ha), - 100.0 mL of the test item /ha (31.9 g of flufenacet + 25.9 g of diflufenican/ha + 3.87 g of penoxsulam/ha), - 200.0 mL of the test item /ha (63.8 g of flufenacet + 51.8 g of diflufenican/ha + 7.74 g of penoxsulam/ha), - 400.0 mL of the test item /ha (127.5 g of flufenacet + 103.6 g of diflufenican/ha + 15.48 g of penoxsulam/ha). .
Volume of deionized water:	volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha.
Test conditions:	temperature: 17.4 – 26.5°C, humidity: 48.2 – 82.7%, lighting: 16 h light : 8 h dark; light intensity: 61.7 – 163.2 µE/m2/s; carbon dioxide concentration: 335 – 372 ppm
Statistical analysis:	ER_{25}, ER_{50} – probit analysis with the linear max. likelihood regression (final number of plants), non-linear regression - 3 parametric normal Distribution Function (CDF) (plant shoot length and plant shoot weight). NOER (plant emergence): - Multiple Sequentially-rejective Fisher Test After Bonferroni – Holm, - Fisher's Exact Binomial Test with Bonferroni Correction, - Tarone's Test Procedure, - Williams Multiple Sequential t-test Procedure were used. NOER (plant shoot length and plant shoot weight): - Shapiro-Wilk's Test on Normal Distribution, - Levene's Test on Variance Homogeneity (with Residuals), - Multiple Sequentially-rejective Welsch-t-test After Bonferroni – Holm, - Step-down Jonckheere-Terpstra Test Procedure, - Williams Multiple Sequential t-test Procedure.
Endpoints:	ER25, ER50, NOER

Results and discuss:

The study, aimed at evaluating the effect CHR/H/PENDIF 599,5 SC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 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 3 (pea, sunflower) or 5 (carrot, flax, onion) or 2 (corn) 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 1 to 2 days to the emergence of 50% of the control seedlings and after then every 2 – 3 days) and visual phyto-toxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, 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 ER25, ER50, and NOER.

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>400.0*	168.5 (99.6 – 421.5**)	280.7 (211.2 – 433.8**)	>400.0*	182.2 (90.2 – 2541.1**)	>400.0*
NOER	≥400.0	50.0	200.0	≥400.0	50.0	≥400.0
Shoot length (plants without roots)						
ER_{50}	337.4 (139.3 – 794.7**)	110.1 (68.2 – 177.4)	213.1 (132.7 – 338.0)	94.0 (49.5 – 184.1)	117.6 (39.8 – 352.6)	>400.0*
NOER	12.5	25.0	25.0	6.3	6.3	100.0
Plant dry weight (plants without roots)						
ER_{50}	714.6** (280.8 – 1745.9**)	121.1 (48.1 – 298.4**)	251.4 (152.9 – 408.2**)	35.8 (16.1 – 82.8)	88.2 (52.7 – 150.5)	>400.0*
NOER	50.0	12.5	50.0	12.5	25.0	≥400.0

The ER_{10}, ER_{25}. ER_{50} and NOER values were calculated using the ToxRat Professional 3.3.0 computer software.

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 200.0 or 400.0 mL/ha

**the value determined as higher than the highest application rate, i.e. 200.0 or 400.0 mL/ha

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>127.5*	53.7 (31.8 – 134.4**)	89.5 (67.3 – 138.3**)	>127.5*	58.1 (28.8 – 810.1**)	>127.5*
NOER	≥127.5	15.9	63.8	≥127.5	15.9	≥127.5
Shoot length (plants without roots)						
ER_{50}	107.6 (44.4 – 253.4**)	35.1 (21.7 – 56.6)	67.9 (42.3 – 107.8)	30.0 (15.8 – 58.7)	37.5 (12.7 – 112.4**)	>127.5*
NOER	3.99	8.0	8.0	2.0	2.0	31.9
Plant dry weight (plants without roots)						
ER_{50}	227.8** (89.5 – 556.6**)	38.6 (15.3 – 95.1**)	80.2 (48.7 – 130.1**)	11.4 (5.1 – 26.4)	28.1 (16.8 – 48.0)	>127.5*
NOER	15.9	4.0	15.9	4.0	8.0	≥127.5

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 63.8 or 127.5 g of flufenacet / ha

**the value determined as higher than the highest application rate, i.e. 63.8 or 127.5 g of flufenacet / ha

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>103.6*	43.6 (25.8 – 109.2**)	72.7 (54.7 – 112.4**)	>103.6*	47.2 (23.4 – 658.1**)	>103.6*
NOER	≥103.6	13.0	51.8	≥103.6	13.0	≥103.6
Shoot length (plants without roots)						
ER_{50}	87.4 (36.1 – 205.8**)	28.5 (17.7 – 46.0)	55.2 (34.4 – 87.5)	24.3 (12.8 – 47.8)	30.5 (10.3 – 91.3)	>103.6*
NOER	3.2	6.5	6.5	1.6	1.6	25.9
Plant dry weight (plants without roots)						
ER_{50}	185.1** (72.7 – 452.2**)	31.4 (12.5 – 77.3**)	65.1 (39.6 – 105.7**)	9.3 (4.2 – 21.4)	22.8 (13.7 – 39.0)	>103.6*
NOER	13.0	3.2	13.0	3.2	6.5	≥103.6

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 51.8 or 103.6 g of diflufenican / ha

**the value determined as higher than the highest application rate, i.e. 51.8 or 103.6 g of diflufenican / ha

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

	Sunflower *Helianthus annuus*	**Flax** *Linum usitatissimum*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	>15.48*	6.52 (3.85 – 16.31**)	10.86 (8.17 – 16.79**)	>15.48*	2.93 (0.98 – 7.69**)	>15.48*
NOER	≥15.48	1.94	7.74	≥15.48	1.94	≥15.48
Shoot length (plants without roots)						
ER_{50}	13.06 (5.39 – 30.75**)	4.26 (2.64 –6.87)	8.25 (5.14 – 13.08)	3.64 (1.92 – 7.12)	4.55 (1.54 – 13.7)	>15.48*
NOER	0.48	0.97	0.97	0.24	0.24	3.87
Plant dry weight (plants without roots)						
ER_{50}	27.66** (10.87 – 67.57**)	4.69 (1.86 – 11.55)	9.73 (5.92 – 15.80**)	1.39 (0.62 – 3.20)	3.41 (2.04 – 5.82)	>15.48*
NOER	1.94	0.48	1.94	0.48	0.97	≥15.48

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 7.74 or 15.48 g of penoxsulam / ha

**the value determined as higher than the highest application rate, i.e. 7.74 or 15.48 g of penoxsulam / ha

On the basis of the obtained results it was proved that the test item i.e. CHR/H/PENDIF 599,5 SC had varied impact on seedling emergence and seedling growth of the test plant species.
1. For the selected application rates, seedling emergence of sunflower, pea and onion was delayed by one day when compared with the control. The death of tested plants was not observed during the experiment for any of the tested plant species.
2. The lowest ER50 value determined on the basis of the plant shoot length at the end of the experiment, was observed for carrot and it was equal to 94.0 mL of the test item/ha.
3. The lowest ER50 value determined on the basis of the plant shoot weight at the end of the experiment, was observed for carrot and it was equal to 35.8 mL of the test item/ha.
4. Significant and moderate inhibition of plant shoot length was observed for carrot, onion, flax, pea and sunflower. Slightly inhibition of plant shoot length could be noticed only for corn.
5. Significant and moderate inhibition of plant shoot weight was observed for carrot, onion, flax, pea and sunflower. No inhibition of plant shoot weight could be noticed only for corn.
6. Phytotoxic symptoms of plants, at selected application rates, were observed during the experiment. It was stunted growth for sunflower and flax, stunted growth and chlorosis for pea, carrot, corn and stunted growth, chlorosis and wiliting for onion plants.
7. The following order of the test plant sensitivity was noticed:
carrot > onion, flax > sunflower, pea > corn.

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/PENDIF 599,5 SC 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% – sunflower,
100.0% – flax,
100.0% – pea,
100.0% – carrot,
100.0% – onion,
100.0% – corn,
- the mean survival of the emerged control seedlings was 100% for sunflower, flax, pea, carrot, onion and corn (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 Vegetative vigour study was conducted to OECD guideline 227 and according to the principles of GLP. In the definitive test all the validity criteria were met. Following deviation from OECD 227 method was noted: - the light intensity, monitored twice during the experiment, between 50 and 400 µE/m2/s (deviation from the OECD Guideline no. 227). This deviation did not affect results of the experiment. The study is accepted and reliable for risk assessment purposes.

~~Reference:~~ ~~KCP 10.6.1/02~~

~~Report~~ ~~CHR/H/PENDIF 599.5 SC Terrestial Plant Test: Vegetative Vigour Test, A. Gierbuszewska, 2021, Study code: G-43-20, 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~~ ~~No~~
~~(if vertebrate study)~~

~~**Materials and methods**~~
~~Test item:~~
~~CHR/H/PENDIF 599,5 SC~~
~~batch number: 042020~~
~~active substances: flufenacet – 318.8 g/L~~
~~diflufenican – 259.0 g/L~~
~~penoxulam – 38.7 g/L~~

~~Test species:~~
~~pea (Pisum sativum), sunflower (Helianthus annuus), carrot (Daucus carota), flax (Linum usitatissimum), onion (Allium cepa), corn (Zea mays)~~
~~Soil: Sandy loam~~
~~Study design: number of rates: 5 + control (pea, flax, corn), 6 + control (onion), 7 + control (sunflower, carrot); number of replicates/rate: 7 (pea, sunflower), 4 (carrot, flax, onion) or 10 (corn). The total number~~

of plants per application rate – 21 (pea, sunflower) or 20 (carrot, flax, onion, corn)
exposure termination: 21 days after spraying
Application
rates:
- 0.0000 (control), 0.0016, 0.0041, 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – sunflower and carrot,
- 0.0000 (control), 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – pea, flax and corn,
- 0.0000 (control), 0.0041, 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – onion.
volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha.
Test conditions:
temperature: 17.2 – 26.5°C, humidity: 48.2 – 78.9%, lighting: 16 h
light : 8 h dark; light intensity: 73.3 – 231.1 µE/m2/s; carbon dioxide
concentration: 335 – 389 ppm

Statistical analysis:
ER25, ER50 – probit analysis using linear max. likelihood regression, Weibull analysis using linear max. likelihood regression, logit analysis using simple linear regression
NOER:
In order to determine the NOER values for the plant number at the end of the experiment of sunflower, carrot, flax, and onion the Fisher's Exact Binomial Test with Bonferroni Correction was used. In order to determine the NOER value for the plant number at the end of the experiment of pea and corn any computations had been perfomed because of no change in mortality of plants. In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) the following statistical tests were used:
Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test
Procedure or Dunnett's Multiple t-test Procedure
In order to determine the NOER values for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:
Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Endpoints: ER25, ER50, NOER

Results and discussion

The study, aimed at evaluating the effect of CHR/H/PENDIF 599,5 SC on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea and sunflower; 10 seeds/pot for carrot, flax, onion and 4 seeds/pot for corn). 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 concentration were:
- pea: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- sunflower: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- flax: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- corn: 2 plants/pot – 20 plants/ application rate (10 pots/ application rate).
The pot is defined as a replicate. The test item was sprayed onto the plants. For tested species, five (pea, flax, corn) or six (onion) or seven (sunflower, carrot) application rates were used. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for carrot, flax and onion; 7 replicates for pea and sunflower; 10 replicates for corn. 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 experiment finished 21 days after the spraying. At the end of the experiment, 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 ER25, ER50 and NOER.~~

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

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Carrot** *Daucus carota*	**Flax** *Linum usitatissimum*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 0.400	0.067 (0.053 – 0.084)	> 0.400	0.120 (0.092 – 0.158)	0.137 (0.106 – 0.178)	> 0.400
NOER	≥ 0.400	0.026	≥ 0.400	0.064	0.064	≥ 0.400
Shoot length (plants without roots)						
ER_{50}	0.100	0.077 (0.063 – 0.109)	0.129 (0.040 – 2.833)	0.045	0.123 (0.085 – 0.237)	> 0.400
NOER	0.026	0.010	0.010	0.010	0.026	≥ 0.400
Plant dry weight (plants without roots)						
ER_{50}	0.100	0.081	0.027 (0.011 – 0.063)	0.030	0.192 (0.113 – 16.379)	> 0.400
NOER	0.026	0.010	0.004	0.010	0.026	≥ 0.400

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

	Pea *Pisum sativum*	Sunflower *Helianthus annuus*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 127.520	21.360 (16.896 – 26.779)	> 127.520	38.256 (29.330 – 50.370)	43.676 (33.793 – 56.746)	> 127.520
NOER	≥ 127.520	8.289	≥ 127.520	20.403	20.403	≥ 127.520
Shoot length (plants without roots)						
ER_{50}	31.880	24.548 (20.084 – 34.749)	41.125 (12.752 – 903.160)	14.346	39.212 (27.098 – 75.556)	> 127.520
NOER	8.289	3.188	3.188	3.188	8.289	≥ 127.520
Plant dry weight (plants without roots)						
ER_{50}	31.880	25.823	8.608 (3.507 – 20.084)	9.564	61.210 (36.024 – 5221.625)	> 127.520
NOER	8.289	3.188	1.275	3.188	8.289	≥ 127.520

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

	Pea *Pisum sativum*	Sunflower *Helianthus annuus*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 103.600	17.353 (13.727 – 21.756)	> 103.600	31.080 (23.828 – 40.922)	35.483 (27.454 – 46.102)	> 103.600
NOER	≥ 103.600	6.734	≥ 103.600	16.576	16.576	≥ 103.600
Shoot length (plants without roots)						
ER_{50}	25.900	19.943 (16.317 – 28.231)	33.411 (10.360 – 733.747)	11.655	31.857 (22.015 – 61.383)	> 103.600
NOER	6.734	2.590	2.590	2.590	6.734	≥ 103.600
Plant dry weight (plants without roots)						
ER_{50}	25.900	20.979	6.993 (2.849 – 16.317)	7.770	49.728 (29.267 – 4242.161)	> 103.600
NOER	6.734	2.590	1.036	2.590	6.734	≥ 103.600

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

	Pea *Pisum sativum*	Sunflower *Helianthus annuus*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 15.480	2.593 (2.051 – 3.251)	> 15.480	4.644 (3.560 – 6.115)	5.302 (4.102 – 6.889)	> 15.480
NOER	≥ 15.480	1.006	≥ 15.480	2.477	2.477	≥ 15.480
Shoot length (plants without roots)						
ER_{50}	3.870	2.980 (2.438 – 4.218)	4.992 (1.548 – 109.637)	1.742	4.760 (3.290 – 9.172)	> 15.480
NOER	1.006	0.387	0.387	0.387	1.006	≥ 15.480
Plant dry weight (plants without roots)						
ER_{50}	3.870	3.135	1.045 (0.426 – 2.438)	1.161	7.430 (4.373 – 633.867)	> 15.480
NOER	1.006	0.387	0.155	0.387	1.006	≥ 15.480

~~The test item, i.e. CHR/H/PENDIF 599,5 SC had an impact on vegetative vigour of pea, sunflower, carrot, flax and onion. The test item had no impact on vegetative vigour of corn.~~
~~The test item caused mortality of sunflower (rates: from 0.0260 to 0.4000 L/ha), carrot (rates: 0.1600 and 0.4000 L/ha), flax (rates: from 0.0640 to 0.4000 L/ha) and onion (rates: from 0.0640 to 0.4000 L/ha).~~
~~On the basis of NOER, ER25 and ER50 values determined from the shoot length and shoot dry weight it was proved that the test item inhibit the process of growth of pea, sunflower, carrot, flax, and onion.~~
~~Some phytotoxic symptoms as stunted growth, deformations, wilting, chlorosis, spots, necrosis and mortality of plants were observed after 21 days of the exposure.The following order of the test plant sensitivity was noticed:~~
~~flax > sunflower > pea, onion > carrot > corn~~

Reference:	KCP 10.6.1/02
Report	CHR/H/PENDIF 599,5 SC Terrestial Plant Test: Vegetative Vigour Test, A. Gierbuszewska, 2021, Study code: G-43-20, 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:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
CHR/H/PENDIF 599,5 SC
batch number: 042020
active substances: flufenacet – 318.8 g/L
diflufenican – 259.0 g/L
penoxulam – 38.7 g/L

Test species:
pea (Pisum sativum), sunflower (Helianthus annuus), carrot (Daucus carota), flax (Linum usitatissimum), onion (Allium cepa), corn (Zea mays)

Soil: Sandy loam

Study design: number of rates: 5 + control (pea, flax, corn), 6 + control (onion), 7 + control (sunflower, carrot); number of replicates/rate: 7 (pea, sunflower), 4 (carrot, flax, onion) or 10 (corn). The total number of plants per application rate – 21 (pea, sunflower) or 20 (carrot, flax, onion, corn)
exposure termination: 21 days after spraying

Application rates:
- 0.0000 (control), 0.0016, 0.0041, 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – sunflower and carrot,
- 0.0000 (control), 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – pea, flax and corn,
- 0.0000 (control), 0.0041, 0.0100, 0.0260, 0.0640, 0.1600 and 0.4000 L of the test item / ha – onion.
volume of deionized water used to prepare the highest rate
corresponded to 300 L spraying liquid/ha..
.

Test conditions:
temperature: 17.2 – 26.5°C, humidity: 48.2 – 78.9%, lighting: 16 h
light : 8 h dark; light intensity: 73.3 – 231.1 µE/m2/s; carbon dioxide
concentration: 335 – 389 ppm

Statistical analysis:
ER25, ER50 – probit analysis using linear max. likelihood regression, Weibull analysis using linear max. likelihood regression, logit analysis using simple linear regression
NOER:
In order to determine the NOER values for the plant number at the end of the experiment of sunflower, carrot, flax, and onion the Fisher's Exact Binomial Test with Bonferroni Correction was used. In order to determine the NOER value for the plant number at the end of the experiment of pea and corn any computations had been perfomed because of no change in mortality of plants. In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) the following statistical tests were used:
Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test
Procedure or Dunnett's Multiple t-test Procedure
In order to determine the NOER values for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:
Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Endpoints: ER25, ER50, NOER

Results and discussion

The study, aimed at evaluating the effect of CHR/H/PENDIF 599,5 SC on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea and sunflower; 10 seeds/pot for carrot, flax, onion and 4 seeds/pot for corn). 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 concentration were:
- pea: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- sunflower: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- flax: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- corn: 2 plants/pot – 20 plants/ application rate (10 pots/ application rate).

The pot is defined as a replicate. The test item was sprayed onto the plants. For tested species, five (pea, flax, corn) or six (onion) or seven (sunflower, carrot) application rates were used. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for carrot, flax and onion; 7 replicates for pea and sunflower; 10 replicates for corn. 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 experiment finished 21 days after the spraying. At the end of the experiment, 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 ER25, ER50 and NOER.

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

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Carrot** *Daucus carota*	**Flax** *Linum usitatissimum*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 0.400	0.067 (0.053 – 0.084)	> 0.400	0.120 (0.092 – 0.158)	0.137 (0.106 – 0.178)	> 0.400
NOER	≥ 0.400	0.026	≥ 0.400	0.064	0.064	≥ 0.400
Shoot length (plants without roots)						
ER_{50}	0.100	0.077 (0.063 – 0.109)	0.129 (0.040 – 2.833)	0.045	0.123 (0.085 – 0.237)	> 0.400
NOER	0.026	0.010	0.010	0.010	0.026	≥ 0.400
Plant dry weight (plants without roots)						
ER_{50}	0.100	0.081	0.027 (0.011 – 0.063)	0.030	0.192 (0.113 – 16.379)	> 0.400
NOER	0.026	0.010	0.004	0.010	0.026	≥ 0.400

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

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Carrot** *Daucus carota*	**Flax** *Linum usitatissimum*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 127.520	21.360 (16.896 – 26.779)	> 127.520	38.256 (29.330 – 50.370)	43.676 (33.793 – 56.746)	> 127.520
NOER	≥ 127.520	8.289	≥ 127.520	20.403	20.403	≥ 127.520
Shoot length (plants without roots)						
ER_{50}	31.880	24.548 (20.084 – 34.749)	41.125 (12.752 – 903.160)	14.346	39.212 (27.098 – 75.556)	> 127.520
NOER	8.289	3.188	3.188	3.188	8.289	≥ 127.520
Plant dry weight (plants without roots)						
ER_{50}	31.880	25.823	8.608 (3.507 – 20.084)	9.564	61.210 (36.024 – 5221.625)	> 127.520
NOER	8.289	3.188	1.275	3.188	8.289	≥ 127.520

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

	Pea *Pisum sativum*	Sunflower *Helianthus annuus*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 103.600	17.353 (13.727 – 21.756)	> 103.600	31.080 (23.828 – 40.922)	35.483 (27.454 – 46.102)	> 103.600
NOER	≥ 103.600	6.734	≥ 103.600	16.576	16.576	≥ 103.600
Shoot length (plants without roots)						
ER_{50}	25.900	19.943 (16.317 – 28.231)	33.411 (10.360 – 733.747)	11.655	31.857 (22.015 – 61.383)	> 103.600
NOER	6.734	2.590	2.590	2.590	6.734	≥ 103.600
Plant dry weight (plants without roots)						
ER_{50}	25.900	20.979	6.993 (2.849 – 16.317)	7.770	49.728 (29.267 – 4242.161)	> 103.600
NOER	6.734	2.590	1.036	2.590	6.734	≥ 103.600

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

	Pea *Pisum sativum*	Sunflower *Helianthus annuus*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{50}	> 15.480	2.593 (2.051 – 3.251)	> 15.480	4.644 (3.560 – 6.115)	5.302 (4.102 – 6.889)	> 15.480
NOER	≥ 15.480	1.006	≥ 15.480	2.477	2.477	≥ 15.480
Shoot length (plants without roots)						
ER_{50}	3.870	2.980 (2.438 – 4.218)	4.992 (1.548 – 109.637)	1.742	4.760 (3.290 – 9.172)	> 15.480
NOER	1.006	0.387	0.387	0.387	1.006	≥ 15.480
Plant dry weight (plants without roots)						
ER_{50}	3.870	3.135	1.045 (0.426 – 2.438)	1.161	7.430 (4.373 – 633.867)	> 15.480
NOER	1.006	0.387	0.155	0.387	1.006	≥ 15.480

The test item, i.e. CHR/H/PENDIF 599,5 SC had an impact on vegetative vigour of pea, sunflower, carrot, flax and onion. The test item had no impact on vegetative vigour of corn.
The test item caused mortality of sunflower (rates: from 0.0260 to 0.4000 L/ha), carrot (rates: 0.1600 and 0.4000 L/ha), flax (rates: from 0.0640 to 0.4000 L/ha) and onion (rates: from 0.0640 to 0.4000 L/ha).
On the basis of NOER, ER25 and ER50 values determined from the shoot length and shoot dry weight it was proved that the test item inhibit the process of growth of pea, sunflower, carrot, flax, and onion.
Some phytotoxic symptoms as stunted growth, deformations, wilting, chlorosis, spots, necrosis and mortality of plants were observed after 21 days of the exposure.
The following order of the test plant sensitivity was noticed:
flax > sunflower > pea, onion > carrot > corn.

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/PENDIF 599,5 SC on vegetative vigour of terrestrial plants were met:
- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
90.5 – 97.6% – pea,
81.0 – 95.2% – sunflower,
90.0 – 95.0% – carrot,
87.5 – 97.5% – flax,
90.0 – 97.5% – onion,
90.0 – 95.0% – corn,
- 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**