FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: FLD-HER 306 SE

Product name(s): -

Chemical active substance(s):

2,4-D, 300 g/L
florasulam, 6.25 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Pestila Spółka z ograniczoną odpowiedzialnością

Submission date: January 2021

MS Finalisation date: 08/2021; 11/2021

Version history

When	What
August 2021	Finalisation of the assessment by zRMS
November 2021	Final Registration Report

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Spring wheat Spring triticale Spring barley Oat	F	Weeds (detailed information is provided in Part B Section 0 and Section 3)	spraying	Spring BBCH 12-32	1	n.a	Spring 0.4-0.6 L/ha	Spring 2.5-3.75 g florasulam 120-180 g 2,4-D	200-300 L/ha	not relevant	not rele-vant							
2	PL	Winter wheat Winter triticale Winter barley Rye	F	Weeds (detailed information is provided in Part B Section 0 and Section 3)	spraying	Spring BBCH 21-32	1	n.a	Spring 0.4-0.6 L/ha	Spring 2.5-3.75 g florasulam 120-180 g 2,4-D	200-300 L/ha	not relevant	not rele-vant							
3	PL	Maize	F	Weeds (detailed information is provided in Part B Section 0 and Section 3)	spraying	Spring BBCH 12-16	1	n.a.	Spring 0.4-0.6 L/ha	Spring 2.5-3.75 g florasulam 120-180 g 2,4-D	200-300 L/ha	not relevant	not rele-vant							
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)																				

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
-	-	-	-	-	-	-	-	-	-	-	-	-	-							
Minor uses according to Article 51 (interzonal uses)																				
-	-	-	-	-	-	-	-	-	-	-	-	-	-							

* 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

9.1.1 Overall conclusions

zRMS's general comments of review of evaluated dRR

This document is based on the information provided by Applicant and reflects the Applicant's opinion. Clarifications and conclusions of the zRMS are presented in the commenting boxes Amendments/corrections by zRMS are **are marked in blue.**
The zRMS has focused the review on the elements which are crucial for the risk assessment and decision-making; hence, minor errors of no importance for the overall conclusion, or the specific phrasing of the text may not have been commented upon.

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

Birds

Effects on birds for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. However further data on FLD-HER 306 SE is not relevant as data for each active substance on toxicity to birds are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for FLD-HER 306 SE with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

The risk assessment for effects on birds was carried out according to the latest guidance for risk assessment for birds and mammals EFSA Journal 2009; 7(12): 1438.

The acute and reproductive risks of FLD-HER 306 SE to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substances, as well as SV_{90} and SV_m.

Drinking water exposure (leaf scenario) has been estimated from studies with active substances and max. concentration of active substances in working solution. Drinking water exposure (puddle scenario) has not been performed since the ratio of effective application rate to relevant endpoint does not exceed 50 (Koc < 500 L/kg).

Exposure for earthworm-eating birds and fish-eating birds via secondary poisoning was assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substances as well as exposure estimated from predicted environmental concentration of 2,4-D and florasulam in earthworms and fishes.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to birds from the proposed use of FLD-HER 306 SE. No risk management measures are required.

Terrestrial vertebrates (other than birds)

Effects on mammals for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. However further data on FLD-HER 306 SE is not relevant as data for each active substance on toxicity to mammals are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for FLD-HER 306 SE with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

The risk assessment for effects on terrestrial vertebrates other than birds was carried out according to the latest guidance for risk assessment for birds and mammals EFSA Journal 2009; 7(12): 1438.

The acute and reproductive risks of FLD-HER 306 SE to terrestrial vertebrates other than birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with 2,4-D and florasulam, as well as SV_{90} and SV_m. Since preliminary reproductive risk assessment failed further calculations were performed taking into account detailed information on crop, rate and BBCH scale during application. The selected focal species used in refined risk assessment was bank vole.

Drinking water exposure (puddle scenario) has not been performed since the ratio of effective application rate to relevant endpoint does not exceed 50 (Koc < 500 L/kg).

Exposure for earthworm-eating mammals and fish-eating mammals via secondary poisoning was assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with 2,4-D and florasulam as well as exposure estimated from predicted environmental concentration of 2,4-D and florasulam in earthworms and fishes.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to mammals from the proposed use. No risk mitigations are required.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Effects on aquatic organisms for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. Acute toxicity studies of FLD-HER 306 SE to invertebrates, algae and aquatic plants as well as litewrature data for metabolite 4-CP were submitted in this dossier.

Risk assessments for FLD-HER 306 SE with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290.

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation. In case of Step 3 and 4 scenarios: D1, D2, D3, D4, D5 and R1 were taken into account. PECsw/RAC values were less than 1 so it can be concluded that the application of FLD-HER 306 SE does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied.

Scenario	Spring cereals	Winter cereals	Maize
D1	5m buffer zone	mitigation at national level	NR
D2	NR	mitigation at national level	NR
D3	5m buffer zone	5m buffer zone	no buffer zone
D4	no buffer zone	no buffer zone	no buffer zone

D5	no buffer zone	no buffer zone	no buffer zone
R1	NR	no buffer zone	5m vegetated buffer zone

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that FLD-HER 306 SE used at max. rate of 0.6 L/ha to protect cereals and maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition that: 5m buffer zone is applied in case of spring and winter cereals and 5m vegetated buffer zone in case of maize.

Classification of FLD-HER 306 SE was done on the basis of formulation test results as well as active substances properties. The proposed classification of the product FLD-HER 306 SE is:

Aquatic Acute 1, H400
Aquatic Chronic 1, H410

9.1.1.3 Effects on bees (KCP 10.3.1)

Effects on bees for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. Toxicity studies of FLD-HER 306 SE to bees were submitted in this dossier.

Risk assessments for FLD-HER 306 SE with the proposed use pattern was carried out according to the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the latest Draft EFSA Guidance for risk assessment for bees EFSA Journal 2013; 11(7):3295.

The risks of FLD-HER 306 SE to honeybees was assessed from Hazard Quotients (HQ) and Exposure Toxicity Ratio (ETR) between toxicity endpoints, estimated from acute oral and contact studies with active ingredient and formulated product as well as the maximum single application rate of 0.6 L/ha.

All the hazard quotients were considerably less than the respective triggers, indicating that FLD-HER 306 SE at maximum rate of 0.6 L/ha poses a low risk to bees. No risk management measures are required.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Effects on non-target arthropods for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. Toxicity studies of FLD-HER 306 SE to non-target arthropods were submitted in this dossier.

Risk assessments for FLD-HER 306 SE with the proposed use pattern was carried out according to the guidance for risk assessment for arthropods "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and in consideration of the recommendations of the guidance document ESCORT 2.

The in-field and off-field risk of FLD-HER 306 SE to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from studies with active ingredient and the formulated product FLD-HER 306 SE as well as in-field and off-field predicted environmental rate. No risk was determined in-field and off-field after application of FLD-HER 306 SE at maximum rate of 0.6 L/ha. No risk management measures are required.

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

Effects on earthworms and other soil micro-organisms for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. The earthworm chronic toxicity study as well as nitrogen transformation test for FLD-HER 306 SE were submitted in this dossier.

Risk assessments for FLD-HER 306 SE with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Earthworms, collembola and *Hypoapsis*
The acute and chronic risk of FLD-HER 306 SE to earthworms, collembola and *Hypoapsis* was assessed from acute toxicity exposure ratios (TERs) between the selected toxicity endpoint for the active ingredient, metabolites and the formulated product FLD-HER 306 SE as well as the maximum soil PECs.

The acute and chronic TER values were greater than the trigger of 10 and 5 respectively, indicating an acceptable risk to earthworms, collembola and *Hypoapsis* following application of FLD-HER 306 SE at maximum rate of 0.6 L/ha. No risk management measures are required.

Micro-organisms
The risk of FLD-HER 306 SE to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substances, metabolites and the formulated product FLD-HER 306 SE with predicted application concentrations (PECs) obtained for active substances, metabolites and the formulation.

According to the performed risk assessment it was assessed that the application of FLD-HER 306 SE at maximum rate of 0.6 L/ha does not pose unacceptable risk to soil micro-organisms. No risk management measures are required.

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

Effects on non-target terrestrial plants for FLD-HER 306 SE were not evaluated as part of the EU review of 2,4-D and florasulam. The studies on seedling emergence and vegetative vigour for FLD-HER 306 SE were submitted in this dossier.

The risk of FLD-HER 306 SE to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation FLD-HER 306 SE and predicted environmental rate. The TER values were greater than the trigger of 5, indicating an acceptable risk to non-target terrestrial plants following application of FLD-HER 306 SE at maximum rate of 0.6 L/ha. No risk management measures are required.

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 FLD-HER 306 SE

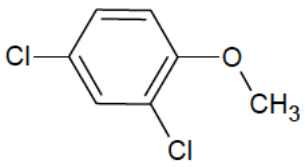
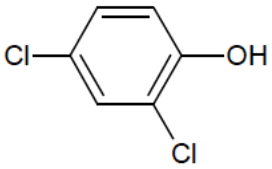
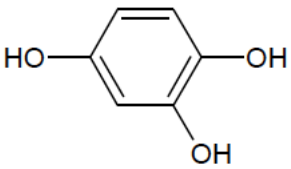
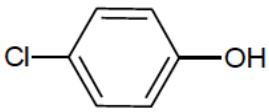
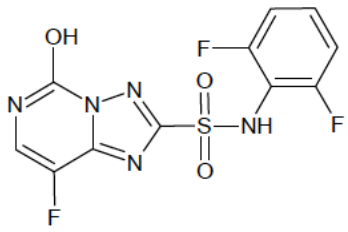
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
risk assessment to birds/mammals acute and long term/reproductive			
cereals/maize	spring cereals, winter cereals, maize	max. application rate	SV_{90}, SV_m
risk assessment to birds - leaf scenario			
cereals/maize	spring cereals, winter cereals, maize	max. concentration of working solution i.e. max. application rate and min. water volume	NR
birds puddle scenario risk assessment			
NR	NR	NR	NR
risk assessment of secondary poisoning for earthworm-eating birds/mammals			
cereals/maize	spring cereals, winter cereals, maize	max. 21-d twa PECs	NR
risk assessment of secondary poisoning for fish-eating birds/mammals			
cereals/maize	spring cereals, winter cereals, maize	max. PECsw	NR
risk assessment to aquatic organisms			
NR	NR	NR	NR
risk assessment to bees			
cereals/maize	spring cereals, winter cereals, maize	max. application rate	NR
risk assessment to non-target arthropods			
cereals/maize	spring cereals, winter cereals, maize	max. application rate	NR
risk assessment to soil macroorganisms			
cereals/maize	spring cereals, winter cereals, maize	max. PECs	NR
risk assessment to soil microorganisms			
cereals/maize	spring cereals, winter cereals, maize	max. PECs	NR

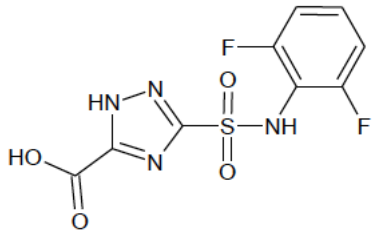
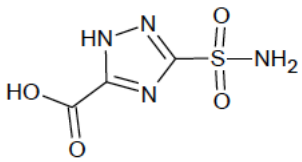
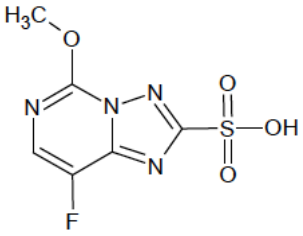
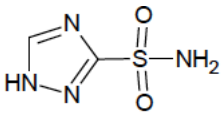
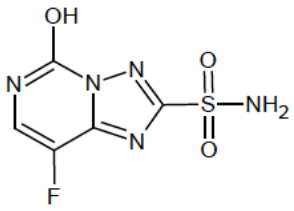
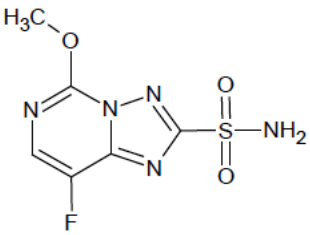
risk assessment to non-target plants			
cereals/maize	spring cereals, winter cereals, maize	max. application rate	NR

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 FLD-HER 306 SE is indicated in the table.

Table 9.1-3 Metabolites of 2,4-D and florasulam

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
2,4-D				
2,4-DCA		177	Soil: 15% Water/sediment: 5.3%	yes
2,4-DCP		163	Soil: 8.7% Water/sediment: 32.1%	yes
1,2,4-benzenetriol		126.1	Soil: NR Water/sediment: 31.7%	yes
4-CP		128.6	Soil: 33% Water/sediment: 6.9%	yes
florasulam				
5-OH Florasu-lam		345.25	Soil: 71.6% Water/sediment: 99%	yes

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
DFP-ASTCA		304.2	Soil: 17.8% Water/sediment: 8.9%	yes
ASTCA		192.13	Soil: 40% Water/sediment: 53.8%	yes
TPSA		248.2	Soil: NR Water/sediment: 58.3%	yes
TSA		148.14	Soil: 15.9% Water/sediment: NR	yes
5-OH-ASTP		233.2	Soil: NR Water/sediment: 28.9%	yes
ASTP		247.2	Soil: 0.0001% Water/sediment: 21.9%	yes

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with 2,4-D and florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of FLD-HER 306 SE were not evaluated as part of the EU assessment. However, the provision of further data on the FLD-HER 306 SE is not considered essential, because it is possible to

extrapolate data from the active substances. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
2,4-D				
Canary (*Serinus canaria*)	2,4-D	Oral, Acute	LD_{50} = 633 mg/kg bw	EFSA Journal 2014;12(9):3812
Japanese quail (*Coturnix coturnix japonica*)	2,4-D	Oral, Acute	LD_{50} = 617.3 mg/kg bw	EFSA Journal 2014;12(9):3812
Bobwhite quail (*Colinus virginianus*)	2,4-D	Oral Acute	LD_{50} = 500 mg/kg bw	EFSA Journal 2014;12(9):3812
-	-	Oral Acute	**LD_{50} = 580.3 mg/kg bw** (geometric mean, n=3)	EFSA Journal 2014;12(9):3812
Northern Bobwhite Mallard duck	2,4-D	Dietary Short-term	LC_{50} > 5620 mg/kg diet	EFSA Journal 2014;12(9):3812
Bobwhite quail (*Colinus virginianus*)	2,4-D	Dietary Long-term	NOEC > 1000 mg/kg diet (NOEL = 100 mg/kg bw/d based on generic conversion factor of 0.1)	EFSA Journal 2014;12(9):3812
Bobwhite quail (*Colinus virginianus*)	2,4-D	Dietary Long-term	NOEC = 1000 mg/kg diet (NOEL > 101 mg/kg bw/d based on study results)	EFSA Journal 2014;12(9):3812
Japanese quail (*Coturnix coturnix japonica*)	2,4-D	Dietary Long-term	NOEC = 1000 mg/kg diet (NOEL = 100 mg/kg bw/d based on study results)	EFSA Journal 2014;12(9):3812
-	-	Dietary Long-term	**NOEL = 58 mg/kg bw/d** (geometric mean LD_{50}/10)	-
Florasulam				
Japanese quail (*Coturnix coturnix japonica*)	florasulam	Oral Acute	**LD_{50} = 1046 mg/kg bw[ab]**	EFSA Journal 2015; 13(1):3984
Mallard duck (*Anas plathyrhynchos*)	FORMULATION EF-1343	Oral Acute	LD_{50} > 2250 mg/kg bw	EFSA Journal 2015; 13(1):3984
Japanese quail *(Coturnix coturnix japonica)*	florasulam	Dietary Short-term	LC_{50} > 938 mg/kg (LC_{50} > 5000 m/kg food)	EFSA Journal 2015; 13(1):3984
Mallard duck (*Anas plathyrhynchos*)	florasulam	Dietary Short-term	LC_{50} > 950 mg/kg bw/d (LC_{50} > 5000 mg/kg food)	EFSA Journal 2015; 13(1):3984
Mallard duck (*Anas plathyrhynchos*)	florasulam	Dietary Long-term	NOEC = 1500 mg/kg food NOEL = 150 mg/kg bw/d (factor: 0.1)	EFSA Journal 2015; 13(1):3984

Japanese quail *(Coturnix coturnix japonica)*	florasulam	Dietary Long-term	NOEC = 1500 mg/kg food NOEL = 150 mg/kg bw/d (factor: 0.1)	EFSA Journal 2015; 13(1):3984
-	-	Dietary Long-term	**NOEL = 104.6 mg/kg bw/d** (LD_{50}/10)	EFSA Journal 2015; 13(1):3984

[a] The worst-case acute oral LD50 value
[b] Endpoint use in long-term risk assessment is LD50 for florasulam of 1046 mg/kg bw divided by 10. The resulting value is lower than the NOEC from reproductive study for florasulam of 1500 mg/kg diet multiplied by a factor 0.1.

zRMS comments:

It should be indicated that for the acute risk assessment the geometric mean value of 580.3 mg/kg bw should be applied (see EFSA conclusion 2014).
The assessment for long-term/reproductive risk should be conducted with the lowest endpoint (58 mg/kg bw based on LD_{50}/10) in accordance with the current EFSA Guidance (2009) (see also EFSA conclusion 2014).

9.2.1.1 Justification for new endpoints

Not relevant. No new endpoints were used.

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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for birds from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FLD-HER 306 SE in cereals and maize (2,4-D endpoints)

Intended uses	spring cereals, winter cereals, maize				
Active substance	2,4-D				
Application rate (kg/ha)	1 × 0.180				
Acute toxicity (mg/kg bw)	580.3				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR, screening	small omnivorous bird	158.8	1	28.6	20.3

Reprod. toxicity (mg/kg bw/d)	58				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR, screening	small omnivorous bird	64.8	1 × 0.53	6.2	9.4

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

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FLD-HER 306 SE in cereals and maize (florasulam endpoints)

Intended use	spring cereals, winter cereals, maize				
Active substance	florasulam				
Application rate (kg/ha)	1 × 0.00375				
Acute toxicity (mg/kg bw)	1046				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR, screening	small omnivorous bird	158.8	1	0.60	1743
Reprod. toxicity (mg/kg bw/d)	104.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR, screening	small omnivorous bird	64.8	1 × 0.53	0.13	804.6

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

zRMS comments:

TER_A values for both active substances 2,4-D Acid and florasulam are higher than the Annex VI trigger value of 10 and 5, when generic focal species are assumed, indicating that active substances poses no acceptable acute and long – term risk to birds following application of FLD-HER306SE at the proposed use rates on cereals and maize in the screening steps.
As it is stated on the GD, no more refinements steps are necessary to be performed.
zRMS verified the combitox toxicity provided by the applicant and presented in the Tables below.

LD_{50} for the mixture of active substances for birds

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	LD_{50} toxicity endpoint (mg as/kg bw)	Fraction of active substance/LD50 for the active substance	LD_{50}mix (mg/kg bw)
florasulam	6.25	0.020	1046	0.00001912	581.69
2.4D	300	0.98	580.3	0.0017	
Total	306.25		sum	0.00171912	

-	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints for FLD-HER 306 SE using the acute toxicity data for birds.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
florasulam	6.25	0.020	1046	52300	581.69	98.89
2.4D	300	0.98	580.3	592.14		1.89
Total	306.25					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

The deviation between the tox per fraction of 2,4-D-2-ethyl-hexyl ester and mixture is < 10 %.
Thus, one active substance (2.4 D) contributes to > 90 % to mixture toxicity.
Consequently, the risk assessment can be performed for the most toxic active substance alone (2.4 D) and the risk from combined exposure from two active substance is covered by 2.4 D risk assessment.

Long-term term toxicity

NOEL for the mixture of active substances for birds.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Long-term toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	NOELmix (mg/kg bw)
florasulam	6.25	0.020	150	0.000133	58.70
2.4D	300	0.98	58	0.0169	
Total	306.25		sum	0.017033	
-					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints for FLD-HER 306 SE using the long-term toxicity data for birds.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Long-termtoxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
florasulam	6.25	0.020	150	7500	58.70	99.22
2.4D	300	0.98	58	59.13		0.73
Total	306.25					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

The deviation between the tox per fraction of 2,4-D-2-ethyl-hexyl ester and mixture is < 10 %. Thus, one active substance contributes to > 90 % to mixture toxicity. Consequently, the risk assessment can be performed for the most toxic active substance alone and the risk from combined exposure from two

active substance is covered by 2.4 D risk assessment.

The product FLD-HER 306 SE is safe for birds when it is used as recommended.

9.2.2.2 Higher-tier risk assessment

Not relevant. First-tier risk assessment confirmed that FLD-HER 306 SE does not pose unacceptable acute and long term/reproductive risk to birds.

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 FLD-HER 306 SE is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario must not 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 58.6 2,4-D belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	180		
Acute toxicity (mg/kg bw)	=	580.3	quotient =	0.31
Reprod. toxicity (mg/kg bw/d)	=	58	quotient =	3.10

With a K(f)oc of 10.35 florasulam belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	3.75		
Acute toxicity (mg/kg bw)	=	1046	quotient =	0.003
Reprod. toxicity (mg/kg bw/d)	=	104.6	quotient =	0.036

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of 2,4-D and florasulam are blow the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However secondary poisoning assessments are required for the two metabolites of 2,4-D for which log P_{ow} are higher than the trigger value of 3 i.e. 3.06 for 2,4-DCP and 3.36 for 2,4-DCA. Risk assessments of secondary poisoning is based on an assumed toxicity i.e. NOEL of 5.8 mg/kg bw/d (ten times higher than toxicity of the parent).

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 group cereals/maize also covers the risk for birds from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.2-4: Assessment of the risk for earthworm-eating birds due to exposure 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCP	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.019	21-TWA PECsoil (ESCAPE v.2)
log P_{ow} / P_{ow}	3.06 / 1148.15	EFSA Journal 2014;12(9):3812
Koc	512	arithmetic mean (n = 7)
foc	0.02	default
BCF_{worm}	1.428	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.027	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.028	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	5.8	value ten times higher than toxicity of the parent
TER_{lt}	203.7	-

TER values shown in bold fall below the relevant trigger.

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCA	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.021	21-TWA PECsoil (ESCAPE v.2)
log P_{ow} / P_{ow}	3.36 / 2290.87	EFSA Journal 2015;13(1): 3984
Koc	1028	arithmetic mean (n = 7)
foc	0.02	default
BCF_{worm}	1.378	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.030	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	5.8	value ten times higher than toxicity of the parent
TER_{lt}	190.89	-

TER values shown in bold fall below the relevant trigger.

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 2,4-DCP and 2,4-DCA in water.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for birds from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.2-6: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCP	comments
PEC_{sw} (mg/L)	0.011	Initial PECsw (wost case)
TWA	0.53	DAR, February 2014
BCF_{fish}	340	EFSA Journal 2014;12(9):3812
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.98	$PEC_{fish} = PEC_{water} \times TWA \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.31	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	5.8	value ten times higher than toxicity of the parent
TER_{lt}	18.7	-

TER values shown in bold fall below the relevant trigger.

Table 9.2-7: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCA	comments
PEC_{sw} (mg/L)	0.004	Initial PECsw (wost case)
TWA	0.53	DAR, February 2014
BCF_{fish}	31	EFSA Journal 2014;12(9):3812
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.07	$PEC_{fish} = PEC_{water} \times TWA \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.01	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	5.8	value ten times higher than toxicity of the parent
TER_{lt}	580	-

TER values shown in bold fall below the relevant trigger.

zRMS comment:

We agree with the calculations provided by the applicant.
The TER_{LT} values exceed the trigger of 5 and an acceptable risk to birds can be concluded for potential exposures to the metabolites arising from bioaccumulation and food chain biomagnification.
The risk for secondary poisoning is considered to be low for 2,4-DCA and 2,4-DCP.

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

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. FLD-HER 306 SE used at max. application rate of 0.6 L/ha with water at amount of 200-300 L/ha to protect cereals and maize according to proposed GAP, does not pose unacceptable risk to birds.

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 2,4-D and florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of FLD-HER 306 SE were not evaluated as part of the EU assessment. However, the provision of further data on the FLD-HER 306 SE is not considered essential, because it is possible to extrapolate data from the active substances. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
2,4-D				
Rat	2,4-D	Oral Acute	LD_{50} = 669 mg/kg bw	EFSA Journal 2014; 12(9):3812
Rat	2,4-D	Oral Acute	LD_{50} = 486 mg/kg bw	EFSA Journal 2014; 12(9):3812
Rat	2,4-D	Oral Acute	LD_{50} > 500 mg/kg bw	EFSA Journal 2014; 12(9):3812
-	-	Oral Acute	**LD_{50} > 554 mg/kg bw** (geometric mean)	EFSA Journal 2014; 12(9):3812
Rat	2,4-D	Dietary Long-term	**NOAEL = 20.6 mg/kg bw/d**	EFSA Journal 2014; 12(9):3812
florasulam				
Mouse	florasulam	Oral Acute	**LD_{50} > 5000 mg/kg bw**	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results	Reference
2,4-D				
Mouse	FORMULATION EF-1343	Oral Acute	LD_{50} > 5000 mg/kg bw	EFSA Journal 2015; 13(1):3984
Rat	florasulam	Dietary Long-term	**NOAEL = 100 mg/kg bw/d**	EFSA Journal 2015; 13(1):3984

9.3.1.1 Justification for new endpoints

Not relevant. No new endpoints were used.

9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for mammals from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of FLD-HER 306 SE in cereals and maize (2,4-D endpoints)

Intended use	spring cereals, winter cereals, maize				
Active substance/product	2,4-D				
Application rate (kg/ha)	1 × 0.180				
Acute toxicity (mg/kg bw)	>554				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR, screening	small herbivorous mammal	136.4	1	24.6	>22.5
Reprod. toxicity (mg/kg bw/d)	20.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR, screening	small herbivorous mammal	72.3	1 × 0.53	6.9	**3**
Cereals BBCH 10-19	small insectivorous mammal "shrew"	4.2	1 × 0.53	0.4	52

Cereals BBCH ≥20	small insectivorous mammal "shrew"	1.9	1 × 0.53	0.2	103
Cereals BBCH 10-29	small omnivorous mammal "mouse"	7.8	1 × 0.53	0.7	29
Cereals BBCH 30-39	small omnivorous mammal "mouse"	3.9	1 × 0.53	0.4	52
Maize BBCH 10-19	small insectivorous mammal "shrew"	4.2	1 × 0.53	0.4	52
Maize BBCH 10-29	small herbivorous mammal "vole"	72.3	1 × 0.53	6.9	**3**
Maize BBCH 10-29	small omnivorous mammal "mouse"	7.8	1 × 0.53	0.7	29

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

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of FLD-HER 306 SE in cereals and maize (florasulam endpoints)

Intended use	spring cereals, winter cereals, maize				
Active substance/product	florasulam				
Application rate (kg/ha)	1 × 0.00375				
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**
NR, screening	small herbivorous mammal	136.4	1	0.51	>9803
Reprod. toxicity (mg/kg bw/d)	100				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR, screening	small herbivorous mammal	72.3	1 × 0.53	0.14	714

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.

9.3.2.2 Higher-tier risk assessment

1st tier long-term risk assessment performed for mammals in maize treated with 2,4-D failed, so further risk mitigations were necessary. Higher tier risk assessment was performed for bank vole (*Myodesglareolus*, previous name *Clethrionomys glareolus*) using reduced TWA value of 0.1065.

In the Tier 1 long-term risk assessment, the default assumption of DT_{50} = 10 days and TWA = 0.53 of 2,4-D on vegetation were used. In order to reduce those values, several decline residue trials in maize were revised and analysed. Details on selected trials and residue results are available in DAR Addendum 2014. According to the results, it was concluded that 2,4-D residues decline rapidly with a mean DT_{50} = 1.55 days and max. DT_{50} = 3.0 days. The mean DT_{50} = 1.55 days and corresponding 21-TWA of 0.1065 were accepted by RMS for refined long-term risk assessment.

Table 9.3-4: Higher-tier assessment of the long-term/reproductive risk for vole due to the use of FLD-HER 306 SE in maize (2,4-D endpoints)

Intended use	maize				
Active substance/product	2,4-D				
Application rate (kg/ha)	1 × 0.180				
Reprod. toxicity (mg/kg bw/d)	20.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	small herbivorous mammal "vole"	72.3	1 × 0.1065	1.385	14.9

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

zRMS comment:

The TER_A values for both active substances are above the trigger of 10, indicating an acceptable risk for mammals.
TER_{LT} value did not exceed the relevant trigger of 5 for cereals and maize from exposure to 2.4 D compound at the screening step, indicating that **an unacceptable** risk to mammals.
Further refinement of the long-term risk based on Tier 1 for use in cereals indicating an acceptable risk but still **an unacceptable** risk for mammals for use in maize.
Therefore, the applicant referred to the residue decline studies provided in maize which were evaluated in the DAR for 2.4 D compound in the process of the renewal of the active substance (EFSA Conclusion 2014). zRMS agrees with refined value f_{twa} of 0.1065 based on DT_{50} value of 1.55 days in maize.
Based on refinement of the risk assessment performed in the Table 9.3-2 above, the TER_{LT} value is above the trigger of 5, indicating an acceptable long-term risk for mammals for use in maize.
It should be indicated that zccording EFSA GD for B&M, 2009 and recommendations given in Central Zone for authorization ppps, the combined risk assessment should be provided for birds and mammals.
zRMS provided the combitox toxicity and presented it in the Tables below.

LD_{50} for the mixture of active substances for mammals.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	LD_{50} toxicity endpoint (mg as/kg bw)	Fraction of active substance/LD50 for the active substance	LD_{50}mix (mg/kg bw)
florasulam	6.25	0.020	5000	0.000004	563.69
2.4D	300	0.98	554	0.00177	
Total	306.25		sum	0.001774	
-					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints for FLD-HER 306 SE using the long-term toxicity data for mammals.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
florasulam	6.25	0.020	5000	250 000	563.69	99.775
2.4D	300	0.98	554	565.303		0.29
Total	306.25					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

The deviation between the tox per fraction of 2,4-D-2-ethyl-hexyl ester and mixture is < 10 %.
Thus, one active substance (2.4 D) contributes to > 90 % to mixture toxicity.
Consequently, the risk assessment can be performed for the most toxic active substance alone (2.4 D) and the risk from combined exposure from two active substance is covered by 2.4 D risk assessment.

Long-term term toxicity

NOEL for the mixture of active substances for mammals.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Long-term toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	NOELmix (mg/kg bw)
florasulam	6.25	0.020	100	0.0002	20.92
2.4D	300	0.98	20.6	0.0476	
Total	306.25		sum	0.0478	
-					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints for FLD-HER 306 SE using the long-term toxicity data for mammals.

Test substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
florasulam	6.25	0.020	100	5000	20.92	99.58
2.4D	300	0.98	20.6	21.02		0.48
Total	306.25					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

The deviation between the tox per fraction of 2,4-D-2-ethyl-hexyl ester and mixture is < 10 %.
Thus, one active substance (2.4 D) contributes to > 90 % to mixture toxicity.
Consequently, the risk assessment can be performed for the most toxic active substance alone (2.4 D) and the risk from combined exposure from two active substance is covered by 2.4 D risk assessment.
In conclusion: The product is safe for FLD-HER 306 SE mammals when it is used as recommended.

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 58.6 2,4-D belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	180		
Acute toxicity (mg/kg bw)	=	554	quotient	= 0.32
Reprod. toxicity (mg/kg bw/d)	=	20.6	quotient	= 8.74

With a K(f)oc of 10.35 florasulam belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	3.75		
Acute toxicity (mg/kg bw)	=	>5000	quotient	= 0.0008
Reprod. toxicity (mg/kg bw/d)	=	100	quotient	= 0.0375

The ratios of effective application rates to relevant endpoints do not exceed 50 so further risk assessment for mammals due to uptake of contaminated drinking water (puddle scenario) is not necessary.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of 2,4-D and florasulam are blow the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However secondary poisoning assessments are required for the two metabolites of 2,4-D for which log P_{ow} are higher than the trigger value of 3 i.e. 3.06 for 2,4-DCP and 3.36 for 2,4-DCA. Risk assessments of secondary poisoning is based on an assumed toxicity i.e. NOEL of 2.06 mg/kg bw/d (ten times higher than toxicity of the parent).

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. Here, the assessment for the use group cereals/maize also covers the risk for mammals from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCP	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.019	21-TWA PECsoil (ESCAPE v.2)
log P_{ow} / P_{ow}	3.06 / 1148.15	EFSA Journal 2014;12(9):3812
Koc	512	Arythmetic mean (n = 7)
foc	0.02	default
BCF_{worm}	1.428	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.027	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.035	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	2.06	value ten times higher than toxicity of the parent
TER_{lt}	59.34	-

TER values shown in bold fall below the relevant trigger.

Table 9.3-6: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and maize

Parameter	2,4-DCA	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.021	21-TWA PECsoil (ESCAPE v.2)
log P_{ow} / P_{ow}	3.36 / 2290.87	EFSA Journal 2015;13(1): 3984
Koc	1028	Arythmetic mean (n = 7)
foc	0.02	Default
BCF_{worm}	1.378	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.037	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	2.06	value ten times higher than toxicity of the parent
TER_{lt}	55.62	-

TER values shown in bold fall below the relevant trigger.

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 2,4-DCP and 2,4-DCA in water.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for mammals from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.3-7: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use cereals and maize

Parameter	2,4-DCP	comments
PEC_{sw} (mg/L)	0.011	Initial PECsw (wost case)
TWA	0.53	DAR, February 2014
BCF_{fish}	340	EFSA Journal 2014;12(9):3812
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.98	$PEC_{fish} = PEC_{water} \times TWA \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.28	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	2.06	value ten times higher than toxicity of the parent
TER_{lt}	7.36	-

TER values shown in bold fall below the relevant trigger.

Table 9.3-8: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use cereals and maize

Parameter	2,4-DCA	comments
PEC_{sw} (mg/L)	0.004	Initial PECsw (wost case)
TWA	0.53	DAR, February 2014
BCF_{fish}	31	EFSA Journal 2014;12(9):3812
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.07	$PEC_{fish} = PEC_{water} \times TWA \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.01	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	2.06	value ten times higher than toxicity of the parent
TER_{lt}	206	-

TER values shown in bold fall below the relevant trigger.

zRMS comment:

We agree with the calculations provided by the applicant.
The TER values exceed the trigger of 5 and an acceptable risk to mammals can be concluded for potential exposures to the metabolites arising from bioaccumulation and food chain biomagnification.
The risk for secondary poisoning is considered to be low for 2,4-DCA and 2,4-DCP.

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

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. FLD-HER 306 SE used at max. application rate of 0.6 L/ha with water at amount of 200-300 L/ha to protect cereals and maize according to proposed GAP, does not pose unacceptable risk to mammals.

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

Not relevant.

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 2,4-D, florasulam and their relevant metabolites. Full details of these studies are provided in the respective EU DAR.

Effects on aquatic organisms of FLD-HER 306 SE were not evaluated as part of the EU assessment. New data for metabolites 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.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – 2,4-D and its metabolites

Species	Substance	Exposure System	Results	Reference
2,4-D				
Pimephales promelas	2,4-D	96 h, f	Mortality **LC_{50}=100 mg/L $_{nom}$**	Y, EFSA Journal 2014;12(9):3812
Pimephales promelas	2,4-D	32 d (ELS), f	Growth **NOEC=63.4mg/L $_{mm}$**	Y, EFSA Journal 2014;12(9):3812
Cyprinus carpio	2,4-D-DMA 600 SL	96 h, s	Mortality LC_{50}>59.9 mg/L $_{mm}$ (LC_{50}>100 mg prod./L $_{nom}$)	Y, EFSA Journal 2014;12(9):3812
Daphnia magna	2,4-D	48 h, s	Mortality **EC_{50} = 134.2 mg/L $_{nom}$**	Y, EFSA Journal 2014;12(9):3812
Daphnia magna	2,4-D	21 d, ss	Reproduction **NOEC = 38.4 mg/L $_{nom}$**	Y, EFSA Journal 2014;12(9):3812

			(NOEC=46.2 mg DMA salt/L $_{nom}$)	
Daphnia magna	2,4-D	21 d, f	Reproduction NOEC = 79 mg/L $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Daphnia magna	2,4-D-DMA 600 SL	48 h, s	Mortality LC_{50}=50.6 mg/L $_{mm}$ (LC_{50}>100 mg prod./L $_{nom}$)	Y, EFSA Journal 2014;12(9):3812
Pseudokirchneriella subcapitata	2,4-D	72 h, s	**Yield: EyC50>78 mg/L $_{mm}$** Growth rate: ErC50>78 mg/L $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Navicula pelliculosa	2,4-D	72 h	Yield: EyC50>100 mg/L $_{nom}$ Growth rate: ErC50>100 mg/L $_{nom}$	Y, EFSA Journal 2014;12(9):3812
Desmodesmus subspicat	2,4-D	72 h	Yield: EyC50>582.2 mg/L $_{mm}$ Growth rate: ErC50>582.2 mg/L $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Skeletonema costatum	2,4-D	72 h, s	**Yield: EyC50= 0.68 mg/L $_{nom}$** Growth rate: ErC50=4.58 mg/L $_{nom}$	Y, EFSA Journal 2014;12(9):3812
Pseudokirchneriella subcapitata	2,4-D-DMA 600 SL	72 h, s	Yield: EyC50>115.35 mg/L (EyC50> 186.65 mg prod./L $_{mm}$) Growth rate: ErC50> 197.8 mg/L (ErC50> 320 mg/L)	Y, EFSA Journal 2014;12(9):3812
Lemna minor	2,4-D	7 d, s	**Fronds, EyC50=10.66 mg/L $_{nom}$** Fronds, ErC50=17.51 mg/L $_{nom}$ Dry weight, EyC50=18.50 mg/L $_{nom}$ Dry weight, ErC50>100 mg/L $_{nom}$	Y, EFSA Journal 2014;12(9):3812
Myriophyllum spicatum	2,4-D	14 d	Total root length, **EC50=0.011 mg/L$^{1}_{nom}$** Total root length, NOEC=0.0047 mg/L$^{1}_{nom}$	Y, EFSA Journal 2014;12(9):3812
Lemna minor	2,4-D-DMA 720 SL	7 d	Fronds, E_yC_{50}=2.7 mg/L$_{nom}$ (E_yC_5=4.6 mg prod./L$_{nom}$) Growth rate, E_yC_{50}= 14.4 mg/L$_{nom}$ (E_yC_{50}= 24.6 mg prod./L$_{nom}$)	Y, EFSA Journal 2014;12(9):3812
2,4-DCA				
Oncorthynchus mykiss	2,4-DCA	96 h	Mortality **LC_{50}>1.4 mg/L $_{mm}$**	Y, EFSA Journal 2014;12(9):3812
Daphnia magna	2,4-DCA	48 h, s	Mortality **LC_{50}=6.4 mg/L $_{mm}$**	Y, EFSA Journal 2014;12(9):3812
Pseudokirchneriella subcapitata	2,4-DCA	72 h, s	**Yield: EyC50= 2.2 mg/L $_{mm}$** Growth rate: ErC50=4.3 mg/L $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Lemna gibba	2,4-DCA	7 d	Fronds, **EC50=2.1 mg/L $_{mm}$**	Y, EFSA Journal 2014;12(9):3812
Myriophyllum aquaticum	2,4-DCA	10 d, s	Shoot length, **EC50=1.16 mg/L $_{mm}$**	Y, EFSA Journal 2014;12(9):3812
2,4-DCP				
Daphnia magna	2,4-DCP	48 h, s	Mortality	Y, EFSA Journal

			LC_{50}=2.8 mg/L $_{nom}$	2014;12(9):3812
Pseudokirchneriella subcapitata	2,4-DCP	72 h, s	Yield: **EyC50= 1.13 mg/L** $_{mm}$ Growth rate: ErC50=3.44 mg/L $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Lemna gibba	2,4-DCP	10 d	Fronds, **EC50=1.5 mg/L** $_{mm}$	Y, EFSA Journal 2014;12(9):3812
Myriophyllum aquaticum	2,4-DCP	10 d, s	Fresh weight, **EC50=12.4 mg/L** $_{mm}$	Y, EFSA Journal 2014;12(9):3812
4-CP				
Oncorhynchus mykiss	4-CP	96 h	**LC_{50} = 1.9 mg/L**	…….. (1984), KCP 10.2.1.1/01
Daphnia magna	4-CP	48 h	**EC_{50} = 2.5 mg/L**	Kühn, R. *et al.* (1989), KCP 10.2.1.2/01
Skeletonema costatum	4-CP	-	**E_rC_{50} = 13.8 mg/L**	Cowgill, U.M. *et al.* (1989), KCP 10.2.1.3/01
Higher-tier studies (micro- or mesocosm studies)				
Not 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

[1]endpoint agreed at the Pesticides Peer Review Meeting 111 (04 – 07 February 2013) and it is the geometric mean value for root length from the available 6 ring test studies with *Myriophyllum*.

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

Test substance/ organism/ time scale	Species	Exposure System	Results	Reference
Florasulam				
Fish Acute	*Oncorhynchus mykiss, Lepomis macrochirus*	96 h	**LC_{50}>100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Invertebrates Acute	*Daphnia magna*	48 h	**EC_{50}>292000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Green Algae	*Scenedesmus subspicatus*	72 h	**EC_{50}= 8.94 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	14 d	**EC_{50}=1.18 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Fish Chronic	*Pimephales promelas*	33 d	**NOEC = 2900 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Invertebrates Chronic	*Daphnia magna*	21 d	**NOEC =23400 µg/L**	Y, EFSA Journal 2015; 13(1):3984

Sediment-dwelling organism Chronic	*Chironomus riparius*	28 d	**NOEC =10000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
5-OH-florasulam				
Fish Acute	*Oncorhynchus mykiss, Lepomis macrochirus*	96 h	**LC_{50}>91000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Invertebrates Acute	*Daphnia magna*	48 h	**EC_{50}>96700 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Green Algae	*Pseudokirchneriella subcapitata*	72 h	**EC_{50}= 21320 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	**EC_{50}=37.8 µg/L**	Y, EFSA Journal 2015; 13(1):3984
DFP-ASTCA				
Invertebrates Acute	*Daphnia magna*	48 h	**EC_{50}>30 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Green Algae	*Pseudokirchneriella subcapitata*	72 h	**EC_{50}= 96000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	EyC_{50}>100000 µg/L **ErC_{50}>100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
ASTCA				
Invertebrates Acute	*Daphnia magna*	48 h	**EC_{50}>30 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Green Algae	*Pseudokirchneriella subcapitata*	72 h & 96 h	EC_{50}> 9200 µg/L E_yC_{50}> 9200 µg/L **E_rC_{50}> 9200 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d & 14 d	**EC_{50}>10200 µg/L**	Y, EFSA Journal 2015; 13(1):3984
TSA				
Invertebrates Acute	*Daphnia magna*	48 h	**EC_{50}=30 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Green Algae	*Pseudokirchneriella subcapitata*	96 h	E_yC_{50}> 94000 µg/L **E_rC_{50}> 94000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
TPSA				
Green Algae	*Pseudokirchneriella subcapitata*	72 h & 96 h	EC_{50}> 100000 µg/L E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984

5-OH-ASTP				
Green Algae	*Pseudokirchneriella subcapitata*	72 h & 96 h	EC_{50}> 100000 µg/L E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
ASTP				
Green Algae	*Pseudokirchneriella subcapitata*	72 h & 96 h	EC_{50}> 100000 µg/L E_yC_{50}> 100000 µg/L **E_rC_{50}> 100000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Aquatic Plants	*Lemna gibba*	7 d	**E_yC_{50}=88000 µg/L**	Y, EFSA Journal 2015; 13(1):3984
Higher-tier studies (micro- or mesocosm studies)				
Not 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 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FLD-HER 306 SE

Species	Substance	Exposure System	Results	Reference
Daphnia magna	FLD-HER 306 SE	48 h, s	**EC_{50} = 21.075 mg/L**	Woźniak A/2019/ Study code: 0005/0068/E, KCP 10.2/04
Pseudokirchneriella subcapitata	FLD-HER 306 SE	72 h, s	E_rC_{50} = 3.332 mg/L **E_yC_{50} = 0.750 mg/L** E_bC_{50} = 4.837 mg/L	Meler A/2019/ Study code: 0005/0069/E, KCP 10.2/05
Lemna gibba	FLD-HER 306 SE	7 d, s	E_rC_{50} = 0.278 mg/L **E_yC_{50} = 0.133 mg/**L	Woźniak A/2019/ Study code: 0005/0070/E, KCP 10.2/06
Myriophyllum spicatum	FLD-HER 306 SE	14 d, s	**Fresh weight** E_rC_{50} = 0.086 mg/l **E_yC_{50} = 0.061 mg/l** **Dry weight** E_rC_{50} = 0.284 mg/l E_yC_{50} = 0.289 mg/l **Shoots length** E_rC_{50} = 0.235 mg/l E_yC_{50} = 0.221 mg/l	Woźniak A/2019/ Study code: 0005/0071/E, KCP 10.2/07
Higher-tier studies (micro- or mesocosm studies)				
Not available.				

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

New endpoints are provided for the formulated product FLD-HER 306 EC and the metabolite 4-CP (literature data). Details of studies and results are included in Table 9.5-1 and Table 9.5-2. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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, 3 and 4 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 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2,4-D for each organism group based on FOCUS Steps 1, 2, 3 and 4 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Algae**	**Higher plants**	**Higher plants**
Test species		***Pimephales promelas***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Skeletonema costatum***	***Lemna minor***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	E_yC_{50}	**E_yC_{50}**	EC_{50}
		100000	63400	134200	38400	78000	680	10660	11
AF		100	10	100	10	10	10	10	10
RAC (µg/L)		1000	6340	1342	3840	7800	68	1066	1.1
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)									
-	57.3071	0.06	0.01	0.04	0.01	0.01	0.84	0.05	52.10
Step 2 – spring cereals (180 g a.s./ha)									
N-Europe	7.2778	0.01	0.00	0.01	0.00	0.00	0.11	0.01	6.62
Step 3 – spring cereals (180 g a.s./ha)									
D1/ditch	1.161	0.00	0.00	0.00	0.00	0.00	0.02	0.00	**1.06**
D1/stream	0.9272	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.84
D3/ditch	1.140	0.00	0.00	0.00	0.00	0.00	0.02	0.00	**1.04**
D4/pond	0.03936	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
D4/stream	0.8757	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.80
D5/pond	0.03936	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
D5/stream	0.9062	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.82
Step 4 – spring cereals (180 g a.s./ha) – 5m buffer zone									
D1/ditch	0.3251	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Algae	Higher plants	Higher plants
D3/ditch	0.3090	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28
Step 1 – winter cereals (180 g a.s./ha)									
-	57.3071	0.06	0.01	0.04	0.01	0.01	0.84	0.05	**52.10**
Step 2 – winter cereals (180 g a.s./ha)									
N-Europe	7.2778	0.01	0.00	0.01	0.00	0.00	0.11	0.01	**6.62**
Step 3 – winter cereals (180 g a.s./ha)									
D1/ditch	8.630	0.01	0.00	0.01	0.00	0.00	0.13	0.01	**7.85**
D1/stream	5.411	0.01	0.00	0.00	0.00	0.00	0.08	0.01	**4.92**
D2/ditch	15.28	0.02	0.00	0.01	0.00	0.00	0.22	0.01	**13.89**
D2/stream	9.819	0.01	0.00	0.01	0.00	0.00	0.14	0.01	**8.93**
D3/ditch	1.139	0.00	0.00	0.00	0.00	0.00	0.02	0.00	**1.04**
D4/pond	0.03935	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
D4/stream	0.8430	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.77
D5/pond	0.03936	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
D5/stream	0.9011	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.82
R1/pond	0.04400	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
R1/stream	0.9838	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.89
Step 4 – winter cereals (180 g a.s./ha) – 5m buffer zone									
D1/ditch	8.630	0.01	0.00	0.01	0.00	0.00	0.13	0.01	**7.85**
D1/stream	5.411	0.01	0.00	0.00	0.00	0.00	0.08	0.01	**4.92**
D2/ditch	15.28	0.02	0.00	0.01	0.00	0.00	0.22	0.01	**13.89**
D2/stream	9.819	0.01	0.00	0.01	0.00	0.00	0.14	0.01	**8.93**
D3/ditch	0.3089	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Algae	Higher plants	Higher plants
Step 1 – maize (180 g a.s./ha)									
-	57.3071	0.06	0.01	0.04	0.01	0.01	0.84	0.05	**52.10**
Step 2 – maize (180 g a.s./ha)									
N-Europe	5.7960	0.01	0.00	0.00	0.00	0.00	0.09	0.01	**5.27**
Step 3 – maize (180 g a.s./ha)									
D3/ditch	0.9446	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.86
D4/pond	0.03814	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
D4/stream	0.8087	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.74
D5/pond	0.03814	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
D5/stream	0.8439	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.77
R1/pond	0.08658	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
R1/stream	1.815	0.00	0.00	0.00	0.00	0.00	0.03	0.00	**1.65**
Step 4 – maize (180 g a.s./ha) – 5m buffer zone									
R1/stream	1.815	0.00	0.00	0.00	0.00	0.00	0.03	0.00	**1.65**
Step 4 – maize (180 g a.s./ha) – 5m vegetated buffer zone									
R1/stream	0.2702	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25

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 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2,4-DCP for each organism group based on FOCUS Steps 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Higher plants**	**Higher plants**
Test species		-	-	***Daphnia magna***	-	***Pseudokirchn. subcapitata***	***Lemna gibba***	***Myriophyllum aquaticum***
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	EC_{50}	EC_{50}
		10000*	-	2800	-	1130	1500	12400
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	-	28	-	113	150	1240
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)								
-	11.1221	0.01	-	0.40	-	0.10	0.07	0.01
Step 1 – winter cereals (180 g a.s./ha)								
-	11.1221	0.01	-	0.40	-	0.10	0.07	0.01
Step 1 – maize (180 g a.s./ha)								
-	11.1221	0.01	-	0.40	-	0.10	0.07	0.01

* The endpoint used for risk assessment for the metabolite 2,4-DCP is the EC_{50} of parent molecule / 10, according to SANCO Guidance Document on Aquatic Ecotoxicology p.49 (European Commission, 2002b)

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2,4-DCA for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Higher plants**	**Higher plants**
Test species		***Oncorthynchus mykiss***	-	***Daphnia magna***	-	***Pseudokirchn. subcapitata***	***Lemna gibba***	***Myriophyllum aquaticum***
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	EC_{50}	EC_{50}
		1400	-	6400	-	2200	2100	1160
AF		100	10	100	10	10	10	10
RAC (µg/L)		14	-	64	-	220	210	116
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)								
-	4.1852	0.30	-	0.07	-	0.02	0.02	0.04
Step 1 – winter cereals (180 g a.s./ha)								
-	4.1852	0.30	-	0.07	-	0.02	0.02	0.04
Step 1 – maize (180 g a.s./ha)								
-	4.1852	0.30	-	0.07	-	0.02	0.02	0.04

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 4-CP for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**-**
Test species		***Oncorthynchus mykiss***	-	***Daphnia magna***	**-**	***Skeletonema costatum***	**-**	**-**
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	EC_{50}	-
		1900	-	2500	-	2200	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		19	-	25	-	220	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	--	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)								
-	6.0368	0.32	-	0.24	-	0.03	-	-
Step 1 – winter cereals (180 g a.s./ha)								
-	6.0368	0.32	-	0.24	-	0.03	-	-
Step 1 – maize (180 g a.s./ha)								
-	6.0368	0.32	-	0.24	-	0.03	-	-

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-benzenetriol for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**-**
Test species		***Pimephales promelas***	**-**	***Daphnia magna***	**-**	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***	**-**
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	EC_{50}	-
		10000	-	13420	-	7800	1.1	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		100	-	134.2	-	780	0.11	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)								
-	10.3651	0.01	-	0.08	-	0.01	**94**	-
Step 2 – spring cereals (180 g a.s./ha)								
N-Europe	1.3163	0.01	-	0.01	-	0.00	**12**	-
Step 3 – spring cereals (180 g a.s./ha)								
D1/ditch	0.2100	0.00	-	0.00	-	0.00	**1.91**	-
D1/stream	0.1677	0.00	-	0.00	-	0.00	**1.52**	-
D3/ditch	0.2062	0.00	-	0.00	-	0.00	**1.87**	-
D4/pond	0.0071	0.00	-	0.00	-	0.00	0.06	-
D4/stream	0.1584	0.00	-	0.00	-	0.00	**1.44**	-
D5/pond	0.0071	0.00	-	0.00	-	0.00	0.06	-
D5/stream	0.1639	0.00	-	0.00	-	0.00	**1.49**	-
Step 4 – spring cereals (180 g a.s./ha) – 5m buffer zone								
D1/ditch	0.0588	0.00	-	0.00	-	0.00	0.53	

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Higher plants	-
D1/stream	0.0623	0.00	-	0.00	-	0.00	0.57	
D3/ditch	0.0559	0.00	-	0.00	-	0.00	0.51	
D4/stream	0.0578	0.00	-	0.00	-	0.00	0.53	
D5/stream	0.0598	0.00	-	0.00	-	0.00	0.54	
Step 1 – winter cereals (180 g a.s./ha)								
-	10.3655	0.01	-	0.08	-	0.01	**94**	-
Step 2 – winter cereals (180 g a.s./ha)								
N-Europe	1.3164	0.01	-	0.01	-	0.00	**12**	-
Step 3 – winter cereals (180 g a.s./ha)								
D1/ditch	1.5610	0.02	-	0.01	-	0.00	**14.19**	-
D1/stream	0.9787	0.01	-	0.01	-	0.00	**8.90**	-
D2/ditch	2.7638	0.03	-	0.02	-	0.00	**25.13**	-
D2/stream	1.7760	0.02	-	0.01	-	0.00	**16.15**	-
D3/ditch	0.2060	0.00	-	0.00	-	0.00	**1.87**	-
D4/pond	0.0071	0.00	-	0.00	-	0.00	0.06	-
D4/stream	0.1525	0.00	-	0.00	-	0.00	**1.39**	-
D5/pond	0.0071	0.00	-	0.00	-	0.00	0.06	-
D5/stream	0.1630	0.00	-	0.00	-	0.00	**1.48**	-
R1/pond	0.0080	0.00	-	0.00	-	0.00	0.07	-
R1/stream	0.1779	0.00	-	0.00	-	0.00	**1.62**	-
Step 4 – winter cereals (180 g a.s./ha) – 5m buffer zone								
D1/ditch	1.5609	0.02	-	0.01	-	0.00	**14.19**	
D1/stream	0.9787	0.01	-	0.01	-	0.00	**8.90**	
D2/ditch	2.7637	0.03	-	0.02	-	0.00	**25.12**	

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Higher plants	-
D2/stream	1.7760	0.02	-	0.01	-	0.00	**16.15**	
D3/ditch	0.0559	0.00	-	0.00	-	0.00	0.51	
D4/stream	0.0557	0.00	-	0.00	-	0.00	0.51	
D5/stream	0.0595	0.00	-	0.00	-	0.00	0.54	
R1/stream	0.1779	0.00	-	0.00	-	0.00	**1.62**	
Step 4 – winter cereals (180 g a.s./ha) – 5m vegetated buffer zone								
R1/stream	0.0496	0.00	-	0.00	-	0.00	0.45	
Step 1 – maize (180 g a.s./ha)								
-	10.3655	0.01	-	0.08	-	0.01	**94**	-
Step 2 – maize (180 g a.s./ha)								
N-Europe	1.0484	0.01	-	0.01	-	0.00	**9.5**	-
Step 3 – maize (180 g a.s./ha)								
D3/ditch	0.1709	0.00	-	0.00	-	0.00	**1.55**	-
D4/pond	0.0069	0.00	-	0.00	-	0.00	0.06	-
D4/stream	0.1463	0.00	-	0.00	-	0.00	**1.33**	-
D5/pond	0.0069	0.00	-	0.00	-	0.00	0.06	-
D5/stream	0.1526	0.00	-	0.00	-	0.00	**1.39**	-
R1/pond	0.0157	0.00	-	0.00	-	0.00	0.14	-
R1/stream	0.3283	0.00	-	0.00	-	0.00	**2.98**	-
Step 4 – maize (180 g a.s./ha) – 5m buffer zone								
D3/ditch	0.0560	0.00	-	0.00	-	0.00	0.51	
D4/stream	0.0616	0.00	-	0.00	-	0.00	0.56	
D5/stream	0.0642	0.00	-	0.00	-	0.00	0.58	
R1/stream	0.3283	0.00	-	0.00	-	0.00	**2.98**	

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Higher plants	-
Step 4 – maize (180 g a.s./ha) – 5m vegetated buffer zone								
R1/stream	0.0489	0.00	-	0.00	-	0.00	0.44	

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for florasulam for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***	***Lemna minor***	**-**
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	-
		100000	2900	292000	23400	8.94	10000	1.18	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	1.2675	0.00	0.00	0.00	0.00	**1.42**	0.00	**10.74**	-
Step 2 – spring cereals (180 g a.s./ha)									
N-Europe	0.0697	0.00	0.00	0.00	0.00	0.08	0.00	0.59	-
Step 1– winter cereals (180 g a.s./ha)									
-	1.2675	0.00	0.00	0.00	0.00	**1.42**	0.00	**10.74**	-
Step 2 – winter cereals (180 g a.s./ha)									
N-Europe	0.0697	0.00	0.00	0.00	0.00	0.08	0.00	0.59	-
Step 1– maize (180 g a.s./ha)									
-	1.2675	0.00	0.00	0.00	0.00	**1.42**	0.00	**10.74**	
Step 2 – maize (180 g a.s./ha)									
N-Europe	0.0594	0.00	0.00	0.00	0.00	0.07	0.00	0.50	-

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 5-OH-florasulam for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		***Oncorhynchus mykiss***	**-**	***Daphnia magna***	**-**	***Pseudokirchneriella subcapitata***	**-**	***Lemna gibba***	**-**
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	-
		91000	-	96700	-	21320	-	37.8	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		910	-	967	-	2132	-	3.78	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	2.0430	0.00	-	0.00	-	0.00	-	0.54	-
Step 1– winter cereals (180 g a.s./ha)									
-	2.0430	0.00	-	0.00	-	0.00	-	0.54	-
Step 1– maize (180 g a.s./ha)									
-	2.0430	0.00	-	0.00	-	0.00	-	0.54	-

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DFP-ASTCA for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		-	-	***Daphnia magna***	-	***Pseudokirchneriella subcapitata***	-	***Lemna gibba***	-
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	-
		-	-	30	-	96000	-	100000	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	0.3	-	9600	-	10000	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	0.2594	-	-	0.86	-	0.00	-	0.00	-
Step 1– winter cereals (180 g a.s./ha)									
-	0.2594	-	-	0.86	-	0.00	-	0.00	-
Step 1– maize (180 g a.s./ha)									
-	0.2594	-	-	0.86	-	0.00	-	0.00	-

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) ASTCA for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		-	-	***Daphnia magna***	-	***Pseudokirchneriella subcapitata***	-	***Lemna gibba***	-
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	-
		-	-	30	-	9200	-	10200	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	0.3	-	920	-	1020	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1 – spring cereals (180 g a.s./ha)									
-	0.5600	-	-	**1.87**	-	0.00	-	0.00	-
Step 2 – spring cereals (180 g a.s./ha)									
N-Europe	0.0661	-	-	0.22	-	0.00	-	0.00	-
Step 1 – winter cereals (180 g a.s./ha)									
-	0.5600	-	-	**1.87**	-	0.00	-	0.00	-
Step 2 – winter cereals (180 g a.s./ha)									
N-Europe	0.0661	-	-	0.22	-	0.00	-	0.00	-
Step 1 – maize (180 g a.s./ha)									
-	0.5600	-	-	**1.87**	-	0.00	-	0.00	-
Step 2 – maize (180 g a.s./ha)									
N-Europe	0.0518	-	-	0.17	-	0.00	-	0.00	-

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) TSA for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		**-**	**-**	***Daphnia magna***	**-**	***Pseudokirchneriella subcapitata***	**-**	***Lemna gibba***	**-**
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	-
		-	-	30	-	94000	-	100000	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	0.3	-	9400	-	10000	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	0.0795	-	-	0.27	-	0.00	-	0.00	-
Step 1– winter cereals (180 g a.s./ha)									
-	0.0795	-	-	0.27	-	0.00	-	0.00	-
Step 1– maize (180 g a.s./ha)									
-	0.0795	-	-	0.27	-	0.00	-	0.00	-

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) TPSA for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		-	-	-	-	***Pseudokirchneriella subcapitata***	-	***Lemna gibba***	-
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	-
		-	-	-	-	100000	-	100000	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	-	-	10000	-	10000	-
FOCUS Scenar-io	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	0.4909	-	-	-	-	0.00	-	0.00	-
Step 1– winter cereals (180 g a.s./ha)									
-	0.4909	-	-	-	-	0.00	-	0.00	-
Step 1– maize (180 g a.s./ha)									
-	0.4909	-	-	-	-	0.00	-	0.00	-

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASTP for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		***Oncorhynchus mykiss***	-	-	-	***Pseudokirchneriella subcapitata***	-	***Lemna gibba***	-
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	-
		-	-	-	-	100000	-	88000	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	-	-	10000	-	8800	-
FOCUS Scenar-io	**PEC $_{gl\text{-}max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	0.1795	-	-	-	-	0.00	-	0.00	-
Step 1– winter cereals (180 g a.s./ha)									
-	0.1795	-	-	-	-	0.00	-	0.00	-
Step 1– maize (180 g a.s./ha)									
-	0.1795	-	-	-	-	0.00	-	0.00	-

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) 5-OH-ASTP for each organism group based on FOCUS Step 1 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**	**-**
Test species		-	-	-	-	***Pseudokirchneriella subcapitata***	-	***Lemna gibba***	-
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	-
		-	-	-	-	100000	-	100000	-
AF		100	10	100	10	10	10	10	-
RAC (µg/L)		-	-	-	-	10000	-	10000	-
FOCUS Scenar-io	**PEC $_{gl-max}$ (µg/L)**	-	-	-	-	-	-	-	-
Step 1– spring cereals (180 g a.s./ha)									
-	0.2189	-	-	-	-	0.00	-	0.00	-
Step 1– winter cereals (180 g a.s./ha)									
-	0.2189	-	-	-	-	0.00	-	0.00	-
Step 1– maize (180 g a.s./ha)									
-	0.2189	-	-	-	-	0.00	-	0.00	-

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for FLD-HER 306 SE for each organism group based on FOCUS Steps 1, 2, 3 and 4 calculations for the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Algae**	**Higher plants**	**Higher plants**
Test species		-	-	***Daphnia magna***	-	***Pseudokirchn. subcapitata***	-	***Lemna gibba***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	E_yC_{50}	E_yC_{50}	E_yC_{50}
		-	-	21075	-	750	-	133	61
AF		100	10	100	10	10	10	10	10
RAC (µg/L)		-	-	210.75	-	75	-	13.3	6.1
Spring cereals (180 g a.s./ha)									
ditch	4.0989	-	-	0.02	-	0.05	-	0.31	0.67
pond	0.1398	-	-	0.00	-	0.00	-	0.01	0.02
stream	3.0419	-	-	0.01	-	0.04	-	0.23	0.50
Winter cereals (180 g a.s./ha)									
ditch	4.0989	-	-	0.02	-	0.05	-	0.31	0.67
pond	0.1398	-	-	0.00	-	0.00	-	0.01	0.02
stream	3.0419	-	-	0.01	-	0.04	-	0.23	0.50
Step 3 – maize (180 g a.s./ha)									
ditch	3.3890	-	-	0.02	-	0.05	-	0.25	0.56
pond	0.1354	-	-	0.00	-	0.00	-	0.01	0.02
stream	2.6397	-	-	0.01	-	0.04	-	0.20	0.43

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, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies. For few of them further assessment at national level is still necessary.

9.5.3 Overall conclusions

PECsw/RAC values were calculated with PECsw values obtained for active substances and their metabolites calculated in Step 1, 2, 3 and 4. In case of Step 3 and 4 scenarios: D1, D2, D3, D4, D5 and R1 were taken into account. Most of the PECsw/RAC values were below 1 for acute and long-term risk thus indicating no unacceptable risk to aquatic organisms from the proposed uses under condition that appropriate risk mitigations were applied:

Scenario	Spring cereals	Winter cereals	Maize
D1	5m buffer zone	mitigation at national level	NR
D2	NR	mitigation at national level	NR
D3	5m buffer zone	5m buffer zone	no buffer zone
D4	no buffer zone	no buffer zone	no buffer zone
D5	no buffer zone	no buffer zone	no buffer zone
R1	NR	no buffer zone	5m vegetated buffer zone

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that FLD-HER 306 SE used at the max. rate of 0.6 L/ha to protect cereals and maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition that: 5m buffer zone is applied in case of spring and winter cereals and 5m vegetated buffer zone in case of maize.

zRMS comments:

We agree with the risk assessment provided for both active substances and their metabolites:
PECsw/RAC values were calculated with PEC_{sw} values obtained for active substances and their metabolites calculated in Step 1, 2, 3 and 4. In case of Step 3 and 4 scenarios: D1, D2, D3, D4, D5 and R1 were taken into account. Most of the PECsw/RAC values were below 1 for acute and long-term risk thus indicating no unacceptable risk to aquatic organisms from the proposed uses under condition that appropriate risk mitigations were applied:

Scenario	Spring cereals	Winter cereals	Maize
D1	5m buffer zone	mitigation at national level	NR
D2	NR	mitigation at national level	NR
D3	5m buffer zone	5m buffer zone	no buffer zone
D4	no buffer zone	no buffer zone	no buffer zone
D5	no buffer zone	no buffer zone	no buffer zone
R1	NR	no buffer zone	5m vegetated buffer zone

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that FLD-HER 306 SE used at the max. rate of 0.6 L/ha to protect cereals and maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition that: 5m buffer zone is applied in case of spring and winter cereals and 5m vegetated buffer zone in case of maize.

In addition, the mixture toxicity assessment is required according to AGD, 2013.
The zRMS provided these calculations and presented below:

Mixture toxicity – Risk assessment approach from simultaneous exposure to two active substances.

The evaluation of potential mixture toxicity is performed under consideration of the current EFSA guidance (2013).

The mixture toxicity assessment was provided by zRMS for the most sensitive organism-Lemna gibba and algae.

A ´toxicity per fraction´ assessment is performed providing information on the relative contribution of the active substances to the overall toxicity of the mixture based on the fractions of active substances as in the formulated product by assuming concentration addition (CA). For detailed explanation of the calculations reference is made to the EFSA birds and mammals guidance (2009) or to the explanations provided in the Birds & Mammals chapters of this submission.

A surrogate endpoint for CA is calculated using the following equation.

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

With:

$EC_{X\ mix-CA}$	surrogate endpoint for additive mixture toxicity
n	number of mixture components
i	index from 1…n mixture components
pi	the ith component as a relative fraction of the mixture composition (Σ pi = 1)
EC_{Xi}	concentration of component I provoking X % effect (or NOECi)

Fractions in the mixture are calculated according to the following equation with the sum of fractions adding up to 1.

$$p_1 = c_1/c_1 + \cdots + c_n$$

Based on active substance concentrations of 6,25 g florasulam/L and 300g of 2.4D/L, fractions (pi) of 0.2, 0.98 respectively are calculated for the composition as in the formulated product.

The surrogate endpoint is related to the measured ECX or NOEC (ECX PPP) from product studies, where available, building the Model Deviation Ratio (MDR).

$$MDR = \frac{EC_{X\ mix-CA}}{EC_{X\ PPP}}$$

With an MDR in the range of 0.2 to 5 the predicted endpoint for CA is interpreted as to be in line with the measured toxicity. Values below 0.2 indicate a potential antagonism (i.e. CA overestimates mixture toxicity), whereas values greater than 5 might indicate a potential synergism (i.e. CA potentially underestimates mixture toxicity).

The EFSA guidance further requests to evaluate the relevance of formulation toxicity data for the active substance composition at PECmix.

$$PEC_{mix} = \sum_{i=1}^{n} PEC_i$$

Measured toxicity data for the product in principal are considered relevant for mixture toxicity assessments only in case the mixture composition in the formulation is similar to the mixture composition at PECmix; i.e. if the ratio of calculated mixture toxicity (based on CA) for both mixture compositions does not deviate by more than 20%, respectively if:

$$\frac{EC_{X\ mix-CA\ for\ PPP}}{EC_{X\ mix-CA\ for\ PEC_{mix}}} = 0.8 - 1.2$$

The ECX mix-CA for PECmix is calculated based on relative proportions of individual actives at PECmix.

The mixture toxicity assessment is presented below:

Summary of results obtained in the studies with the formulated product FLD-HER 306 SE and comparison of calculated and measured mixture toxicity – *Lemna* and algae.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of FLD-HER 306 SE ($LC_{50\ Konik}$ or $EC_{50\ CKonik}$) (mg/L)	Measured toxicity of FLD-HER 306 SE (converted to be a.i. based) ($LC_{50\ Konik}$ or $EC_{50\ Konik}$) (mg a.s./L)	Calculated mixture toxicity[a] $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$	Model deviation ratio (MDR = $EC_{50\ mix-CA}$ / $EC_{50Konik}$)
L. gibba	7d E_rC_{50}	0.086	0.088	0.058	0.66
Algea	7d E_rC_{50}	3.32	1.017	0.401	0.39

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Florasulam 6.25 g/L*)* and 2,D (300 g/L) within the formulation.

Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in FLD-HER 306 SE) $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$	Calculated mixture toxicity (a.s. in PEC_{mix})[b] $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$ at lower exposure tier	Factors ($EC_{50\ mix-CA}$ (a.s. in FLD-HER 306 SE)/$EC_{50\ mix-CA}$ (a.s. in PEC_{mix})) at lower exposure tier
P. subcapitata	7d E_rC_{50}	0.058	0.124	0.512
L. gibba	7d E_rC_{50}	0.401	0.783	0.467

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Florasulam (6.25 g/L*)* and 2.4 D (300 g/L) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Florasulam (0.0000697 mg/L at Step 2 for NEU scenario) and 2,4 D (0.00727 mg/L at Step 2 for NEU scenario).

Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test	LC_{50} / EC_{50} [mg/L]

	system	Calculated mixture toxicity (a.s. in Konik) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated toxicity per fraction of Konik (based on each a.s.) $(1/TU_i)^a$	Deviation from mixture toxicity $(1\text{-}EC_{x\ mix\text{-}CA} \times (1/EC_{x\ mix\text{-}CA} - TU_i))$ [%]
P. subcapitata	E_rC_{50}, static, 72 h	0.058	Florasulam 0.438 2,4 D: 4.675	Florasulam: 91.4% 2.4 D: 8.6 %
L. gibba	E_rC_{50}, semi static 7d	0.401	Florasulam: 0.058 2,4 D: 17.875	Florasulam: 99.7% 2.4D: 17.875 %

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ Konik}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of Florasulam n (6.25 g/L*)* and 2.4 D (300 g/L) within the formulation.

For algae and macrophytes, the product **FLD-HER 306 SE** does not show an increased toxicity and the assumed mixture toxicity is driven by a single active substance, in this case florasulam. Therefore, mixture toxicity assessments are also not deemed necessary for these organism groups when accounting for the active substance ratios as in the formulated product. No additional calculation are required.

Overall conclusion of the risk assessment for aquatic organism:

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that FLD-HER 306 SE used at the max. rate of 0.6 L/ha does not pose unacceptable risk to aquatic organisms under condition that:
-5m buffer zone is applied in case of spring and winter cereals and
-5m vegetated buffer zone in case of maize.

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 2,4-D and florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of FLD-HER 306 SE were not evaluated as part of the EU assessment. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
2,4-D				
Bees	2,4-D	Oral, 48h	LD_{50} = 94 µg/bee	EFSA Journal 2014; 12(9):3812
Bees	2,4-D	Contact, 48h	LD_{50} > 100 µg/bee	EFSA Journal 2014; 12(9):3812
florasulam				

Bees	florasulam	Oral, 48h	LD_{50} > 100 µg/bee	EFSA Journal 2015; 13(1):3984
Bees	florasulam	Contact, 48h	LD_{50} > 100 µg/bee	EFSA Journal 2015; 13(1):3984
FLD-HER 306 SE				
Bees	FLD-HER 306 SE	Oral, 48h	LD_{50} > 100 µg/bee	Orzechowska U/2019/Study code: 0005/0072/E
Bees	FLD-HER 306 SE	Contact, 48h	LD_{50} > 100 µg/bee	Orzechowska U/2019/Study code: 0005/0073/E
Bees	FLD-HER 306 SE	Feeding, 10d	LDD_{50}>86.939 µg/bee/day	Orzechowska U/2019/Study code: 0005/0075/E
Higher-tier studies (tunnel test, field studies)				
Not available.				

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product FLD-HER 306 EC. Details of studies and results are included in Table 9.6-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.6.2 Risk assessment

The evaluation of the acute 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). Since for chronic exposure no adopted guidelines are available, chronic risk assessment was performed according to Draft EFSA Guidance (EFSA Journal 2013;11(7):3295) - although this has not been adopted.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for bees from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Acute risk to adult honeybees

The acute risk to honeybees from use of FLD-HER 306 SE was assessed using the maximum single application rate and the oral and contact LD_{50} values. A Hazard Quotient (HQ) of less than 50 indicates a low risk to bees.

Table 9.6-2: First-tier acute assessment of the risk for bees due to the use of FLD-HER 306 SE in cereals and maize (2,4-D endpoints)

Intended use	spring cereals, winter cereals, maize

Active substance	2,4-D		
Application rate (kg/ha)	1 × 0.180		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Trigger HQ ≤ 50**
Oral toxicity	94	180	1.9
Contact toxicity	>100		<1.8

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

Table 9.6-3: First-tier acute assessment of the risk for bees due to the use of FLD-HER 306 SE in cereals and maize (florasulam endpoints)

Intended use	spring cereals, winter cereals, maize		
Active substance	florasulam		
Application rate (kg/ha)	1 × 0.00375		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Trigger HQ ≤ 50**
Oral toxicity	>100	3.75	<0.04
Contact toxicity	>100		<0.04

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

Table 9.6-4: First-tier acute assessment of the risk for bees due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use	spring cereals, winter cereals, maize		
Product	FLD-HER 306 SE		
Application rate (kg/ha)	1 × 0.638* ml		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Trigger HQ ≤ 50**
Oral toxicity	>100	638	<6.38
Contact toxicity	>100		<6.38

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* application rate calculated on the basis of density 1.0641 g/ml (see dRR Part B 0,1-4)

All Hazard Quotients are considerably less than 50, indicating that the active substances and formulated product FLD-HER 306 SE does not pose an unacceptable acute risk to bees.

zRMS comments:

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).
The submitted risk assessment based on laboratory studies has been accepted by zRMS.
HQ values are below trigger indicating an acceptable risk to bees.

Chronic risk to adult honeybees

The chronic risk to honeybees from use of FLD-HER 306 SE was assessed using the maximum single application rate and the chronic LDD_{50} value. An Exposure Toxicity Ratio (ETR) of less than 0.03 indicates a low risk to bees.

Table 9.6-5: Screening chronic assessment of the risk for adult bees due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use **Product** **Application rate (kg/ha)**	spring cereals, winter cereals, maize FLD-HER 306 SE 1 × 0.638*		
10 Day LDD_{50} (µg product/bee/day)	>86.939		
Scenario	**SV**	**ETR**	**Trigger ≤ ETR**
NR	7.6	**0.055**	0.03

The Exposure Toxicity Ratio value was considerably higher than 0.03, indicating that the formulated product FLD-HER 306 SE may pose an unacceptable chronic risk to bees hence first-tier risk assessment is required. The first-tier risk assessment was performed for five scenarios: foraging on the treated crop, foraging on an adjacent crop, foraging on weeds in the treated field, foraging in the field margin and foraging the following year on a permanent crop or on a succeeding crop for annual crops.

Table 9.6-6: First-tier chronic assessment of the risk for adult bees due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use **Product** **Application rate (kg/ha)**	spring cereals, winter cereals, maize FLD-HER 306 SE 1 × 0.638*				
10 Day LDD_{50} (µg product/bee/day)	>86.939				
Scenario	**Ef**	**SV**	**TWA**	**ETR**	**Trigger ≤ ETR**
Treated Crop, cereals	1	0.92	0.72	0.001	0.03
Treated Crop, maize	1	5.8	0.72	0.030	0.03
Adjacent Crop	0.0033	5.8	0.72	0.000	0.03
Weeds in crop, cereals	1	2.9	0.72	0.015	0.03
Weeds in crop, maize	1	2.9	0.72	0.015	0.03
Field Margin	0.0092	2.9	0.72	0.000	0.03
Following year	1	0.54	0.72	0.003	0.03

* application rate calculated on the basis of density 1.0641 g/ml (see dRR Part B 0,1-4)

All Exposure Toxicity Ratios are considerably less than 0.03, indicating that the formulated product FLD-HER 306 SE does not pose an unacceptable chronic risk to bees.

Chronic risk to bee larvae

The chronic risk to bee larvae from use of FLD-HER 306 SE was assessed using the maximum single application rate and the chronic NOE~~L~~D value. An Exposure Toxicity Ratio (ETR) of less than 0.2 indicates a low risk to bee larvae.

Table 9.6-7: Screening chronic assessment of the risk for bee larvae due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use	spring cereals, winter cereals, maize		
Product	FLD-HER 306 SE		
Application rate (kg/ha)	1 × 0.638*		
NOEC (µg product/larva/day)	>100		
Scenario	**SV**	**ETR**	**Trigger ≤ ETR**
NR	4.4	0.028	0.2

* application rate calculated on the basis of density 1.0641 g/ml (see dRR Part B 0,1-4)

The Exposure Toxicity Ratio was considerably less than 0.2, indicating that the formulated product FLD-HER 306 SE does not pose an unacceptable chronic risk to bee larvae.

zRMS comment:

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees) \ the chronic test on bees and chronic test for larvae for formulated product are required.
The risk assessment provided by the applicant based on the 10 Day LDD_{50} of 86.939 (µg product/bee/day) at Tier 1 indicated acceptable risk as the ETR value < trigger of 0.03.
In case of chronic risk for larvae based on NOED of 100 µg product/larva/day value it can be concluded acceptable risk as the ETR value at Tier 1 trigger <0.2.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

The acute risk of FLD-HER 306 EC to honeybees was assessed from HQ between toxicity endpoints, estimated from acute oral and contact studies with active ingredients and formulated product as well as the maximum single application rate. The HQ values were considerably less than 5 that means product FLD-HER 306 SE does not pose unacceptable acute oral and contact risk to honeybees.

The chronic risk of FLD-HER 306 EC to honeybees was assessed from ETR between exposure and chronic toxicity endpoint, estimated from 10d chronic study with formulated product FLD-HER 306 SE.

The ETR values were considerably less than 0.03. Results indicate that the product does not pose unacceptable chronic risk to bees.

The chronic risk of FLD-HER 306 EC to bee larvae was assessed from ETR between exposure and chronic toxicity endpoint, estimated from bee larvae chronic toxicity study with formulated product FLD-HER 306 SE. The ETR values were considerably less than 0.2. Results indicate that the product does not pose unacceptable chronic risk to bee larvae.

It can be concluded that FLD-HER 306 SE used at max. application rate of 0.6 L/ha to protect cereals and maize according to proposed GAP, does not pose unacceptable risk to bees and bee larvae. No risk mitigations are required.

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 2,4-D and florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of FLD-HER 306 SE were not evaluated as part of the EU assessment. 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.

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

Species	Substance	Exposure System	Results	Reference
2,4-D				
Aphidius rhopalosiphi	2,4-D DMA 600SL	Laboratory test, glass cover slides (2D)	LR_{50} > 3000 g as/ha	EFSA Journal 2014;12(9):3812
Typhlodromus pyri	2,4-D DMA 600SL	Laboratory test, glass plates (2D)	LR_{50} > 3000 g as/ha	EFSA Journal 2014;12(9):3812
Poecilus cupreus	Herbizid Marks	Laboratory test, Arenas containing sand, plastic trays 14 days (3D)	Mortality 0% and 29.6% effect on feeding at rate 1000 g as/ha	EFSA Journal 2014;12(9):3812
Aleochara bilineata	Herbizid Marks	Laboratory test, Arenas containing sand, glass Beakers 4 weeks + 5 weeks (3D)	Mortality 0% and 1.3% effect on beneficial capacity at rate 1000 g as/ha	EFSA Journal 2014;12(9):3812
Pardosa ssp.	Herbizid Marks	Laboratory test, Arenas containing sand, plastic containers	Mortality 5% and 0% effect on food consumption at rate 1000 g as/ha	EFSA Journal 2014;12(9):3812

		14 days (3D)		
florasulam				
Aphidius rhopalosiphi	Formulation EF-1343	Laboratory test (2D)	LR_{50} > 15 g as/ha	EFSA Journal 2015; 13(1):3984
Typhlodromus pyri	Formulation EF-1343	Laboratory test (2D)	LR_{50} > 15 g as/ha	EFSA Journal 2015; 13(1):3984
Poecilus cupreus	Formulation EF-1343	Laboratory test (2D)	Mortality 0% and sublethal effects 1.07% at rate 0 g as/ha. Mortality 0% and sublethal effects 1.12% at rate 7.5 g as/ha. Mortality 0% and sublethal effects 1.33% at rate 15 g as/ha.	EFSA Journal 2015; 13(1):3984
Chrysoperla carnea	Formulation EF-1343	Laboratory test (2D)	Mortality 14.7% and sublethal effects 19.8% at rate 0 g as/ha. Mortality 6.9% and sublethal effects 4.4% at rate 7.5 g as/ha. Mortality 21.4% and sublethal effects 0% at rate 15 g as/ha.	EFSA Journal 2015; 13(1):3984
Episyrphus balteatus	Formulation EF-1343	Extended laboratory test (3D)	Mortality 4% and sublethal effects 29.6% at rate 0 g as/ha. Mortality 2% and sublethal effects 32% at rate 7.5 g as/ha. Mortality 2% and sublethal effects 25.4% at rate 15 g as/ha.	EFSA Journal 2015; 13(1):3984
FLD-HER 306 SE				
Aphidius rhopalosiphi	FLD-HER 306 SE	Extended laboratory test (3D)	Mortality: LR_{50} = 2658.4 ml/ha Fecundity: NOER = 1080 ml/ha	Kręglewska M/2019/ Study code: 0005/0078/E
Typhlodromus pyri	FLD-HER 306 SE	Extended laboratory test (~~3~~2D)	Mortality: LR_{50} = 5383.3 ml/ha Reproduction: LR_{50} = 5805.3 ml/ha	Kręglewska M/2019/ Study code: 0005/0077/E
Coccinella septempunctata	FLD-HER 306 SE	Extended laboratory test (~~3~~2D)	Mortality: LR_{50} = 5796.30 ml/ha	Rovetto I/2019/ Study code: 1075-

			Fecundity: no effect up to rate 1200 ml/ha	1075HSAG19/r
Chrysoperla carnea	FLD-HER 306 SE	Extended laboratory test (3D, 2D)	Mortality: LR_{50} = 107913.80 mL/ha Fecundity: ER_{50} = 983.40 ml/ha	Rovetto I/2019/ Study code: 1074-1074HSAG19/r
Field or semi-field tests				
Not available.				

9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product FLD-HER 306 EC. Details of studies and results are included in Table 9.7-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for non-target arthropods from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (2,4-D endpoints)

Intended use	spring cereals, winter cereals, maize
Active substance	2,4-D
Application rate (kg/ha)	1 × 0.180
MAF	1

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>3000	180	<0.06
Aphidius rhopalosiphi	>3000		<0.06
Test species Tier I	**Rate with ≤ 50 % effect* (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	1000	180	yes
Aleochara bilineata	1000	180	yes
Pardosa ssp.	1000	180	yes

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.

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (florasulam end-points)

Intended use	spring cereals, winter cereals, maize		
Active substance	florasulam		
Application rate (kg/ha)	1 × 0.00375		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>15	3.75	<0.25
Aphidius rhopalosiphi	>15		<0.25
Test species Tier I	**Rate with ≤ 50 % effect* (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	15	3.75	yes
Chrysoperla carnea	15	3.75	yes
Episyrphus balteatus	15	3.75	yes

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.

Table 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use	spring cereals, winter cereals, maize		
Product	FLD-HER 306 SE		
Application rate (kg/ha)	1 × 0.638* (0.6 L/ha)		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	5728.4* (5383.3 ml/ha)	638 (600 ml/ha)	0.11
Aphidius rhopalosiphi	2828.8* (2658.4 ml/ha)		0.23
Coccinella septempunctata	6167.8* (5796.30 ml/ha)		0.10
Chrysoperla carnea	114831.1* (107913.80 ml/ha)		0.006

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.
* application rate and LR_{50} calculated on the basis of density 1.0641 g/ml (see dRR Part B 0,1-4)

zRMS comment:

The risk assessment provided by the applicant was verified by zRMS according to recommendation given in Escort 2. Therefore, the own zRMS's assessment are provided in the Tables below:

Higher-tier assessment of the in-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use	spring cereals, winter cereals, maize
Product	FLD-HER 306 SE
Application rate (kg/ha)	1 × 0.638* (0.6 L/ha)
MAF	1

Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**$PER_{in-field}$ (L f.p./ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?****
Typhlodromus pyri	5728.4* (5383.3 ml/ha)	638 (600 ml/ha)	Yes
Aphidius rhopalosiphi	2828.8* (2658.4 ml/ha)		Yes
Coccinella septempunc-tata	6167.8* (5796.30 ml/ha)		Yes
Chrysoperla carnea	114831.1* (107913.80 ml/ha)		Yes

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.
* application rate and LR_{50} calculated on the basis of density 1.0641 g/ml (see dRR Part B 0,1-4)
** 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.

The calculations of the risk assessment for in – field for **FLD-HER** for two indicator species and two additional species are acceptable.
The PER-in (based on the extended laboratory studies) are below the rate with ≤ 50 % effects.
Therefore, this assessment indicates that **FLD-HER** poses low risk to in-field to non-target arthropods following application according to the proposed use patterns.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for non-target arthropods from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (2,4-D endpoints)

Intended use	spring cereals, winter cereals, maize
Active substance	2,4-D
Application rate (kg/ha)	1 × 0.180
MAF	1
vdf	10 (Tier 1)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>3000	2.77	0.50	10	<0.002
Aphidius rhopalosiphi	>3000				<0.002

Test species Tier II	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	1000	2.77	0.50	NR	yes
Aleochara bilineata	1000				yes
Pardosa ssp.	1000				yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-6: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (florasulam endpoints)

Intended use	spring cereals, winter cereals, maize
Active substance	florasulam
Application rate (kg/ha)	1 × 0.00375
MAF	1
vdf	10 (Tier 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 ≤ 2**
Typhlodromus pyri	>15	2.77	0.01	10	<0.007
Aphidius rhopalosiphi	>15				<0.007

Test species Tier II	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	15	2.77	0.01	NR	yes
Chrysoperla carnea	15				yes
Episyrphus balteatus	15				yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-7: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FLD-HER 306 SE in cereals and maize (FLD-HER 306 SE endpoints)

Intended use	spring cereals, winter cereals, maize
Active substance/product	FLD-HER 306 SE
Application rate (kg/ha)	1 × ~~0.~~638* (0.6 L/ha)
MAF	1
vdf	10 (Tier 1)

Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Aphidius rhopalosiphi	5728.4* (5383.3 ml/ha)	2.77	1.77 (1.66 ml/ha)	10	0.003

Typhlodromus pyri	2828.8* (2658.4 ml/ha)				0.006
Coccinella septempunctata	6167.8* (5796.30 ml/ha)				0.003
Chrysoperla carnea	114831.1* (107913.80 ml/ha)				0.0002

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.

zRMS comment:

The risk assessment provided by the applicant was verified by zRMS according to recommendation given in Escort 2. Therefore, the own risk assessment zRMS are provided in the Tables below:

Intended use	spring cereals, winter cereals, maize
Active substance/product	FLD-HER 306 SE
Application rate (g/ha)	1 × 0.638* (0.6 L/ha)
MAF	1
vdf	10 (Tier 1) for 2D, 1 for 3D

Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>10.20		1.77		yes
Aphidius rhopalosiphi	>8.00		17		yes
Coccinella septempunctata	6167.8* (5796.30 ml/ha)		1.77	5	yes
Chrysoperla carnea	114831.1* (107913.80 ml/ha)		1.77		yes

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.

The PER_{off}-field corrected for two indicator species T.Pyri and A. rhopalosiphi and two additional species (based on the extended laboratory studies) are below the rate with ≤ 50 % effects. Therefore, this assessment indicates that poses low risk to off-field non-target arthropods following application according to the proposed use patterns.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk of FLD-HER 306 EC to non-target arthropods was assessed from in-field and off-field HQ between toxicity endpoints, estimated from extended laboratory studies with active ingredients and formulated product as well as the maximum single application rate. The HQ values were considerably less than 2, indicating that the product poses a low risk to non-target arthropods. It can be concluded that FLD-HER 306 SE used at max. application rate of 0.6 L/ha to protect cereals and maize according to proposed GAP, does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk mitigations are required.

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 2,4-D and florasulam their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FLD-HER 306 SE were not evaluated as part of the EU assessment. 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.

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
2,4-D				
Eisenia fetida	2,4-D	14 d, acute	**LC_{50} = 350 mg/kg dw soil**	EFSA Journal 2014;12(9):3812
Eisenia fetida	2,4-D	56 d, chronic	**NOEC = 62.5 mg as/kg dw soil**	EFSA Journal 2014;12(9):3812
Eisenia fetida	Aminopielik Standard 600 SL	14 d, acute	LC_{50} > 618 mg as/kg dw soil	EFSA Journal 2014;12(9):3812
Eisenia fetida	2,4-DCA	14 d, acute	LC50 > 101.8 mg/kg soil **LC50corr > 50.9 mg/kg soil**	EFSA Journal 2014;12(9):3812
Eisenia fetida	2,4-DCA	56 d, chronic	NOEC = 10 mg/kg soil **NOECcorr = 5 mg/kg soil**	EFSA Journal 2014;12(9):3812
Eisenia fetida	2,4-DCP	56 d, chronic	NOEC = 10 mg/kg soil **NOECcorr = 5 mg/kg soil**	EFSA Journal 2014;12(9):3812
Hypoaspis aculeifer	2,4-DCA	chronic	NOEC = 10 mg/kg dw soil **NOECcorr = 5 mg/kg soil**	EFSA Journal 2014;12(9):3812
Hypoaspis aculeifer	2,4-DCP	chronic	NOEC = 5 mg/kg dw soil **NOECcorr = 2.5 mg/kg soil**	EFSA Journal 2014;12(9):3812
Folsomia candida	2,4-DCA	chronic	NOEC = 10 mg/kg dw soil **NOECcorr = 5 mg/kg soil**	EFSA Journal 2014;12(9):3812

Folsomia candida	2,4-DCP	chronic	NOEC = 1.25 mg/kg dw soil **NOECcorr = 0.625 mg/kg soil**	EFSA Journal 2014;12(9):3812
florasulam				
Eisenia fetida	formulation EF-1343	14 d, acute	LC50 > 1033 mg as/kg dw soil	EFSA Journal 2015; 13(1):3984
Eisenia fetida	formulation EF-1343	56 d, chronic	NOEC = 4.14 mg as/kg dw soil	EFSA Journal 2015; 13(1):3984
Eisenia fetida	florasulam	14 d, acute	**LC_{50} > 1320 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	florasulam	chronic	**NOEC = 0.203 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	5-OH-florasulam	14 d, acute	**LC50 > 1120 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	5-OH-florasulam	chronic	**NOEC = 0.14 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	DFP-ASTCA	14 d, acute	**LC50 > 0.1 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	DFP-ASTCA	chronic	**NOEC = 0.0304 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	ASTCA	14 d, acute	**LC50 > 100 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	ASTCA	chronic	**NOEC = 1.0 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	TSA	14 d, acute	**LC50 > 0.1 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Eisenia fetida	TSA	chronic	**NOEC = 10.0 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	5-OH-florasulam	chronic	**NOEC = 1.25 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	DFP-ASTCA	chronic	**NOEC = 10 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	ASTCA	chronic	**NOEC = 100 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	TSA	chronic	**NOEC = 50 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Folsomia candida	5-OH-florasulam	28 d, chronic	**NOEC = 2.5 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Folsomia candida	DFP-ASTCA	28 d, chronic	**NOEC = 10 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Folsomia candida	ASTCA	28 d, chronic	**NOEC = 12.5 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
Folsomia candida	TSA	28 d, chronic	**NOEC = 50 mg/kg dw soil**	EFSA Journal 2015; 13(1):3984
FLD-HER 306 SE				

Eisenia fetida	FLD-HER 306 SE	56 d, chronic	NOEC ≥1000 mg/kg dw soil (off spring numer) **NOEC = 390.63 mg/kg dw soil (weight of adult)**	Woźniak A/2019; Study Code: 0005/0080/E
Field studies				
Not available.				
Litter bag test				
Not available.				

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

9.8.1.1 Justification for new endpoints

New endpoints are provided for the formulated product FLD-HER 306 EC. Details of studies and results are included in Table 9.8-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for soil organisms from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

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 FLD-HER 306 SE in spring cereals, winter cereals and maize

Intended uses	spring cereals, winter cereals, maize		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**$PEC_{soil, accum}$(worst case) (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
2,4-D	350	0.258	1357
2,4-DCA	> 50.9	0.029	1755
florasulam	> 1320	0.0050	264000
5-OH-florasulam	> 1120	0.0034	329412
DFP-ASTCA	> 0.1	0.0012	83
ASTCA	> 100	0.0027	37037
TSA	> 0.1	0.0010	100

Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
2,4-D	62.5	0.258	242
2,4-DCA	5	0.029	172
2,4-DCP	5	0.021	238
florasulam	0.203	0.0050	41
5-OH-florasulam	0.14	0.0034	41
DFP-ASTCA	0.0304	0.0012	25
ASTCA	1	0.0027	370
TSA	10	0.0010	10000
FLD-HER 306 SE	390.63	0.851	459
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)**
2,4-DCA	5	0.029	172
2,4-DCP	2.5	0.021	119
5-OH-florasulam	1.25	0.0034	368
DFP-ASTCA	10	0.0012	8333
ASTCA	100	0.0027	37037
TSA	50	0.0010	50000
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)**
2,4-DCA	5	0.029	172
2,4-DCP	0.625	0.021	30
5-OH-florasulam	2.5	0.0034	735
DFP-ASTCA	10	0.0012	8333
ASTCA	12.5	0.0027	4630
TSA	50	0.0010	50000

TER values shown in bold fall below the relevant trigger.

zRMS comments:

The long-term risks to earthworms and soil meso - and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}.

The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).

For earthworms the risk provided for the active substances and their substances, for formulation product **FLD-HER** indicated an acceptable risk as TER_{LT} values are above trigger of 5.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk of FLD-HER 306 SE to soil macro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substances, metabolites and FLD-HER 306 SE with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of FLD-HER 306 SE at maximum rate of 0.6 L/ha does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

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 2,4-D and florasulam their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of FLD-HER 306 SE were not evaluated as part of the EU assessment. 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.

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

Endpoint	Substance	Exposure System	Results	Reference
2,4-D				
N-mineralisation	2,4-D	-	**No effect at 3 mg/kg soil**	EFSA Journal 2014;12(9):3812
N-mineralisation	LAF-74	56 days	No effect at 29.9 mg as/kg soil	EFSA Journal 2014;12(9):3812
N-mineralisation	2,4-DCA	28 days	**No effect at 5 mg/kg soil**	EFSA Journal 2014;12(9):3812
N-mineralisation	2,4-DCP	42 days	**No effect at 5 mg/kg soil**	EFSA Journal 2014;12(9):3812
C-mineralisation	2,4-D	-	No effect at 3 mg a.s./kg soil	EFSA Journal 2014;12(9):3812
C-mineralisation	LAF-74	28 days	No effect at 29.9 mg a.s./kg soil	EFSA Journal 2014;12(9):3812
C-mineralisation	2,4-DCA	28 days	No effect at 5 mg a.s./kg soil	EFSA Journal 2014;12(9):3812
C-mineralisation	2,4-DCP	28 days	No effect at 5 mg a.s./kg soil	EFSA Journal 2014;12(9):3812

florasulam				
N-mineralisation & C-mineralisation	formulation EF-1343	100 days	Treatment causing <25% deviation from control: 1.03 mg/kg dry soil	EFSA Journal 2015; 13(1):3984
N-mineralisation & C-mineralisation	florasulam	100 days	Treatment causing <25% deviation from control**: 0.05 mg/kg dry soil**	EFSA Journal 2015; 13(1):3984
N-mineralisation & C-mineralisation	5-OH-florasulam	100 days	Treatment causing <25% deviation from control: **0.036 mg/kg dry soil**	EFSA Journal 2015; 13(1):3984
N-mineralisation & C-mineralisation	DFP-ASTCA	100 days	Treatment causing <25% deviation from control: **0.00760 mg/kg dry soil**	EFSA Journal 2015; 13(1):3984
N-mineralisation & C-mineralisation	ASTCA	100 days	Treatment causing <25% deviation from control: **1.0 mg/kg dry soil**	EFSA Journal 2015; 13(1):3984
N-mineralisation & C-mineralisation	TSA	100 days	Treatment causing <25% deviation from control: **0.05 mg/kg dry soil**	EFSA Journal 2015; 13(1):3984
FLD-HER 306 SE				
N-mineralisation	FLD-HER 306 SE	28 days	Effect 1.1% at **0.9982 mg of test item/kg soil** Effect -9.6% at **4.9910 mg of test item/kg soil**	Parma P/2019/ Study Code: 0005/0083/E

zRMS comments:

FLD-HER has no significant effect on soil micro-organisms at 4.9910 mg a.s./kg dry soil. Based on it, can be concluded that under field conditions, use at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product FLD-HER 306 EC. Details of studies and results are included in Table 9.9-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for soil microorganisms from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Intended use	spring cereals, winter cereals, maize		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
2,4-D	3	0.258	yes
2,4-DCA	5	0.029	yes
2,4-DCP	5	0.021	yes
florasulam	0.05	0.0050	yes
5-OH-florasulam	0.036	0.0034	yes
DFP-ASTCA	0.00760	0.0012	yes
ASTCA	1	0.0027	yes
TSA	0.05	0.0010	yes
FLD-HER 306 SE	0.9982 & 4.9910	0.851	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
2,4-D	3	0.258	yes
2,4-DCA	5	0.029	yes
2,4-DCP	5	0.021	Yes
florasulam	0.05	0.0050	yes
5-OH-florasulam	0.036	0.0034	yes
DFP-ASTCA	0.00760	0.0012	yes
ASTCA	1	0.0027	yes
TSA	0.05	0.0010	yes

9.9.3 Overall conclusions

The risk of FLD-HER 306 EC to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substances, metabolites and FLD-HER 306 SE with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of FLD-HER 306 SE at maximum rate of 0.6 L/ha does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

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 2,4-D and florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of FLD-HER 306 SE were not evaluated as part of the EU assessment. 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.

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

Species	Substance	Exposure System	Results	Reference
2,4-D				
Lettuce Lactuca sativa	formulation LAF-74	Vegetative vigour & Seedling emergence	Vegetative vigour: ER_{50} = 19 g a.s./ha Emergence: ER_{50} = 27 g a.s./ha	EFSA Journal 2014;12(9):3812
florasulam				
Oilseed rape	formulation EF-1343	Seedling emergence	ER_{50} = 0.596 g a.s./ha	EFSA Journal 2015; 13(1):3984
Sunflower	formulation EF-1343	Vegetative vigour	ER_{50} = 0.222 g a.s./ha	EFSA Journal 2015; 13(1):3984
FLD-HER 306 SE				
Carrot *Daucus carota* Cucumber *Cucumis dativus* Oilseed rape *Brassica napus* Lettuce *Lactuca sativa* Onion *Allium cepa* Perennial ryegrass *Lolium perenne*	FLD-HER 306 SE	21 d Vegetative vigour	Carrot *Daucus carota* ER_{50} = 0.288 L/ha Cucumber *Cucumis dativus* ER_{50} > 0.7 L/ha Oilseed rape *Brassica napus* ER_{50} = 0.255 L/ha Lettuce *Lactuca sativa* **ER_{50} = 0.110 L/ha** Onion *Allium cepa* ER_{50} = 0.380 L/ha Perennial ryegrass *Lolium perenne* ER_{50} = 0.536 L/ha	Parma P/2019/ Study Code: 0005/0082/E
Carrot *Daucus carota* Cucumber *Cucumis dativus* Oilseed rape *Brassica napus* Lettuce *Lactuca sativa*	FLD-HER 306 SE	21 d Seedling emergence	Carrot *Daucus carota* ER_{50} > 0.7 L/ha Cucumber *Cucumis dativus* ER_{50} > 0.7 L/ha Oilseed rape *Brassica napus* ER_{50} > 0.7 L/ha Lettuce *Lactuca sativa*	Parma P/2019/ Study Code: 0005/0081/E

Onion *Allium cepa* Perennial ryegrass *Lolium perenne*			ER_{50} > 0.7 L/ha Onion *Allium cepa* ER_{50} > 0.7 L/ha Perennial ryegrass *Lolium perenne* ER_{50} > 0.7 L/ha	

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product FLD-HER 306 EC. Details of studies and results are included in Table 9.10-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals/maize also covers the risk for non-target terrestrial plants from all other intended uses i.e. spring cereals, winter cereals and maize (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of FLD-HER 306 SE in spring cereals, winter cereals and maize

Intended uses	spring cereals, winter cereals, maize			
Product	FLD-HER 306 SE			
Application rate (L/ha)	1 × 0.6			
MAF	1			
Test species	**ER_{50} (L/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Lettuce *Lactuca sativa*	0.110	2.77	0.017	6.5

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

zRMS comments:

The risk assessment is based on the "Guidance Document 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.
zRMS accepted the calculations of the deterministic risk assessment provided by the applicant.
Based on these results TER_{LT} was above trigger values of 5, indicated an acceptable rik for non-target plants.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risk of FLD-HER 306 SE to non-target plants was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation with application rate of FLD-HER 306 SE. According to the performed risk assessment it was assessed that the application of FLD-HER 306 SE in maximum rate of 0.6 L/ha does not pose unacceptable risk to non-target plants. No risk mitigations are required.

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

Not available.

9.12 Monitoring data (KCP 10.8)

Not available.

9.13 Classification and Labelling

Justified proposals for classification and labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for the formulation:

Table 9.13-1: Justified proposals for classification and labelling for FLD-HER 306 SE according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS09

Signal word:	Warning
Hazard statement(s):	Very toxic to aquatic life. [H400] Very toxic to aquatic life with long lasting effects. [H410]
Precautionary statement(s):	-
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] 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). [SP 1] To protect aquatic organisms respect an 5m vegetated unsprayed buffer zone of to surface water bodies. [SPe 3] Collect spillage [P391]

Table 9.13-2: Summary of evaluation of the ecotoxicological studies for FLD-HER 306 EC

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	E_yC_{50} = 0.061 mg/L		Aquatic Acute 1, H400	Woźniak A/ 2019/ Study code: 0005/0071/E
Chronic toxicity to aquatic organisms	no data, extrapolation form active substance data		Aquatic Chronic 1, H410	-

zRMS comment:

We agree with the risk assessment provided by the applicant.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	Owner
KCP 10.2.1.1/01	.	1984	Measurement of median lethal dose as a rapid indication of contaminant toxicity to fish Environmental Toxicology and Chemistry, Vol. 3, pp. 243-254, 1984 GLP: N Published: Y	Y	NR
KCP 10.2.1.2/01	Kühn, R. *et al.*	1989	Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to D*aphnia magna* Wat. Res. Vol. 23, No. 4, pp. 495-499, 1989 GLP: N Published: Y		
KCP 10.2.1.2/02	Woźniak A	2019	Daphnia acute immobilization test according to OECD 202 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0068/E GLP: Y Published: N	N	Pestila*
KCP 10.2.1.3/01	Cowgill, U. *et al.*	1989	Toxicity of nine benchmark chemicals to *Skeletonema costatum*, a marine diatom Environmenlal Toxicology and Chemisrry, Vol. 8, pp. 451-455, 1989 GLP: N Published: Y	N	NR
KCP 10.2.1.3/02	Woźniak A	2019	Freshwater algae growth inhibition test according to OECD No 201 SORBBOLAB Research Laboratory LLC, Poznań, Poland	N	Pestila*

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**
			Study code: 0005/0069/E GLP: Y Published: N		
KCP 10.2.1.4/01	Woźniak A	2019	*Lemna gibba* growth inhibition test according to OECD 221 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0070/E GLP: Y Published: N	N	Pestila*
KCP 10.2.1.4/02	Woźniak A	2019	Water-sediment *Myriophyllum spicatum* toxicity test according to OECD 239 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0071/E GLP: Y Published: N	N	Pestila*
KCP 10.3.1.1.1/01	Orzechowska U	2019	Honeybees, Acute Oral Toxicity Test according to OECD 213 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0072/E GLP: Y Published: N	N	Pestila*
KCP 10.3.1.1.2/01	Orzechowska U	2019	Honeybees, Acute Contact Toxicity Test according to OECD 214 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0073/E GLP: Y Published: N	N	Pestila*
KCP 10.3.1.2/01	Orzechowska U	2019	Honey Bee, Chronic Oral Toxicity Test according to OECD 245 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0075/E GLP: Y Published: N	N	Pestila*
KCP	Orzechowska U	2019	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239		

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**
10.3.1.4/01			SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0076/E GLP: Y Published: N		
KCP 10.3.2.2/01	Kręglewska M	2019	Extended laboratory test (Tier2) for the impact assessment on the parasitic wasp *Aphidius rhopalosiphi;* SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0078/E GLP: Y Published: N	N	Pestila*
KCP 10.3.2.2/02	Kręglewska M	2019	Extended laboratory test (Tier2) for evaluating the effects on the predatory mites *Typhlodromus pyri* (Scheuten) SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0077/E GLP: Y Published: N	N	Pestila*
KCP 10.3.2.2/03	Rovetto I	2019	Effects of FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) on *Coccinella septempunctata* in the laboratory – Extended laboratory test – Year 2019 SAGEA Centro di Saggio s.r.l. Study code: 1075-1075HSAG19/r GLP: Y Published: N	N	Pestila*
KCP 10.3.2.2/04	Rovetto I	2019	Effects of FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) on *Coccinella septempunctata* in the laboratory – Extended laboratory test – Year 2019 SAGEA Centro di Saggio s.r.l. Study code: 1074-1074HSAG19/r GLP: Y Published: N	N	Pestila*
KCP 10.4.1.1/01	Woźniak A	2019	Earthworm reproduction test according to OECD 222 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0080/E	N	Pestila*

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: Y Published: N		
KCP 10.5/01	Parma P	2019	Study of impact on soil microorganisms - nitrogen transformation according OECD 216 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0083/E GLP: Y Published: N	N	Pestila*
KCP 10.6.2/01	Parma P	2019	Seedling emergence and seedling growth test according to OECD 208 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0081/E GLP: Y Published: N	N	Pestila*
KCP 10.6.2/02	Parma P	2019	Vegetative Vigour Test according to OECD 227 SORBBOLAB Research Laboratory LLC, Poznań, Poland Study code: 0005/0082/E GLP: Y Published: N	N	Pestila*

* Pestila Spółka z ograniczoną odpowiedzialnością.

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**
-	-	-	-	-	-

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

Not relevant. No studies submitted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

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 KCP 10.2.1.1 Acute toxicity to fish

Comments of zRMS: The study is considered valid.

Agreed endpoints:

Tested sub-stance	Test period in h	LC_{50}
p-chlorophenol	96	14.8 µmol/L corresponding to 1.9 mg/L

Reference:	KCP 10.2.1.1/01
Report	Measurement of median lethal dose as a rapid indication of contaminant toxicity to fish,……., 1984 Environmental Toxicology and Chemistry, Vol. 3, pp. 243-254, 1984
Guideline(s):	No
Deviations:	Not relevant
GLP:	No

Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

A new method was developed to measure the toxicity of chemicals to fish over 96 h. Tested substances were dissolved in 5% ethanol in saline or in cod-liver oil and injected at a rate of 1.0 ml per 100 g of fish. The results of parallel bioassays to measure toxicity by oral intubation (OI-LD_{50}) or aqueous exposure (LC_{50}) were closely linked to IP-LD_{50} values. In this summary only data of the aqueous exposure test on p-chlorophenol are included.

MATERIALS AND METHODS

Test material:

Test item:	p-Chlorophenol (= 4-chlorophenol)
Molecular weight	128.6 g/mol
Description:	analytical
Lot/Batch no.:	2450950
Purity:	not stated
Source:	BDH Chemicals
Vehicle and/or positive control:	no
Test system:	
Organism (Species):	rainbow trout (S*almo gairdneri / Oncorhynchus mykiss*)
Age:	not stated
Size:	4.6-6.4 cm
Body weight of the animals:	1.2 - 3.8g
Source:	Goosen's Trout Farm, R.R. #1, Otterville, ON, Canada
Diet/Food:	daily with Ewos trout pellets except on weekends
Acclimatisation period:	at least 1 week
Medium:	water from Lake Ontario, dechlorinated to less than 10 µg Cl/L with specific composition: acid capacity $K_{S4,3}$ 0f 0.8 mmol L^{-1}, total hardness of 2,4 mmol/L, a calcium to magnesium ratio of 4 : 1, a sodium to potassium ratio of 10 : 1
Environmental conditions:	
Temperature:	14.1 – 16.5 °C
Photoperiod:	16 h light and 8 h dark
pH:	7.6 – 8.19
Dissolved oxygen:	5.6 – 9.4
Conductivity:	340 µmhos/cm²
Hardness:	86 mg $CaCO_3$/L

STUDY DESIGN

Based on the results of a preliminary range finding test at 1.0, 10, 100 and 1,000 mg/L, a definite test was performed using concentrations of 0 (control), 10, 18, 32, 56 and 100% of the maximum test concentration. Ten fish were exposed to each concentration and the bioassay was repeated three times (n= 10 fishes x 6 concentrations x 3 replicates = 180). Chemicals were added by a Hamilton syringe pump and dilutions were achieved by a Mount-Brungs diluter. Each bioassay tank contains 14 L of medium and flow per tank varied between tests from 21 to 111 mL/min. Size of test fish was chosen such that the flow rate was always greater than 2 L per gram of fish per day. During bioassay tanks were not aerated.

Observations
After 96 h the number of animals in the control and test solutions was assessed for mortality.

Statistical calculations

LC_{50} values were calculated from records of percent mortality by computerized probit analysis. When the number of partial mortalities was too low for probit analysis, a graphical method was chosen.
LC_{50} values are based on mean measured concentration.

RESULTS AND DISCUSSION

Findings

The determined LC values for rainbow trout are given in the table below.

Table KCP 10.2.1.1-1: Determined LC_{50} values for p-chlorophenol on rainbow trout

Tested substance	Test period in h	LC_{50}
p-chlorophenol	96	14.8 µmol/L corresponding to 1.9 mg/L

CONCLUSION

Taking into account the obtained results, toxicity of p-chlorophenol to rainbow trout resulted in an 96 h LC_{50} of 1.9 mg/L.

A 2.2.1.2 KCP 10.2.1.2 Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study is considered valid. **Agreed endpoints:**

Test period in h	EC_{50} [mg/L]
24	3.4
48	2.5

Reference:	KCP 10.2.1.2/01
Report	Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to D*aphnia magna* , Kühn, R. *et al.*, 1989 Wat. Res. Vol. 23, No. 4, pp. 495-499, 1989
Guideline(s):	DIN 38412, part II
Deviations:	Not relevant
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

In the acute *Daphnia* test, the EC_{50}, EC_0 and EC_{100} after 24 and 48 h were determined for 70 substances. The results of the tests are given in three tables according to substance group. Evaluation showed that the toxicity of the substances may be higher or even substantially greater when the test period is extended from 24 to 48 h. In this summary only data of the aqueous exposure test on p-chlorophenol are included.

MATERIALS AND METHODS

Test material:

Test item:	p-Chlorophenol (= 4-chlorophenol)
Description:	analytical
Lot/Batch no.:	not stated
Purity:	not stated
Source:	not stated
Vehicle and/or positive control:	none
Test system:	
Organism (Species):	*Daphnia magna*
Source:	own culture
Medium:	water with specific composition: acid capacity $K_{S4,3}$ 0f 0.8 mmol L^{-1}, total hardness of 2,4 mmol L^{-1}, a calcium to magnesium ratio of 4:1, a sodium to potassium ratio of 10:1

Environmental conditions:

Temperature:	20°C in an incubator
Photoperiod:	not stated
pH:	start of the test: 8.0 ± 0.2 end of the test: > 7.0
Dissolved oxygen:	end of the test: > 4.0 mg o²/l
Conductivity:	not stated

STUDY DESIGN

The effects of p-chlorophenol on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (2 replicates of ten 6-24 h old animals per test beaker) were exposed per concentration and control. Concentrations were selected so that 3-4 EC values were in the range between EC_0 and EC_{100}, with at least one value below and one above EC_{50}. The ratio between concentrations was 1:1.4.

Observations
After 24 h and 48 h, the number of animals in the control and test solutions that could still swim were counted.

Statistical calculations
Not stated how data were statistical evaluated.

RESULTS AND DISCUSSION

Findings
The determined EC values for *Daphnia magna* are given in the table below.

Table KCP 10.2.1.2-1 Determined EC values for p-chlorophenol on *Daphnia magna*

Test period in h	EC_{50} [mg/L]	EC_0 [mg/L]	EC_{100} [mg/L]
24	3.4	1.5	11
48	2.5	1.5	4

CONCLUSION
Taking into account the obtained results, the toxicity of p-chlorophenol to *Daphnia magna* is 48 h EC_{50} = 2.5 mg/L.

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

- in the control the number of immobilized daphnia at the end of the test was 0% (required: ≤10%)
- the lowest oxygen concentration at the end of the test in the control and the tested concentrations was 8.23 mg/L (required: ≥3 mg/L).

Agreed endpoints:

Final results calculated by ToxRat Professional		
Parameter	Time of measurement	
	24 h	48 h
EC_{10} [mg/L]	12.721 (n.d. – n.d.)*	13.675 (n.d. – n.d.)*
EC_{50} [mg/L]	25.823 (n.d. – n.d.)*	21.075 (n.d. – n.d.)*
EC_{95} [mg/L]	64.071 (n.d. – n.d.)*	36.717 (n.d. – n.d.)*
LOEC [mg/L]	25.0	25.0
NOEC [mg/L]	12.5	12.5

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

Reference:	KCP 10.2.1.2/02
Report	Daphnia acute immobilization test according to OECD 202, Woźniak A.; 2019; Study code: 0005/0068/E
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	Stability test was carried out for the following test item concentrations: 1 mg/L and 100 mg/L in M7 medium. The analysis was performed in comparison to a control sample (light and darkness), prepared and maintained under the same conditions as the tested samples but without the test item.

2. Vehicle and/or positive control: vehicle control: M7 medium,
positive control: potassium dichromate

3. Test organism

Species:	*Daphnia magna* Straus
Source:	own culture of the SORBOLAB Research Laboratory
Age:	no older than 24 h, being not the first brood progeny
Feeding:	during the test daphnia were not fed
Test units:	glass beakers of volume 100 mL

4. Environmental conditions:

Medium:	M7 (one of the recommended by OECD Guideline 202), before using the medium was saturated with air through intense aeration for about 1 hour
Medium temperature:	the test was conducted at an average temperature of 20.269°C, minimal temperature 19.80°C, maximal temperature 20.60°C

Lighting:	fluorescent lighting on a daily cycle 16 hours of light with an intensity of 1100 lux (requirements according to OECD 202: 1000-1500 lux) and 8 hours of darkness or complete darkness

STUDY DESIGN AND METHOD

The acute *Daphnia magna* immobilisation test for FLD-HER 306 SE was conducted according to OECD Guideline 202. The aim of the study was to evaluate of test item effect on daphnia immobilization. Test endpoints were EC_x, NOEC and LOEC values calculated for the particular observed immobilization of test organisms after 24 and 48 hours. The study was conducted for *Daphnia magna* collected from own culture. *Daphnia magna* was grown in laboratory conditions: temperature 20 ± 2ºC, light intensity 1000 – 1500 lux in a daily cycle – 16h day and 8h night in M7 medium. Medium used in the culture was the same which was later used during the experiment. Daphnia were fed with green algae *Pseudokirchneriella subcapitata* from own culture. In the study daphnia, no older than 24h, being not the first brood progeny were used. The daphnia culture showed no signs of stress like high mortality, delay in the production of the first brood, discolouring or presence of males and ephippia. Good condition of daphnia culture was confirmed by the study with the reference substance, potassium dichromate.

Test design:	definitive test: control and tested concentration prepared in 4 replicates each, with 5 daphnia introduced into each replicate
Type of the exposure:	static
Exposure time:	2 days (48 hours)
Tested concentrations, definitive test:	control (0 mg/l), 6.25 mg/l; 12.5 mg/l; 25.0 mg/l; 50.0 mg/l; 100.0 mg/l
Dates:	start of the study 04.06.2019 start of the experimental part: 26.08.2019 end of the experimental part: 03.10.2019 end of the study: 15.10.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

Based on the obtained results, it was assumed that the tested material is stable in the test conditions. In the final finding test, the following parameters were observed and recorded:
- the number of immobilized individuals in each test vessel after 24 and 48 hours from the beginning of the test
- symptoms of intoxication: changes in the appearance and behaviour of animals.

Table KCP 10.2.1.2-2: Immobilization of daphnia after 24 h – definitive test

Concentration [mg/L]	Introduced daphnia [pcs.]	24 h		
		Immobilized daphnia [pcs]	Immobilized daphnia [%]	Statistical significance*)
control	20	0	0	not applicable
6.25	20	0	0	-
12.5	20	0	0	-
25.0	20	15	75	+
50.0	20	15	75	+
100.0	20	20	100	+

\- not statistically significant
\+ statistically significant
*) values calculated by ToxRat Professional using the Cochran-Armitrage test at the significance level p> 0.05

Table KCP 10.2.1.2-3: Immobilization of daphnia after 48 h – definitive test

Concentration [mg/L]	Introduced daphnia [pcs.]	48 h		
		Immobilized daphnia [pcs]	Immobilized daphnia [%]	Statistical significance*)
control	20	0	0	not applicable
6.25	20	0	0	-
12.5	20	0	0	-
25.0	20	17	85	+
50.0	20	19	95	+
100.0	20	20	100	+

\- not statistically significant
\+ statistically significant
*) values calculated by ToxRat Professional using Cochran-Armitrage test at the significance level of p>0.05

Table KCP 10.2.1.2-4: Symptoms of intoxication – definitive test

Concentration [mg/L]	Time of observations	
	24 h	48 h
control	none	none
6.25	none	none
12.5	none	none
25.0	none	none
50.0	none	none
100.0	none	none

The tested item affects the immobilization of daphnia after 24 h and 48 h exposure in the concentration range from 25.0 mg/L to 100 mg/L. The value of EC_{50} after 48 hours of exposure was **21.075** mg/l.

Table KCP 10.2.1.2-5: Results of *Daphnia magna* immobilisation test

Final results calculated by ToxRat Professional		
Parameter	Time of measurement	
	24 h	48 h
EC_{10} [mg/L]	12.721 (n.d. – n.d.)*	13.675 (n.d. – n.d.)*
EC_{50} [mg/L]	25.823 (n.d. – n.d.)*	21.075 (n.d. – n.d.)*
EC_{95} [mg/L]	64.071 (n.d. – n.d.)*	36.717 (n.d. – n.d.)*
LOEC [mg/L]	25.0	25.0
NOEC [mg/L]	12.5	12.5

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

A 2.2.1.3 KCP 10.2.1.3 Effects on aquatic algae

Comments of zRMS: The study is considered valid.
All validity criteria were met.

Agreed endponts:

4 - chlorophenol	Total cell count	Total cell volume
120 EC_{50} (95 % CI)	13.8 mg/L (-16.0, 43.5)	11.6 mg/L (-18.9, 42.2)
120 NOEL	1.08 mg/L	0.39 mg/L

Reference:	KCP 10.2.1.3/01
Report	Toxicity of nine benchmark chemicals to *Skeletonema costatum*, a marine diatom, Cowgill, U., 1989 Environmenlal Toxicology and Chemisrry, Vol. 8, pp. 451-455, 1989
Guideline(s):	No
Deviations:	Not relevant
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

The purpose of this study was to determine the sensitivity of a *Skeletonema costatum* to eight common chemicals and one herbicide. The 50% reduction in the number of cells per milliliter and that of total cell volume × 10^4 μm^3/ml was estimated in relation to each of the nine chemicals. Nominal concentrations of triclopyr triethylamine salt (Garlon 3A), $K_2Cr_2O_7$, 4—chlorophenol and phenol were slightly toxic (>10 mg/L) according to the U.S. Environmental Protection Agency classificatory scheme, while diethanolamine, chlorobenzene, chloroform, acetone and ethanol were classified as practically nontoxic (>100 mg/L). No observed effect levels were found for each of the two cell measurements in relation to each of

the chemicals tested for the 5—d period of the test. The range was found to be from 1 to 6,000 mg/L for total cell count and from 0.65 to 6,000 mg/L for total cell volume. Data on the sensitivities of other organisms to the group of common chemicals are also included. The marine diatom proved to be less sensitive to $K_2Cr_2O_7$ and diethanolamine than the green alga *Selenastrum capricornutum*. In this summary only data of the aqueous exposure test on p-chlorophenol are included.

MATERIALS AND METHODS

Test material:

Test item:	4 - chlorophenol
Description:	analytical
Lot:	AOD
Purity:	Not stated
Source:	Eastman Kodak, CAS No. 106-48-9

Vehicle and/or positive control: none

Test system:

Organism:	marine diatom (*Skeletonema costatum*)
Source:	Bigelow Laboratory for Ocean Sciences at West Boothbay Harbor, Maine
Medium:	Provasoli, revised ASP 12 medium. Revision consists of addition of SE (Na_2SeO_4, 0.00479 g/L) and Cu ($CuCl_2$ $2H_2O$, 0.06707 g/L) and doubling of the amount of cyanocobalamine (0.00040 g/L)

Environmental conditions:

Temperature:	19.5-20.6 °C
Light:	4,296-4,318 lux
Photoperiod:	14h light/ 10 h dark

STUDY DESIGN

At the beginning of the experiment a range finding test was conducted. Concentrations were set an order of magnitude apart, 0.1, 1, 10, 100, 1,000 mg/L and so on. Cell density was 100,000 cells/mL at the beginning of the range finding test and in the definitive test. The definitive test consisted of 5 or more concentrations and a control replicated three times. For each concentration a counting blank was included. Total cell count and cell volume were measured by the use of a cell counter. Additionally, initial and final pHs of control, low, middle and high-test item rates were measured. Each test lasted 5 days.

Observations
In all tests temperature and light intensity was assessed daily until day 5.

Statistical calculations
Not stated how data were statistical evaluated.

RESULTS AND DISCUSSION

Findings
The determined EC_{50} and NOEL values for *Skeletonema costatum* are given in the table below.

Table KCP 10.2.1.3-1 Determined EC_{50} and NOEL values for 4 - chlorophenol

4 - chlorophenol	Total cell count	Total cell volume
120 EC_{50} (95 % CI)	13.8 mg/L (-16.0, 43.5)	11.6 mg/L (-18.9, 42.2)
120 NOEL	1.08 mg/L	0.39 mg/L

CONCLUSION

The toxicity of 4 - chlorophenol to *Skeletonema costatum* is EC_{50} = 13.8 mg/L and NOEL = 1.08 mg/L for total cell count and EC_{50} = 11.6 mg/L and NOEL = 0.39 mg/L.

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

- yield in control during 72 hours of test increased exponentially 168.3 times (requirements according to OECD 201: ≥16)
- the coefficient of variance for the average specific growth rate for all repetitions of the control culture over the entire time of the test was 2.2% (requirements according to OECD 201: <7%)
- the average coefficient of variance for a specific growth rate day after day (0-24 h, 24-48 h, 48-72 h) for the control culture was 27.4% (requirements according to OECD 201: <35%).

Agreed endpoints:

Parameter	Yield	Average specific growth rate	Sectional growth rate
EC_{10} – 72 h [mg/L]	0.192 (0.152-0.231)*	1.091 (0.848-1.313)*	1.256 (0.853-1.688)*
EC_{20} – 72 h [mg/L]	0.307 (0.258-0.353)*	1.600 (1.332-1.842)*	1.995 (1.463-2.609)*
EC_{50} – 72 h [mg/L]	0.750 (0.679-0.829)*	3.332 (3.013-3.686)*	4.837 (3.689-6.674)*
LOEC – 72 h [mg/L]	0.300	0.970	10.000
NOEC – 72 h [mg/L]	0.090	0.300	3.125

*) the lower and upper 95% confidence limits are given in brackets

Reference:	KCP 10.2.1.3/02
Report	Freshwater algae growth inhibition test according to OECD No 201; Meler A.; 2019; Study Code: 0005/0069/E
Guideline(s):	Yes, OECD 201
Deviations:	Yes: The experimental part ended in November 2019, which is a deviation from scheduled date. During the definitive test, the temperature increased above the temperature recommended by the Guideline. The test was carried out at an average temperature of 24.099°C, minimal temperature 22.7°C, maximal temperature 24.6°C (requirements according to OECD 201: 21-24°C ±2°C). The deviations had no effect on the course of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	The stability test was carried out for the following concentrations of the test item: 1 mg/L and 100 mg/L in the AAP medium.
2. Vehicle and/or positive control:	vehicle control: APP medium, positive control: 3,5-dichlorophenol
3. Test organism	
Species:	*Pseudokirchneriella subcapitata* (equivalent name *Raphidocelis subcapitata*)
Source:	own culture of the SORBOLAB Research Laboratory
Age:	three days prior to the start of the test
Test units:	conical flask of a volume 250 mL
4. Environmental conditions:	
Medium:	APP (one of the recommended by OECD Guideline 201) prepared using deionized water with ≤1 μS·cm-1 conductivity
Medium temperature:	average temperature 24.099ºC (min. temperature 22.70ºC, max. temperature 24.20ºC);
Lighting:	continuous fluorescent light 6990 – 7200 lux; the pH value in the test vessels was maintained at 7.09-7.43 at the beginning of the test and 7.15-7.26 at the end of the test

STUDY DESIGN AND METHOD

The aim of the study was to determine the influence of test item FLD-HER 306 SE on growth algae (*Pseudokirchneriella subcapitata*) expressed in yield, average specific growth rate, sectional specific growth rate based on OECD 201. The endpoints of the experiment are EC10, EC20 and EC50 values for the mentioned parameters based on measurements of the number of algae cells. The NOEC and LOEC values were also statistically determined. The study was conducted for freshwater algae (*Pseudokirchneriella subcapitata*) obtained from culture from Laboratory of Ecotoxicology in SORBOLAB Research Laboratory. Algae were cultured in a laboratory incubator at 21-24°C±2°C, with constant lighting in the range of 4400-8800 lux and shaking at 90 rpm. As a culture medium, the AAP medium was used. As an inoculum, the algae in the logarithmic growth stage was used to start the test. To check the condition of the test system 3,5-dichlorophenol as a reference item was used.

In the course of the range finding test, absorbance measurements at wavelength λ670 nm were performed on each day for each repeat of the concentration and control tested. Measurements were carried out 3 times for each sample in cuvettes with an optical length of 10 mm. The number of algae cells was determined based on the prepared nomogram. Additionally, microscopic observations were made on the day of

the end of the experiment to verify the abnormal appearance of the cells at any concentration. Detailed results are included in study report.

Test design:	tested concentrations in three replicates, control in six replicates, flasks arranged randomly
Type of the exposure:	static
Exposure time:	72 hours
Inoculum:	104 cells/mL
Tested concentrations, definitive test:	control (0 mg/L), 0.09 mg/L; 0.3 mg/L; 0.97 mg/L; 3.125 mg/L; 10 mg/L
Dates:	start of the study 14.08.2019 start of the experimental part: 19.08.2019 end of the experimental part: 21.11.2019 end of the study: 26.11.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

In the course of the test the test item FLD-HER 306 SE showed toxic effects on yield of algae *Pseudo-kirchneriella subcapitata* in concentrations: 0.3; 0.97; 3.125 and 10 mg/L, on average specific growth rate in concentrations: 0.97; 3.125; and 10 mg/L and on sectional growth rate in concentration 10 mg/L.

Table KCP 10.2.1.3-1: Freshwater alga growth inhibition test – final results calculated by ToxRat Professional

Parameter	Yield	Average specific growth rate	Sectional growth rate
EC_{10} – 72 h [mg/L]	0.192 (0.152-0.231)*	1.091 (0.848-1.313)*	1.256 (0.853-1.688)*
EC_{20} – 72 h [mg/L]	0.307 (0.258-0.353)*	1.600 (1.332-1.842)*	1.995 (1.463-2.609)*
EC_{50} – 72 h [mg/L]	0.750 (0.679-0.829)*	3.332 (3.013-3.686)*	4.837 (3.689-6.674)*
LOEC – 72 h [mg/L]	0.300	0.970	10.000
NOEC – 72 h [mg/L]	0.090	0.300	3.125

*) the lower and upper 95% confidence limits are given in brackets

A 2.2.1.4 KCP 10.2.1.4 Effects on aquatic macrophytes

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

– duckweed frond number doubling time in control: 2.0 day (required according to OECD 221: <2.5 days).
– average growth rate was 0.354/day (required according to OECD 221: >0.275/day).

Agreed endpoints:

Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	LOEC [mg/l]	NOEC [mg/l]
Frond number yield	0.035 (0.022-0.054)*	0.055 (0.037-0.083)*	0.133 (0.081-0.218)*	≤0.062	<0.062
Frond number growth rate	0.066 (0.045-0.095)*	0.108 (0.077-0.153)*	0.278 (0.183-0.425)*	≤0.062	<0.062
Frond number selectional growth rate	0.129 (0.069-0.242)*	0.165 (0.092-0.299)*	0.262 (0.127-0.541)*	0.250	0.125
Dry weight yield	0.061 ((0.017-0.218)*	0.122 (0.037-0.421)*	0.464 (0.099-2.088)*	≤0.062	<0.062
Dry weight growth rate	0.156 (0.067-0.363)*	0.356 (0.152-0.859)*	1.718 (0.458-6.049)*	≤0.062	<0.062
Dry weight selectional growth rate	0.156 (0.067-0.363)*	0.356 (0.152-0.859)*	1.718 (0.458-6.049)*	≤0.062	<0.062

* the lower and upper 95% confidence limits

Reference:	KCP 10.2.1.4/01
Report	*Lemna gibba* growth inhibition test according to OECD 221; Woźniak A.; 2019; Study Code: 0005/0070/E
Guideline(s):	Yes, OECD 221
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid

Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	In order to determine the photolytic stability of the test item, a stability test was performed. The stability test was carried out for the following concentrations of the test item: 1 mg/L and 100 mg/L and control (0 mg/L). The test vessel for each concentration and control was illuminated with constant fluorescent light at 6500-10000 lux or kept in the dark.
2. Vehicle and/or positive control:	vehicle: SIS medium, positive control: 3,5-dichlorophenol
3. Test organism	
Species:	gibbous duckweed (*Lemna gibba*)
Source:	from the University of Waterloo in Canada and obtained from the Canadian Phycological Culture Center (CPCC)
Test units:	250 ml glass crystallizers, volume of test solution 100 mL, vessels covered with cling film to reduce evaporation
4. Environmental conditions:	
Medium:	SIS (one of the recommended by OECD Guideline 221) prepared using deionized water with ≤1 μS·cm-1 conductivity
Medium temperature:	average temperature 24.371ºC (min. temperature 24.20ºC, max. temperature 24.70ºC)
pH:	the pH value in the test vessels was maintained at 6.32-6.52 at the beginning of the test and 7.32-7.81 at the end of the test and did not fluctuate by more than 1.5 units
Lighting:	continuous fluorescent light: 8000-9000 lux

STUDY DESIGN AND METHOD

Lemna gibba growth inhibition test was performed according to OECD Guideline No 221. The aim of the study was to determine the influence of test item FLD-HER 306 SE on growth of gibbous duckweed *Lemna gibba* expressed in yield, growth rate and sectional growth rate based on the frond number and dry weight. The values of EC10, EC20 and EC50 as well as NOEC and LOEC for the mentioned parameters were also statistically determined.

Test design:	tested concentrations in three replicates, control in six replicates, 9 fronds on every replicate
Type of the exposure:	static
Exposure time:	7 days (168 hours)

Tested concentrations, definitive test:	control (0 mg/l), 0.0625 mg/l; 0.125 mg/l; 0.25 mg/l; 0.5 mg/l, 1.0 mg/l
Dates:	start of the study 02.09.2019 start of the experimental part: 19.09.2019 end of the experimental part: 03.12.2019 end of the study: 12.12.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

The test item – FLD-HER 306 SE in the course of test showed a toxic effect on the yield of frond, frond number growth rate and sectional dry weight growth rate of gibbous duckweed *Lemna gibba* in the concentration range from 0.062 mg/L to 1 mg/L as well as on the frond number sectional growth rate in the concentration range from 0.25 mg/L to 1 mg/L.

Table KCP 10.2.1.4-1: *Lemna gibba* growth inhibition test-final results calculated by ToxRat Professional

Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	LOEC [mg/l]	NOEC [mg/l]
Frond number yield	0.035 (0.022-0.054)*	0.055 (0.037-0.083)*	0.133 (0.081-0.218)*	≤0.062	<0.062
Frond number growth rate	0.066 (0.045-0.095)*	0.108 (0.077-0.153)*	0.278 (0.183-0.425)*	≤0.062	<0.062
Frond number selectional growth rate	0.129 (0.069-0.242)*	0.165 (0.092-0.299)*	0.262 (0.127-0.541)*	0.250	0.125
Dry weight yield	0.061 ((0.017-0.218)*	0.122 (0.037-0.421)*	0.464 (0.099-2.088)*	≤0.062	<0.062
Dry weight growth rate	0.156 (0.067-0.363)*	0.356 (0.152-0.859)*	1.718 (0.458-6.049)*	≤0.062	<0.062
Dry weight selectional growth rate	0.156 (0.067-0.363)*	0.356 (0.152-0.859)*	1.718 (0.458-6.049)*	≤0.062	<0.062

* the lower and upper 95% confidence limits

Comments of zRMS:

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

- fresh weight yield factor for control plants was 3.7 (required in OECD 239: ≥2.0)
- total shoots length factor for control plants was 2.5 (required in OECD 239: ≥2.0)
- variation coefficient for biomass based on the fresh weight was 28.5% (required in OECD 239: ≤35%).

Agreed endpoints:

Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	NOEC [mg/l]	LOEC [mg/l]
Fresh weight yield	0.007 (0.00-0.372)*	0.014 (0.000-0.652)*	0.061 (0.001-5.989)*	<0.063	≤0.063
Fresh weight growth rate	0.014 (0.001-0.325)*	0.026 (0.001-0.516)*	0.086 (0.003-3.001)*	0.063	0.125
Dry weight yield	0.026 (nd.-nd.)*	0.060 (nd.-nd.)*	0.289 (nd.-nd.)*	0.5	1.0
Dry weight growth rate	0.027 (nd.-nd.)*	0.060 (nd.-nd.)*	0.284 (nd.-nd.)*	0.5	1.0

	Total shoots length yield after 14 days	0.028 (0.002-0.315)*	0.057 (0.006-0.585)*	0.221 (0.013-3.615)*	0.063	0.125
	Total shoots length growth rate after 14 days	0.017 (0.003-0.090)*	0.042 (0.009-0.212)*	0.235 (0.033-1.622)*	0.063	0.125
	* lower and upper 95% confidence limits are given in brackets nd. impossible to determine due to mathematical reasons					

Reference:	KCP 10.2.1.4/02
Report	Water-sediment *Myriophyllum spicatum* toxicity test according to OECD 239; Woźniak A.; 2019; Study Code: 0005/0071/E
Guideline(s):	Yes, OECD 239
Deviations:	Yes: During range-finding test and definitive test, the temperature decreased below the temperature recommended by the OECD 239 Guideline 18.0°C. It was a short-timed that did not affect the condition of the test system. The deviation had no effect on the course of the study and the reliability of the results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	To establish test item photolytic stability, test vessels were constantly lighted with fluorescent light of intensity 8800-11800 lux and in parallel kept in inactive lighting. Stability test was conducted for following test item concentrations: 1 mg/L and 100 mg/L and control (without test item addition). Test item concentrations analysis was performed at the beginning, after 7 days and at the end of the test (14 days).

2. Vehicle and/or positive control: vehicle: Smart & Barko medium
positive control: 3,5-dichlorophenol

3. Test organism

Species: *Myriophyllum spicatum*

Source:	commercial farm, acclimated and then grown in the SORBOLAB Research Laboratory
Test units:	glass beakers of 2L volume, medium volume 1.7 L, milfoil shoots were planted in the pot containing 0.5 kg of artificial sediment and covered with quartz sand

4. Environmental conditions:

Medium:	Smart & Barko (one of the recommended by OECD Guideline 239) prepared using deionized water with ≤1 μS·cm-1 conductivity
Medium temperature:	average temperature 18.884ºC (min. temperature 17.40ºC, max. temperature 20.80ºC)
pH:	pH value in test vessels was kept at the level of 7.77-7.94 at the beginning of the test; 7.88--9.00 at the end of the test and fluctuated by no more than 1.5 unit during the test
Dissolved oxygen:	dissolved oxygen concentration was kept within the level 81.22–90.43% at the beginning of the test and 86.20–100.21% at the end of the test
Lighting:	lighting in daily cycle 16 hours light of intensity 10400-10880 lux (required in OECD 239 Guideline: 8800-11800 lux) and 8 hours darkness and in parallel complete darkness

STUDY DESIGN AND METHOD

Water-sediment *Myriophyllum spicatum* toxicity test was performed according to OECD Guideline No 239. The aim of the study was to determine the effect of the test item FLD-HER 306 SE on growth of spiked water-milfoil *Myriophyllum spicatum*, expressed as biomass increase and fresh and dry weight growth rate and total shoot length after 7 and 14 days of test. Endpoints of the study were ECx values for tested parameters. Also statistically were determined NOEC and LOEC values.

During test duration, following parameters were observed and registered: representative plants parameters (length of main shoots, fresh and dry weight of plants), fresh weight of plants (day 0, day 14) and biomass yield and growth rate based on plant fresh weight, dry weight of plants (day 0, day 14) and biomass yield and growth rate based on plant dry weight, length of main shoots (day 0, day 7, day 14), number of lateral shoots (day 0, day 7, day 14), total lateral shoots length of each plant (day 0, day 7, day 14), yotal length of the main and lateral shoots of each plant (day 0, day 7, day 14) and biomass yield and growth rate based on total length of the main and lateral shoots, morphological observations of plants (day 14). Details of observations are included in study report.

Test design:	all test item concentrations were performed in four replicates and control in six
Test type:	test type B – one pot with three spiked water-milfoil shoots per one test vessel
Type of the exposure:	static

Exposure time:	10 days establishment phase, 14 days test item exposure phase
Tested concentrations, definitive test:	0.063 mg/L; 0.125 mg/L; 0.25 mg/L; 1.0 mg/L
Dates:	start of the study 14.08.2019 start of the experimental part: 19.08.2019 end of the experimental part: 02.12.2019 end of the study: 12.12.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

The test item – FLD-HER 306 SE effects statistically significantly the fresh weight yield in concentrations 0.063 mg/L, 0.125 mg/L, 0.25 mg/L, 0.5 mg/L, 1.0 mg/L and fresh weight growth rate, total shoots length yield and growth rate after 14 days in concentrations 0.125 mg/L, 0.25 mg/L, 0.5 mg/L, 1.0 mg/L, also dry weight yield and growth yield in concentration 1.0 mg/L.

Table KCP 10.2.1.3-2: *Myriophyllum spicatum* toxicity test -final results calculated by ToxRat Professional

Rated value	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	NOEC [mg/l]	LOEC [mg/l]
Fresh weight yield	0.007 (0.00-0.372)*	0.014 (0.000-0.652)*	0.061 (0.001-5.989)*	<0.063	≤0.063
Fresh weight growth rate	0.014 (0.001-0.325)*	0.026 (0.001-0.516)*	0.086 (0.003-3.001)*	0.063	0.125
Dry weight yield	0.026 (nd.-nd.)*	0.060 (nd.-nd.)*	0.289 (nd.-nd.)*	0.5	1.0
Dry weight growth rate	0.027 (nd.-nd.)*	0.060 (nd.-nd.)*	0.284 (nd.-nd.)*	0.5	1.0
Total shoots length yield after 14 days	0.028 (0.002-0.315)*	0.057 (0.006-0.585)*	0.221 (0.013-3.615)*	0.063	0.125
Total shoots length growth rate after 14 days	0.017 (0.003-0.090)*	0.042 (0.009-0.212)*	0.235 (0.033-1.622)*	0.063	0.125

* lower and upper 95% confidence limits are given in brackets
nd. impossible to determine due to mathematical reasons

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

Not relevant. No studies submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant. No studies submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

- bee mortality in control group was 0% (required: not more than 10%)
- LD_{50} value for reference item after 24 hours was 0.123 µg/bee (required: 0.10-0.35 µg/bee).

Agreed endpoints:

Parameter	Exposure time 48h
LD_{10} [µg/bee]	>100
LD_{20} [µg/bee]	>100
LD_{50} [µg/bee]	>100
NOED [µg/bee]	>100
LOED [µg/bee]	>100

Reference: KCP 10.3.1.1.1/01

Report: Honeybees, Acute Oral Toxicity Test according to OECD 213; Orzechowska U.; 2019; Study Code: 0005/0072/E

Guideline(s): Yes, OECD 213

Deviations: Yes: Due to experience, information obtained from literature and OECD Guideline 245, the temperature in the course of the experiment was in the range of 33±2°C, which allowed to obtain reliable results, instead of the required by the 213 OECD Guideline the range of 25±2°C. Furthermore, during range-finding test average humidity was 76.069% and during definitive test, average humidity was 49.147%, instead of the required by the 213 OECD Guideline the range of 50-70%. During the reference test, the observations were recorded only after 24 hours, with accordance to OECD Guideline 213.The above Deviations had no effect on the results of the test. The test met the validity criteria.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: 50% sucrose solution
positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	registered breeding of Mr. Sebastian Zieliński in Poznań, entered in the register under veterinary number 302171856
Age:	about 3-5 weeks after pupation
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	50% sucrose solution
Test units:	cages 20x20x20cm

4. Environmental conditions:

Temperature:	average temperature 32.759ºC (min. temperature 31.8ºC, max. temperature 33.5ºC)
Relative humidity:	average air humidity was 49.147% (minimal humidity 33.2%, maximal humidity 57.6%)
Photoperiod:	during the experimental phase, the bees were kept in darkness

STUDY DESIGN AND METHOD

The aim of the study was to determine the dose that would cause mortality of 50, 20 and 10 % of the population ($LD_{50,}$ $LD_{20\ and}$ LD_{10} value) as well as NOED and LOED after 4, 24 and 48 hours. The experiment was carried out in accordance with OECD Guideline No 213. The study was conducted on honeybee, *Apis mellifera*. Quarantine of the bees was not carried out, because within a month before the beginning of the study, insects were not treated with chemicals compounds. The tested bees were collected at the start day, in the morning hours, in order to compensate for the condition of the bees. The bees were then provided with 50% sucrose solution and appropriate environmental conditions. The study employed adult honeybees workers at a similar age (about 3-5 weeks after pupation) from a healthy, well-maintained family with a fertile mother.

Test design:	tested dose and control in five repetitions, 10 bees per repeat
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	100 µg/bee (10g/L) (limit test)
Dates:	start of the study 30.05.2019 start of the experimental part: 08.07.2019 end of the experimental part: 10.07.2019 end of the study: 20.09.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

Based on results of the experiment, observations and statistical analysis of results, it is concluded that the test material – FLD-HER 306 SE does not have toxic effects for bee mortality over 48 hours after oral exposure.
Due to conducting the definitive test as a limit test the LD_{50}, LD_{20} and LD_{10} values were not determined. No bee mortality was observed in tested dose as well as in control, in effect no statistically significant differences for bee mortality in regard to the control (ToxRat Professional Software). On the basis of data analysis, the values LOED and NOED were determined at the level of >100 µg/bee (>10 g/L).

Table KCP 10.3.1.1.1-1: *Apis mellifera* acute oral toxicity test -final results calculated by ToxRat Professional

Parameter	Exposure time 48h
LD_{10} [µg/bee]	>100
LD_{20} [µg/bee]	>100
LD_{50} [µg/bee]	>100
NOED [µg/bee]	>100
LOED [µg/bee]	>100

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

- bee mortality in control group was 0% (required: not more than 10%)
- LD_{50} value for reference item after 24 hours was 0.133 µg/bee (required: 0.10-0.30 µg/bee).

Agreed endpoints:

Parameter	Exposure time 48h
LD_{10} [µg/bee]	>100
LD_{20} [µg/bee]	>100
LD_{50} [µg/bee]	>100
NOED [µg/bee]	>100
LOED [µg/bee]	>100

Reference:	KCP 10.3.1.1.2/01
Report	Honeybees, Acute Contact Toxicity Test according to OECD 214; Orzechowska U.; 2019; Study Code: 0005/0073/E
Guideline(s):	Yes, OECD 214
Deviations:	Yes: Due to experience, information obtained from literature and OECD Guideline 245, the temperature in the course of the experiment was in the range of 33±2°C, which allowed to obtain reliable results, instead of the required by the 214 OECD Guideline the range of 25±2°C. Furthermore, during range-finding test average humidity was 48.3% and during definitive test, average humidity was 76.2%, instead of the required by the 214 OECD Guideline the range of 50-70%. The test was performed in July, which poses a deviation from months, June and July, appointed in the Study Plan. Above Deviations had no effect of the test results. The experiment fulfilled the validity criteria.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: water
positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	registered breeding of Mr. Sebastian Zieliński in Poznań, entered in the register under veterinary number 302171856
Age:	no data
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	50% sucrose solution
Test units:	cages 20x20x20cm

4. Environmental conditions:

Temperature:	average temperature 33.040 (min. temperature 31.6ºC, max. temperature 33.3ºC)
Relative humidity:	average air humidity was 60.668% (minimal humidity 58.1%, maximal humidity 76.2%)
Photoperiod:	during the experimental phase, the bees were kept in darkness

STUDY DESIGN AND METHOD

The aim of the study was to determine the dose that would cause mortality of 50, 20 and 10% of the population (LD_{50}, LD_{20} and LD_{10}) as well as NOED and LOED after 4, 24 and 48 hours. The study was carried out in accordance with OECD Guideline No 214. The study was conducted on honeybee, *Apis mellifera*. Quarantine of the bees was not carried out, because within a month before the beginning of the study, insects were not treated with chemicals compounds. The tested bees were collected at the start day of the test in the morning, in order to compensate for the condition of the bees. The bees were then provided with 50% sucrose solution and appropriate environmental conditions. The study employed adult honeybees workers at a similar age from a healthy, well-maintained family with a fertile mother.

Test design:	tested dose and control in five replicates, 10 bees per replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	100 µg/bee (100 g/L) (limit test)
Dates:	start of the study 30.05.2019 start of the experimental part: 17.07.2019 end of the experimental part: 19.07.2019 end of the study: 12.09.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

Based on results of the experiment, observations and statistical analysis of results, it is concluded that the test material – FLD-HER 306 SE does not have toxic effects for bee mortality over 48 hours after contact exposure. Due to conducting the definitive test as a limit test the LD_{50}, LD_{20} and LD_{10} values were not determined. Mortality of the bees was observed in tested dose in comparison to the control. No signs of intoxication were recorded. On the basis of data analysis, the values LOED and NOED were determined at the level of >100 µg/bee (>100 g/L) (ToxRat Professional Software).

Table KCP 10.3.1.1.2-1: *Apis mellifera* acute contact toxicity test - final results calculated by ToxRat Professional

Parameter	Exposure time 48h
LD_{10} [µg/bee]	>100
LD_{20} [µg/bee]	>100
LD_{50} [µg/bee]	>100
NOED [µg/bee]	>100
LOED [µg/bee]	>100

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. – bee mortality in control after 10 days was 8.0% (required: ≤15%), Table 5. – bee mortality in the reference test after 10 days was 50.0% (required: ≥50%), **Agreed endpoints:** NOEC >2500 mg/kg; NOED >86.939 µg/bee/day (nominal dose 100 µg/bee/day); LC50 >2500 mg/kg LDD50 >86.939 µg/bee/day (nominal dose 100 µg/bee/day)

Reference:	KCP 10.3.1.2/01
Report	Honey Bee, Chronic Oral Toxicity Test according to OECD 245; Orzechowska U.; 2019; Study Code: 0005/0075/E
Guideline(s):	Yes, OECD 245
Deviations:	Yes: During the range-finding test, one-time, brief decrease 29.6% of humidity and its increase to 73.1% was noted. It did not affect the condition of research system nor reliability of the results. During the definitive test one-time brief decrease of temperature to 29.7°C was noted (required: 33±2). The average air humidity was 75.565% (required: 50-70%). The changes were minor, which did not affect the condition of the research system nor reliability of the results. The experimental part was finished in September, which poses a deviation from months, June – August, scheduled in the Study Plan.
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study) No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: 50% sucrose solution
positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	registered breeding of Mr. Wiesław Londzin in Poznań, entered into the register under veterinary number 3021711077
Age:	2-day old
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	50% sucrose solution
Test units:	cages 20x20x20cm

4. Environmental conditions:

Temperature:	average temperature 31.498ºC (min. temperature 29.7ºC, max. temperature 34.4ºC)
Relative humidity:	average air humidity was 75.565% (minimal humidity 38.7%, maximal humidity 93.9%)
Photoperiod:	during the experimental phase, the bees were kept in darkness

STUDY DESIGN AND METHOD

The aim of the study was to determine the concentration (value LC_{50}) and the dose (value LDD_{50}) that would cause mortality of 50% of the population after 10 days. The study was carried out in accordance with OECD Guideline No 245. The study was conducted on honeybee, *Apis mellifera L*. Quarantine of the bees was not carried out, because within a month before the beginning of the study, insects were not treated with chemicals compounds including antibiotics or anti-varroa treatment. In the study, young bees

being in the similar age (approx. 2 days old) were used, originating from a healthy and well maintained breeding. Bees were placed in a test room in experiment conditions one day before the beginning of the experiment.

Test design:	tested dose and control in five replicates, 10 bees per replicate
Exposure time:	chronic test, 10 days
Tested concentrations, definitive test:	2500 mg/kg of food (86.939 µg/bee/day) (limit test)
Dates:	start of the study 05.07.2019 start of the experimental part: 11.09.2019 end of the experimental part: 21.09.2019 end of the study: 30.09.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

Based on results of the experiment, observations and statistical analysis of results, it is concluded that the test material – FLD-HER 306 SE does not have toxic effects for bee mortality over 10days experiment.
Due to the fact of conducting the definitive test for terminal concentration (limit test), concentration causing mortality of 50% of the population in the study (LC50 value) and dose of test item per bee causing mortality of 50% of the population after 10 days (LDD50 value) were not determined, as well as NOEC and NOEDD values.
On the basis of data analysis, NOEC value was determined as >2500 mg/kg; NOEDD value was determined as >86.939 µg/bee/day (nominal dose 100 µg/bee/day); LC50 value was determined as >2500 mg/kg and LDD50 value was determined as >86.939 µg/bee/day (nominal dose 100 µg/bee/day)

Table KCP 10.3.1.2-1: Honeybees, Chronic Oral Toxicity Test – final results

Test item concentration [mg/kg of food]	Mortality [pcs.]	Mortality [%]	Statistical significance in comparison to the control*
Control	4	8	not applicable
2500	3	6	-

* - statistically insignificant (for statistical calculation was used Fisher's Test using ToxRat Professional Software)

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

Not relevant. No studies submitted.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. – cumulative larval mortality in control in days 3-8 was 2.8% (required: ≤15%), – the adults emergence rate in control at day 22 was 75.0% (required: ≥70%), – the adults emergence rate in reference test at day 22 was 5.6% (required: ≤20%)

Agreed endpoints:

Parameter	Concentration [mg/kg of food]	Parameter	Dose [µg/larva]
LC_{10}	n.d.** (n.d. – n.d.)*	LD_{10}	n.d.*** (n.d. – n.d.)*
LC_{20}	n.d.** (n.d. – n.d.)*	LD_{20}	n.d.*** (n.d. – n.d.)*
LC_{50}	n.d.** (n.d. – n.d.)*	LD_{50}	n.d.*** (n.d. – n.d.)*
NOEC	≥650	NOED	≥100

* upper and lower confidence limits (95%)
** based on the results analysis, value was defined as >650 mg/kg of food
*** based on the results analysis, value was defined as >100 µg/larva
n.d. not determined

Reference:	KCP 10.3.1.4/01
Report	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239; Orzechowska U.; 2019; Study Code: 0005/0076/E
Guideline(s):	Yes, OECD GD 239
Deviations:	Yes: At day 8 of the test, desiccator with larvae was transferred to test room (temperature 34-35°C, humidity 60-80%), what poses a deviation from Standard Experimental Procedure and Study Plan. The alteration was introduced due to observable improvement of larvae development outside incubator. In course of the definitive test, periodic decreases of temperature (required: 34-35°C) and humidity (required: 50-100%) occurred. It resulted from daily feedings and observations. These drops were shorttermed, did not affect the condition of the test system. The deviations had no effect of the test results. The test met the validity criteria..
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: 50% sucrose solution
positive control: fenoxycarb

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	registered breeding of Mr. Wiesław Londzin in Poznań, entered into the register under veterinary number 3021711077
Age:	1-day old larvae of honey bee originated from 3 different, healthy, well-maintained breeding
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	Larval diets were adjusted depending on the developmental stage (all solutions were prepared in weight percentage): Food A: 50% fresh royal jelly + 50% aqueous solution containing 2% yeast extract/ 12% glucose/12% fructose; Food B: 50% fresh royal jelly + 50% aqueous solution containing 3% yeast extract / 15% glucose /15% fructose; Food C: 50% fresh royal jelly + 50% aqueous solution containing 4% yeast extract / 18% glucose /18% fructose. Following the above, prepared food should have density around 1.1 mg/µL (20 µL of food corresponds to 22 mg of food). Before administration, food was warmed to 35°C. It was provided using automatic pipette, with caution to avoid touching a larva or drowning it in food liquid. From the emergence phase (D15 - D22) as food was used: 50% aqueous solution of sucrose, pine pollen.
Test units:	48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator; since day 15 of the test – transparent plastic boxes placed in test room

4. Environmental conditions:

Temperature:	average temperature 34.071ºC (min. temperature 31.7ºC, max. temperature 35.4ºC)
Relative humidity:	average air humidity was 85.458% (minimal humidity 46.2%, maximal humidity 99.3%)
Photoperiod:	during the experimental phase, the bees were kept in darkness

STUDY DESIGN AND METHOD

The aim of the study was determination of the concentration causing 50% mortality of population (LC50 value) and the dose of the test item causing mortality of 50% of the population after 22 days (LD50 value). Values NOEC and NOED were determined for each following developmental stages for honey bee. The study was carried out in accordance with OECD GD 239. The study was conducted on honeybee, *Apis mellifera L*. Quarantine of the bees was not carried out, because within a month before the beginning of the study, insects were not treated with chemicals compounds including antibiotics or anti-varroa

treatment. The study used 1-day larvae of honey bee originated from 3 different, healthy, well-maintained breeding. The physiological condition of the bees was tested during the test with a reference item (fenoxycarb), chosen on the base of results of the range-finding test.
3 days before the beginning of the test (D-3), in each family, queen bee was isolated using one-frame isolator. After max. 30 hours (D-2), queens were released from the isolator (after conforming the presence of freshly laid eggs). The frame containing the eggs remains in the isolator, placed next to the frame containing brood, for 3 days, until the hatching (D1). At day 1 (D1), the frames with freshly brooded larvae are transferred from the hive to laboratory in the temperature optimal for larvae (above 20°C). Frames are placed under the inactive laminar-flow hood. For the study are chosen larvae, which has not yet formed C-shape or the ones laying on the top of royal jelly. The larvae were carefully placed in the same position at the bottom of queen-cell cup filled with diet A placed in breeding plate's well. Larvae are collected in excess to provide minimal amount of larvae (12 larvae from each colony) required for the beginning of test item exposition at third day of the test.

The weight of the test item was dissolved in a deionized water. Then final solutions of test item were prepared by adding given volume of stock solution to following portions of food (larval diets). Fresh diets were prepared daily.

During the definitive test, test item was administrated in food during 4-day exposition. The observations of mortality and behavioural changes were recorded daily during 22 days of the test. Parallel to definitive test, reference test was performed using fenoxycarb as reference item.

Test design:	tested concentrations and control in one replicate; 36 larvae per replicate
Exposure time:	chronic test, exposition: 4 days (from D3 to D6), duration of the test: 22 days
Tested concentrations, definitive test:	16.64 mg/kg; 41.6 mg/kg; 104 mg/kg; 260 mg/kg; 650 mg/kg of food
Dates:	start of the study 05.07.2019 start of the experimental part: 12.05.2020 end of the experimental part: 23.06.2020 end of the study: 24.06.2020
Statistic:	ToxRat Professional statistical program

During the test, following measurements and observations were performed:
- larval mortality from day 4 to day 8 and on day 15, observations recorded during feeding periods; immobile or non-reacting larva was noted as dead; on day 15, larvae, which have not transformed into pupae were recorded as dead; during the feeding, dead individuals were removed for sanitary reasons,
- on day 22 mortality of adults and pupae – amount of emerged or non-emerged,
- on day 22 emerged adults, alive or dead,
- at the end of the test was determined percentage of emerged adults (by comparing the number of bees emerged on day 22 to the number of larvae on day 3); pupal mortality (calculated in percentage by comparing the number of pupae failed to emerge, including those without emergence on day 22 and dead pupae remove during pupa stage from day 8 to day 22 to the number pf bees entering pre-pupa stage on day 8); the larval mortality (percentage calculated by comparing the number of bees died during larvae stage - from day 3 to day 8 - to the number of larvae on day 3),
- on day 8, presence of uneaten food,
- temperature and humidity during definitive test were recorded continuously using temperature and humidity recorder,

- other observations (for larvae, pupae and adults: appearance, size, behaviour, morphological differences).
Details concerning observations are included in the study report.

During definitive test, no statistically significant final mortality was observed in all tested concentrations. Larvae shown slight signs of intoxication, represented by stunted development, i.e. inhibited food intake, smaller size, decreased mobility. Similar signs of intoxication were recorded in case of pupae. On day 8 of the test, the presence of food uneaten by larvae was recorded. The amount of food left was minor, resulted mainly from retarded development of larvae.

CONCLUSION

In course of the experiment, the test item has shown no apitoxic effect in mortality of following developmental stages of bees after 22 days of the test. At the end of the study, the concentration and the dose causing 50% mortality of the population in the test (LC_{50} and LD_{50} values) were not determined, however NOEC and NOED values were determined at 22 day.

Table KCP 10.3.1.4-1: Honeybees, Chronic Oral Toxicity Test – final results

Parameter	Concentration [mg/kg of food]	Parameter	Dose [µg/larva]
LC_{10}	n.d.** (n.d. – n.d.)*	LD_{10}	n.d.*** (n.d. – n.d.)*
LC_{20}	n.d.** (n.d. – n.d.)*	LD_{20}	n.d.*** (n.d. – n.d.)*
LC_{50}	n.d.** (n.d. – n.d.)*	LD_{50}	n.d.*** (n.d. – n.d.)*
NOEC	≥650	NOED	≥100

* upper and lower confidence limits (95%)
** based on the results analysis, value was defined as >650 mg/kg of food
*** based on the results analysis, value was defined as >100 µg/larva
n.d. not determined

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not relevant. No studies submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not relevant. No studies submitted.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

No new studies provided.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. – mortality in the negative control was 3.3% (should not exceed 10%) – mortality in the toxic reference treatment was 100% (should not be less than 50%) – fecundity in the negative control (mean number of mummies per female per day) was 11.2. (the minimum average mummy value per female should be 5.0) – none of the surviving wasps in the negative control produced zero mummies (for the test to be considered valid, no more than two of the surviving wasps should produce zero values). **Agreed endpoints:** **Mortality** LR_{10} = 251.3 mL $_{TI}$/ha, LR_{20} = 564.8 mL $_{TI}$/ha and LR_{50} = 2658.4 mL $_{TI}$/ha NOER value = 360 mL $_{TI}$/ha and LOER = 1080 mL $_{TI}$/ha. **Fecundity:** LOER = 3240 ml $_{TI}$/ha a NOER = 1080 mL $_{TI}$/ha and ER_{10} = 1479.6 ml $_{TI}$/ha were determined.

Reference:	KCP 10.3.2.2/01
Report	Extended laboratory test (Tier2) for the impact assessment on the parasitic wasp *Aphidius rhopalosiphi;* Kręglewska M.; 2019; Study Code: 0005/0078/E
Guideline(s):	Yes, SETAC; ESCORT I, ESCORT II; IOBC/BART/EPPO
Deviations:	Yes: It was possible to maximally increase illumination it the laboratory up to 2700 lux in the reproduction phase, instead of recommended from 3000 to 20000 lux in the protocol. Temperature was outside the required range (20±2°C) in two prolonged periods of time during the fecundity phase of the Definitive test, but it was most of the time only 0.5°C above its the upper limit. Deviation did not affect on the study results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019

Production date:	04.2019
Expiration date:	04.2021
2. Vehicle and/or positive control:	vehicle: distilled water positive control: dimetholate 400 g /L EC
3. Test organism	
Species:	parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Source:	purchased as synchronized aphid mummies *Rhopalosiphum padi* from breeder which has a certificate confirming their species (Katz Biotech AG);
Age:	adult females (1-2 days old)
Acclimation period:	1-2 days under test conditions
Diet:	cotton wool pad soaked with 10% sugar solution
Test units:	Barley seeds (Hordeum vulgare var. lancelot) were planted approximately 7 days prior to start of the test into pots filled with gardening substrate (Agro SC, Czech Republic; top surface area 10x10cm). The mortality phase was conducted in pots included 8-10 barley seeds. Treatments application was conducted when the seedlings were 10-12 cm high at the 2nd leaf growth stage. For the fecundity phase, pots containing 10-40 seedlings were infested with > 100 cereal aphids/pot
4. Environmental conditions:	
Temperature:	mean temperature in mortality phase 21.6°C (minimal 21.0°C, maximal 22.5°C); mean temperature in fecundity phase 20.6°C (minimal 19.7°C, maximal 21.7°C)
Relative humidity:	mean relative humidity in mortality phase 60% (minimal 56%, maximal 65%); mean relative humidity in fecundity phase 61% (minimal 58%, maximal 68%)
Photoperiod:	lighting: 460-530 lux; after the wasps were removed from the cylinders, after 24 h parasitation period lighting was increased to 2600-2700 lux

STUDY DESIGN AND METHOD

The study was conducted to assess the effects of the test item FLD-HER 306 SE on mortality and reproductive performance of parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). The aim of the study was to determine the lethal rate of the test item producing 50% mortality of the population in the study (value LR50) after 48 hours and the impact on the reproduction of *Aphidius rhopalosiphi*.
Adult female wasps were introduced to the test units within 1 h from the test item application to barley plants. Observation of settling behaviour were made during initial 2.5 hours of exposure. The aims were to determine repellent effects of FLD-HER 306 SE and to check if the tests insects had contact with barley plants sprayed with the test item. Settling behaviour of the wasps from each replicate was observed five times. Mortality assessments were made 2, 24 and 48 hours after the introduction of the wasps to the test units. At each assessment, number of wasps was recorded according to define criteria.

Fecundity assessment of female wasps was carried out for control and doses 120, 360, 1080, 3240 mL $_{TI}$/ha, where above 50% of female survived the mortality phase. 15 female wasps per treatment were individually exposed to aphid-infested plants using clear cylinders placed over the plants in each of the pots (Figure 2.). After 24 hours the female wasps were removed from the cylinders. The parasitized aphids within the reproduction cylinders were left to develop *in situ* and the number of aphid mummies that developed were recorded 11 days later.
To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e. Danadim Progress (dimetholate 400 g /L EC) was used as a reference item. The rate of the reference item was 20 g/ha. The control group was treated with distilled water.

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Test design:	tested concentrations and control in 6 replications, reference in 4 replications, number of females: 5 females/replicate for test and reference item
Exposure time:	14 days (mortality phase: 48 hours + fecundity phase : 12 days)
Tested concentrations, definitive test:	120, 360, 1080, 3240 and 9720 mLTI/ha
Dates:	start of the study: 06.06.2019 start of the experimental part: 24.06.2019 end of the experimental part: 19.08.2019 end of the study: 10.10.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

The test item FLD-HER 306 SE affected survival of *Aphidius rhopalosiphi*. Following values were determined: LR_{10} = 251.3 mL $_{TI}$/ha, LR_{20} = 564.8 mL $_{TI}$/ha and LR_{50} = 2658.4 mL $_{TI}$/ha and values of NOER value = 360 mL $_{TI}$/ha and LOER = 1080 mL $_{TI}$/ha. In the tested dose range, the test item affected fecundity of *Aphidius rhopalosiphi* females. The reduction of mummies number in dose 3240 mL $_{IT}$/ha was 64,3%. The values of LOER = 3240 ml $_{TI}$/ha and NOER = 1080 mL $_{TI}$/ha and ER10 = 1479.6 ml $_{TI}$/ha were determined.

Table KCP 10.3.2.2-1: The effects of FLD-HER 306 SE on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test

Dose [mL $_{TI}$/200 L water/ha]	**Impact of the test item on survival**					
	mortality (corrected by Abbott's formula)	**LR10**	**LR20**	**LR50**	**NOER**	**LOER**
	[%]	**[mL TI/200 L water/ha]**				
Control	0	251.3	564.8	2658.4	360	1080
120	6.9					
360	6.9					
1080	44.8					
3240	37.9					
9720	82.8					
Dose	**Impact of the test item on fecundity**					

[mL TI/200 L water/ha]	reduction in production of mummies /female in comparison to control	LR10	NOER	LOER
	[%]	[mL TI/200 L water/ha]		
Control	not applicable	1479.6	1080	3240
120	-1.2			
360	7.7			
1080	4.8			
3240	64.3			

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. – mortality of predatory mites *Typhlodromus pyri* in control amounted to 5.0% (requirements: not more than 20%) – reproduction averaged 4.7 eggs/female (requirements: ≥ 4 eggs/female). Observations of mortality were made after 7 days from the beginning of the study. The reference item caused 91,3% mortality of tested mites after 7 days towards required validity criterion: 50-100% mortality. **Agreed endpoints:** **Mortality:** LR_{50} = 5383.3 ml BM/200 l water/ha LOER = 1500 mL TI/200 L water/ha NOER = 750 mL TI/200 L water/ha. **Fecundity:** ER_{50} = 5805.3 mL TI/200 L NOER =1500 mL TI/200 L LOER was 3000 mL TI/200 L water/ha.

Reference:	KCP 10.3.2.2/02
Report	Extended laboratory test (Tier2) for evaluating the effects on the predatory mites *Typhlodromus pyri* (Scheuten); Kręglewska M.; 2019; Study Code: 0005/0077/E
Guideline(s):	Yes, IOBC, BART, EPPO
Deviations:	Yes, In the definitive study, deviations of temperature and relative humidity from assumed in Study Plan values were noted. This deviation did not effect on the study results. The validity criteria of the test were met.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: distilled water
positive control: dimetholate 400 g /L EC

3. Test organism

Species:	predatory mite *Typhlodromus pyri*
Source:	purchased as synchronized eggs from breeder which has a certificate confirming their species, ie. Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Age:	young mites, 24-hours individuals (protonymphs)
Acclimation period:	the mites eggs were acclimatized for 1-2 days in experimental conditions
Diet:	every 3 days with pine pollen
Test units:	test area marked on the bean leaf surface. The test area was a form of square with dimensions of 3.5 x 3.5 cm, which is 12,25 cm^2 of test area. After spraying and drying of the test item, the test area was secured by using a special glue which protect mites against escape

4. Environmental conditions:

Temperature:	mean temperature 25.190°C (minimal 23.50°C, maximal 27.40°C)
Relative humidity:	mean relative humidity 77.912% (minimal 56.20%, maximal 94.20%)
Photoperiod:	daily cycle 16 h day/8 h night; lighting: 495 lux

STUDY DESIGN AND METHOD

The study was conducted to assess the potentially negative impact of the test item FLD-HER 306 SE on survival and fecundity of predatory mite *Typhlodromus pyri.* The end points of the test were values LR50 and ER50 for mentioned parameters. The NOER and LOER values were also statistically determined.
The study consisted in two steps: the assessment of the mortality of young mites and evaluation of reproductive ability of organisms. The first step involved the exposure of young mites to dose range of the test for 7 days. The test item was applied to the designated area of leaf. One hour after test item application, on the leaf surface with a small brush a small amount of pine pollen was introduced as a source of nutrition for mites and next young 24-hours mites were. The organisms mortality was assessed after 7 days. In

the second part of the study, in reproductive phase, reproductive ability was observed for a further 7 days on dosage levels in which min. 50% of the initial number of organisms survived. At that time, the adult mites were laying eggs. After 10, 12, 14 days from the beginning of the study, the number of eggs and larvae in specified doses of the test item was checked, as well as the number of females.

Test design:	tested concentrations, reference item and control in 4 replications, number of mites: 20 young/replicate for test and reference item
Exposure time:	14 days (7 days of mortality phase + 7 days of fecundity test)
Tested concentrations, definitive test:	187.5, 375, 1500 and 3000 mL_{TI}/200 L water /ha
Dates:	start of the study: 04.06.2019 start of the experimental part: 19.07.2019 end of the experimental part: 30.08.2019 end of the study: 15.11.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

The test item FLD-HER 306 SE in extended laboratory study showed the statistically significant impact on survival of predatory mites *Typhlodromus pyri* at doses 1500 mL $_{TI}$/200 L water/ha and 3000 mL $_{TI}$/200 L water/ha. It was determined that values of LR_{50} = 5383.3 ml $_{BM}$/200 l water/ha, LOER = 1500 mL $_{TI}$/200 L water/ha and NOER = 750 mL $_{TI}$/200 L water/ha.
The test item show an effect on the reproduction of predatory mites *Typhlodromus pyri* at dose 3000 ml $_{TI}$/200 L water/ha. The value of ER_{50} = 5805.3 mL $_{TI}$/200 L water/ha was determined. Value of NOER of the test item was 1500 mL $_{TI}$/200 L water/ha and LOER was 3000 mL $_{TI}$/200 L water/ha.

Table KCP 10.3.2.2-2: The effects of FLD-HER 306 SE on mortality and reproduction of *Typhlodromus pyri* in in the extended laboratory test

Dose [mL $_{TI}$/200 L water/ha]	**Impact of test item on survival**			
	Abbott's corrected mortality	**LR_{50}**	**NOER**	**LOER**
	[%]	**[mL $_{TI}$/200 L water/ha]**		
Control	0.0	5383.3 (2249.2 – n.d.)*	750	1500
187.5	5.3			
375	3.9			
750	5.3			
1500	14.5			
3000	40.8			
Dose [mL $_{TI}$/200 L water/ha]	**Impact of test item on reproduction**			
	Average number of offspring per female	**LR_{50}**	**NOER**	**LOER**

	[psc.]	[mL TI/200 L water/ha]		
Control	4.7	5805.3 (912.4 – 34571.4)*	1500	3000
187.5	4.2			
375	4.3			
750	4.2			
1500	4.2			
3000	3.4			

*) - the lower and upper 95% confidence limits are given in brackets
n.d. - Not determined due to mathematical reasons or value is beyond the tested rates by more than factor 1000.

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

The following criteria should be satisfied in the control for a test result to be considered valid:

- mean mortality of larvae in the water treated control should not exceed 30%;
- mean mortality of larvae in the toxic reference treatment should be higher than 40%;
- no.2 fertile eggs per viable female per day.

During the study the following criteria were met:

Pre-imaginal mortality in control check	Mortality was 22.50% and so the validity criterion was met.
Reproduction in control check	The mean number of eggs per female per day was 41.02, so this validity criterion was met.
Pre-imaginal mortality in reference	Mortality was 87.50% in the toxic reference treatment and so the validity criterion was met.

Agreed endpoints:

Mortality:

LOER =600 mL/ha

NOER =300 mL/ha

LR_{50} =5796.30 mL/ha.

Reproduction:

NOER >1200 mL/ha.
ER_{50}>1200 mL/ha

Reference: KCP 10.3.2.2/03

Report: Effects of FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) on *Coccinella septempunctata* in the laboratory – Extended laboratory test – Year 2019; Rovetto I.; 2020; Study Code: 1075-1075HSAG19/r

Guideline(s): Yes, IOBC, BART, EPPO

Deviations: No

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

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: distilled water
positive control: dimetholate 400 g /L EC

3. Test organism

Species:	*Coccinella septempunctata*
Source:	Katz Biotech AG, Baruth, Germany
Age:	first instar larvae, 3-day old
Acclimation period:	3 days under test conditions (same feeding)
Diet:	-mortality assessments - larvae were fed *ad libitum* with aphids (*Acyrthosiphon pisum* Mordv.) of mixed stages. -fecundity assessment - individuals were fed daily with honey and 3-4 aphid infested (*Acyrthosiphon pisum*) broad bean stems. Plastic tubes filled with water and closed with a cotton wool were also provided for water supply, water was provided permanently in a reservoir and replaced at least once a week

Test units:	-mortality assessment - transparent plastic cylinder (45.8 mm Ø × 50 mm) with PTFE (politetrafluoroetilene) on the internal side closed with a perforated lid (28 mm Ø) and with an insect proof net, the cylinder was inserted into a plate (57 mm Ø) where a bean leaf disc (50 mm Ø) was placed on a filter paper layer, the cylinder was blocked on the lid by two rubber bands, arranged in a cross design -fecundity assessment - transparent plastic boxes (145 × 135 × 85 mm) with a perforated lid provided with an insect proof net for aeration, at the box bottom one layer of filter paper, while inside, three pieces of bubble wrap (PE) and a dark plastic cylinder, as oviposition substrate, egg clutches were stored in individually labelled plastic dishes (60 mL in volume) until larval hatch, over a wet layer of filter paper

4. Environmental conditions:

Temperature:	25.515°C
Relative humidity:	80.1%
Photoperiod:	16h light (1514.29 lux): 8h dark

STUDY DESIGN AND METHOD

The aim of the study was to determine the effect of FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) on the vitality and reproduction of *Coccinella septempunctata* under laboratory conditions.. *Coccinella septempunctata* eggs were kept in the containers provided by the supplier Katz Biotech AG, Baruth, Germany, and stored in a chamber under mean controlled conditions until hatching. Afterwards, first instar larvae were left to acclimatize for 3 days before test start under test conditions. Larvae were fed *ad libitum* with aphids (*Acyrthosiphon pisum* Mordv.) The bean plants were treated with FLD-HER 306 SE applied once at 1200, 600, 300, 150 and 75 mL/ha; 40 replicates for each treatment were set up. Application was performed in spray chamber, with a spraying surface of 2 m^2 (length: 2 m, width: 1 m).

The larvae were exposed to dried residues on treated bean leaf discs and daily observed for mortality until the adult's emergence. At the end of this period, the observations consisted in recording percent mortality; when 90% of the survived pupae was hatch in the control, females and males (adults) were sexed, assessed for their reproductive performance and transferred to the mass-rearing units. The reproduction test started one week after the first egg laying observation. Insects oviposition was checked daily for up to 15 days. The eggs-hatching was assessed.

Test design:	tested 5 concentrations, reference item and control in 4 replications, 40 replicates per treatment group for the mortality assessments, the number of replicates per treatment for the fecundity assessments depended on the number of survived adults i.e. 1 larva per replicate for the mortality assessments and from 4 to 12 adults per fecundity box for the fecundity assessments

Exposure time:	mortality exposure time - until beetle emergence fecundity exposure time - 15 days (starting one week after the first egg-laying)
Tested concentrations, definitive test:	1200, 600, 300, 150 and 75 mL of FLD-HER 306 SE /ha
Dates:	study Director Study Plan Approval: 14 May 2019 start of the experimental phase: 17 May July 2019 (range finding test application) end of the experimental phase: 18 October 2019
Statistic:	software used for statistical analysis was "RStudio", version 3.0.2., mortality data were processed using the Fisher's Exact Test, $\alpha \leq 0.05$ and LR_{50} was calculated, correction for control mortality was processed using Schneider-Orelli's formula, the different regression tests were compared each other and it was selected the ones with the best: pseudoR^2, AIC and lack of fit values, therefore, the goodness of fit was also calculated.

RESULTS

The mortality percentage for the test item ranged between 30.00% in T2 (FLD-HER 306 SE at 75 mL/ha, corrected mortality: 9.70%) and 45.00% in T6 (FLD-HER 306 SE at 1200 mL/ha, corrected mortality: 29.00%). Significant differences were noticed between the treatments T5 and T6 (FLD-HER 306 SE at 600 mL/ha and 1200 mL/ha respectively, corrected mortality: 25.80% and 29.00%) and the control, showing a 22.50% of mortality.
The mean number of eggs per female per day ranged from 22.04 (T6 – FLD-HER 306 SE at 1200 mL/ha) to 28.56 (T2 – FLD-HER 306 SE at 75 mL/ha) in comparison to the untreated control where 41.02 eggs per female per day were recorded.
The hatching rate ranged from 69.16% in treatment T2 (FLD-HER 306 SE at 75 mL/ha) to 74.53% in treatment T4 (FLD-HER 306 SE at 300 mL/ha), while in the control it was equal to 74.94%. The mean number of eggs counted in 15 days was between 70.52 in treatment T2 (FLD-HER 306 SE at 75 mL/ha) and 128.06 in treatment T4 (FLD-HER 306 SE at 300 mL/ha).

Table KCP 10.3.2.2-3: The effects of FLD-HER 306 SE on mortality and fecundity efficiency of *Coccinella septempunctata* in in the extended laboratory test

Treatment	**T1 Control**	**T2 FLD-HER 306 SE 75 mL/ha**	**T3 FLD-HER 306 SE 150 mL/ha**	**T4 FLD-HER 306 SE 300 mL/ha**	**T5 FLD-HER 306 SE 600 mL/ha**	**T6 FLD-HER 306 SE 1200 mL/ha**	**T7 ROGOR L 40 ST 15 mL/ha**
Mortality [mean %]	22.50	30.00	32.50	35.00	42.50	45.00	87.50
Significance [a]	-	-	-	-	*	*	***
Corrected mortality [mean %] [b]	-	9.70	12.90	16.10	25.80	29.00	82.10
Reproduction [mean eggs/female/day]	41.02	28.56	26.72	28.38	27.70	22.04	-
% egg-hatching	74.94	69.16	71.36	74.53	73.96	74.07	-
Endpoint (mL/ha)							

LR_{25}	695.92
LR_{50}	5796.30
NOER (Mortality)	300
LOER (Mortality)	600
Fecundity	No effect

-, not applicable
n.s., not significantly different compared to the control,
[a], Fisher's Exact Test, α=0.05 *
[b], Schneider-Orelli's formula

Conclusion

A dose-response effect on *Coccinella septempunctata* mortality was observed; therefore, the calculated LOER matched the test item rate of 600 mL/ha (i.e., treatment T5), while the NOER value was 300 mL/ha (matching the application rate in treatment T4) and the estimated LR_{50} was 5796.30 mL/ha.

There was no treatment related effect on the reproductive performance of *Coccinella septempunctata.* Therefore, it can be assumed that there is no effect on reproduction up to and including 1200 mL/ha.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. The following criteria should be satisfied in the control for a test result to be considered valid: - mortality in the water control ≤ 20% (dead larvae, pupae and adults during emergence); - mean number of eggs per female per day in the water control ≥ 15; - mean hatching rate ≥ 70; - mortality in the reference group ≥ 50 %. During the study the following criteria were met: Mortality in control check: Mortality was 3.33 % and so the validity criterion was met. Reproduction in control check: The mean number of eggs per female per day was 31.21, so this validity criterion was met. Hatching rate in control check: The hatching rate was 84.05% and so the validity criterion was met. Mortality in reference: Mortality was 60.00% in the toxic reference treatment and so the validity criterion was met. **Agreed endpoints:** **Mortality:** LOER =16200 mL/ha NOER =5400 mL/ha LR_{50} =107913.80 mL/ha. **Fecundity:** LOER =600 mL/ha NOER =200 mL/ha

	ER_{50} = 983.40 mL/ha.

Reference:	KCP 10.3.2.2/04
Report	Effects of FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) on foliage dwelling predator *Chrysoperla carnea* in the laboratory – Extended laboratory test – Year 2019; Rovetto I.; 2020; Study Code: 1074-1074HSAG19/r
Guideline(s):	Yes, IOBC, BART, EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: distilled water
positive control: dimetholate

3. Test organism

Species:	*Chrysoperla carnea*
Source:	Katz Biotech AG, Baruth, Germany
Age:	first instar larvae, 2-3 days old
Acclimation period:	2 days under test conditions (same feeding)
Diet:	mixture of 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g dried brewer's yeast, 50 g wheatgerm and 45 mL drinking water, this food was available continuously and replaced at least twice, preferably three times per week, water was provided permanently in a reservoir and replaced at least once a week

Test units:	-mortality assessment - cylinder (45.8 mm Ø x 50 mm) of transparent plastic provided with PTFE (politetrafluoroetilene), a perforated lid (28 mm Ø) and with an insect proof net. The cylinder was inserted into a plate (57 mm Ø) provided with a leaf disc of bean (50 mm Ø) leaning on two filter paper layers and blocked to it by rubbers, arranged in a cross design -fecundity assessment - glass cylinder (5000 cc, 16 cm Ø, 29 cm) provided with an insect proof net, blocked to it by rubbers

4. Environmental conditions:

Temperature:	26.021°C
Relative humidity:	77.4%
Photoperiod:	16 h light: 8 h dark; light intensity: 1553.33 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the LR_{50} of test item FLD-HER 306 SE (florasulam 6.5 g/L + 2,4-D-etexyl 297.5 g/L) by assessing *Chrysoperla carnea* mortality over time and to evaluate the sub-lethal effects on insect fecundity subsequent to their exposure to the test item applied once on bean plants compared to a water-treated control and to a reference item. For this purpose, a Range Finding test was initially performed followed by the Definitive Test. The Definitive Test rates were established taking into consideration the Range Finding test results and according to the maximum dosage rate expected from the GAP.

The study encompassed 7 treatments (5 rates of the test item, control, reference item) with 30 replicates each containing 1 larva. The larvae were exposed to dried residues on treated bean leaf discs and observed regularly, three times per week. At the end of this period, the observations consisted in giving percent mortality; ≥ 50% of larvae exposed to the test item survived and successfully completed their metamorphosis, females and males (adults) were sexed, assessed for their reproductive performance and transferred to the mass-rearing units. The reproduction test started one week after the first egg laying observation. Samples of two laid eggs, covering an oviposition period of 24 hours, were taken in one week. Larvae hatching and lacewings survival were assessed.

Mortality of exposed individuals; LR_{50}: lethal rate producing 50% mortality after exposure. Additionally, reproductive capacity for survivors was assessed.

Test design:	5 rates of the test item, control, reference item with 30 replicates per treatment group for the mortality assessment and 2 replicates per treatment for the fecundity assessment
Exposure time:	mortality exposure time - until hatching of adult lacewings
Tested concentrations, definitive test:	200, 600, 1800, 5400 and 16200 of FLD-HER 306 SE /ha

Dates:	study Director Study Plan Approval: 14 May 2019 start of the experimental phase: 16 May July 2019 (range finding test application) end of the experimental phase: 22 August 2019
Statistic:	software used for statistical analysis was "RStudio", version 3.0.2., mortality data were processed using the Fisher's Exact Test, $\alpha \leq 0.05$ and LR_{50} was calculated, correction for control mortality was processed using Schneider-Orelli's formula, the different regression tests were compared each other and it was selected the ones with the best: pseudoR^2, AIC and lack of fit values, therefore, the goodness of fit was also calculated.

RESULTS

The mortality percentage for the test item ranged between 6.67% in T2 (FLD-HER 306 SE at 200 mL/ha, corrected mortality: 3.45%) and 33.33% in T6 (FLD-HER 306 SE at 16200 mL/ha, corrected mortality: 31.03%). Significant differences were noticed between the treatment T6 (FLD-HER 306 SE at 16200 mL/ha) and the control, showing a 3.33% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 60.00% (corrected mortality: 58.62%). A dose-response effect on *Chrysoperla carnea* mortality was observed; therefore, the calculated LOER matched the test item rate of 16200 mL/ha (i.e., treatment T6), while the NOER value was 5400 mL/ha (i.e., treatment T5). The estimated LR_{25} of FLD-HER 306 SE was 9791.19 mL/ha (95% confidence intervals are 3086.90 – 31056.21 mL/ha) and the estimated LR_{50} was 107913.80 mL/ha (95% confidence intervals are 10445.61 – 1114860.07 mL/ha).

One week after the first egg laying had been observed, the mean number of eggs laid per female per day and the hatching rate of eggs was assessed. The mean number of eggs per female per day ranged from 18.04 (T62 – FLD-HER 306 SE at 200 mL/ha) to 10.18 (T6 – FLD-HER 306 SE at 16200 mL/ha) in comparison to the untreated control where 31.21 eggs per female per day were recorded. Significant differences were noticed between the treatment T3 (FLD-HER 306 SE at 600 mL/ha) and the control, showing a mean number of eggs per female per day of 31.21. The hatching rate ranged from 80.14% in treatment T2 (FLD-HER 306 SE at 200 mL/ha) to 75.45% in treatment T6 (FLD-HER 306 SE at 16200 mL/ha), while in the control it was equal to 84.05%. A dose-response effect on *Chrysoperla carnea* reproductive performance was observed; therefore, the calculated LOER matched the test item rate of 600 mL/ha (i.e., treatment T3), while the NOER value was 200 mL/ha (i.e., T2). The estimated ER_{25} of FLD-HER 306 SE was 327.80 mL/ha (95% confidence intervals are 76.90 – 578.70 mL/ha) and the estimated ER_{50} was 983.40 mL/ha (95% confidence intervals are 230.69 – 1736.11 mL/ha).

Table KCP 10.3.2.2-3: The effects of FLD-HER 306 SE on mortality and fecundity efficiency of *Chrysoperla carnea* in in the extended laboratory test

Treatment	T1 Control	T2 FLD-HER 306 SE 200 mL/ha	T3 FLD-HER 306 SE 600 mL/ha	T4 FLD-HER 306 SE 1800 mL/ha	T5 FLD-HER 306 SE 5400 mL/ha	T6 FLD-HER 306 SE 16200 mL/ha	T7 ROGOR L 40 ST 90 mL/ha
Mortality [mean %]	3.33	6.67	10.00	20.00	20.00	33.33	60.00
Significance [a]	-	n.s	n.s	n.s	n.s	*	***
Corrected mortality [mean %] [b]	-	3.45	6.90	17.24	17.24	31.03	58.62

Reproduction [mean eggs/female]	31.21	18.04	16.39	15.21	11.54	10.18	-
Significance [c]	-	n.s	*	*	*	*	-

Endpoint (mL/ha)	
LR_{25}	9791.19
LR_{50}	107913.80
NOER (Mortality)	5400
LOER (Mortality)	16200
NOER (Reproduction)	200
LOER (Reproduction)	600
ER_{25}	327.80
ER_{50}	983.40

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.05 *, 0.01 **, 0.001 ***, with Bonferroni-Holm's correction
[b], Schneider-Orelli's formula
[c], Anova, α=0.01

CONCLUSION

The test item FLD-HER 306 SE showed a dose-response effect on *Chrysoperla carnea* mortality and reproductive capacity.

About mortality, significant differences were noticed between the treatment T6 (FLD-HER 306 SE at 16200 mL/ha) and the control, therefore, the calculated LOER matched the test item rate of 16200 mL/ha (i.e., treatment T6), while the NOER value was 5400 mL/ha (i.e., treatment T5) and the estimated LR_{50} was 107913.80 mL/ha.

About fecundity, significant differences were noticed between the treatment T3 (FLD-HER 306 SE at 600 mL/ha) and the control, therefore, the calculated LOER matched the test item rate of 600 mL/ha (i.e., treatment T3), while the NOER value was 200 mL/ha (i.e., treatment T2). The estimated ER_{50} was 983.40 mL/ha.

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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. - adult mortality at the end of the study in control was 0.0% (requirements: ≤10%).

Agreed endpoints:

Parameter	EC_{10} [mg/kg dry weight of soil]	EC_{20} [mg/kg dry weight of soil]	EC_{50} [mg/kg dry weight of soil]	LOEC [mg/kg dry weight of soil]	NOEC [mg/kg dry weight of soil]
Offspring number	989.804 (n.d.-n.d.)*	1022.116 (n.d.-n.d.)*	1086.899 (n.d.-n.d.)*	>1000.0	≥1000.0
Weight of adult individuals	77.409 (0.00-n.d.)*	n.d. (n.d.-n.d.)*	n.d. (n.d.-n.d.)*	625.0	390.63
Parameter	**LC_{10} [mg/kg dry weight of soil]**	**LC_{20} [mg/kg dry weight of soil]**	**LC_{50} [mg/kg dry weight of soil]**	**LOEC [mg/kg dry weight of soil]**	**NOEC [mg/kg dry weight of soil]**
Survival	0.0 (0.0-0.0)*	0.0 (0.0-0.0)*	0.0 (0.0-0.0)*	n.d.**	n.d.**

*) the lower and upper 95% confidence limits are given in brackets
**) based on the analysis of results, this value was determined as >1000.0 mg/kg dry weight of soil
n.d. impossible to determine for mathematical reasons

Reference:	KCP 10.4.1.1/01
Report	Earthworm reproduction test according to OECD 222; Woźniak A.; 2019; Study Code: 0005/0080/E
Guideline(s):	Yes, OECD 222
Deviations:	Yes: The OECD 222 guideline assumes a dose of 5 g of horse manure for each aquarium. Based on the experience gained during the cultivation of earthworm cultures, it was found to grow properly at a dose of 60 g, with the addition of cellulose in a form free of impurities cardboard. Therefore, during the experiment, 60 g food was given. During the definitive test the temperature increased several times to a maximum of 23.70° (requirements: 20°C±2). The deviations did not have a negative impact on the course of the study, the reliability criteria were met.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control:	vehicle: deionized water positive control: carbendazim
3. Test organism	
Species:	earthworm *Eisenia fetida*
Source:	own culture of SORBOLAB Research Laboratory, and former obtained from commercial culture
Age:	3 months old
Acclimation period:	1-day acclimatization
Diet:	dried and pasteurized horse manure
Test units:	glass aquarium, size about 14.5x14.5x14.5 cm and cross-section area about 210cm^2
4. Environmental conditions:	
Temperature:	mean temperature 20.682°C (minimal temperature 18.80°C, maximal temperature 23.70°C)
Soil:	artificial soil in an amount corresponding to 440 g of its dry weight and 60 g of horse manure; pH: at the beginning and end of the test, the pH in the control and in the soil with the test item was 6.5; water content: in artificial soil, calculated on the dry weight, was 20.01-22.75% before the test and 20.25-24.21% at the end of the test, which corresponds to 40.8-45.49% of the maximum water capacity before the test and 40.49-47.41% of the maximum water capacity at the end of the test
Photoperiod:	lighting: daily cycle 16h light to 8h darkness, light intensity of 560-630 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the effects of the test item FLD-HER 306 SE on earthworms *Eisenia fetida* reproduction. During the test, the impact of the test item on the number of offspring, weight and survival of the parental individuals was determined and compared to the control group. The study was conducted on the earthworm *Eisenia fetida* originating from healthy and properly maintained own culture, and former obtained from commercial culture. The test consisted of placing earthworms in an artificial control substrate containing the tested item. Observations were made on day 28 of the test (week 4) on adults, parental individuals and on day 56 of the test (week 8) on the offspring. Animals, after 1-day acclimatization in artificial soil, were rinsed in deionized water, dried and weighted. First, 20 earthworms, randomly chosen, were weighted individually to ensure homogeneity of the population. Weight ranged between 300-600 mg/individual. Next, earthworms were weighted in groups of 10 individuals. Number of groups complied the number of all replicated concentrations and control. On day 28 of the experiment, the entire contents of the test vessels were transferred to a metal, stainless tray. Adults were removed, and then the artificial medium was gently placed back into the test vessels. Live adult worms removed from the soil were weighed in groups and observations were made. Morphological and behavioral changes were assessed. On day 56 of test, juveniles were recovered, animals were extracted from the soil manually.

Test design:	control in 8 replicates with 10 earthworms for each replication; tested concentrations in 4 replicates with 10 earthworms for each replication
Exposure time:	56 days
Tested concentrations, definitive test:	37.33, 59.71, 95.54, 152.59, 244.14, 390.63, 625.0, 1000.0 mg/kg dry weight of soil
Dates:	start of the study: 22.02.2019 start of the experimental part: 03.07.2019 end of the experimental part: 12.11.2019 end of the study: 22.11.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

In the course of the test and on the basis of statistical calculations made, it was shown that the tested item FLD-HER 306 SE did not have a statistically significant effect on the number of offspring and survival of adults in the concentrations range used in the study from 37.33 mg/kg dry weight of soil to 1000.0 mg/kg dry weight of soil. The tested item has a statistically significant effect on the weight of individuals at a concentrations of 625.0 mg/kg dry weight of soil and 1000.0 mg/kg dry weight of soil.

Table KCP 10.4.1.1-1: Earthworm reproduction test – final results calculated by ToxRat Professional

Parameter	**EC_{10} [mg/kg dry weight of soil]**	**EC_{20} [mg/kg dry weight of soil]**	**EC_{50} [mg/kg dry weight of soil]**	**LOEC [mg/kg dry weight of soil]**	**NOEC [mg/kg dry weight of soil]**
Offspring number	989.804 (n.d.-n.d.)*	1022.116 (n.d.-n.d.)*	1086.899 (n.d.-n.d.)*	>1000.0	≥1000.0
Weight of adult individuals	77.409 (0.00-n.d.)*	n.d. (n.d.-n.d.)*	n.d. (n.d.-n.d.)*	625.0	390.63
Parameter	**LC_{10} [mg/kg dry weight of soil]**	**LC_{20} [mg/kg dry weight of soil]**	**LC_{50} [mg/kg dry weight of soil]**	**LOEC [mg/kg dry weight of soil]**	**NOEC [mg/kg dry weight of soil]**
Survival	0.0 (0.0-0.0)*	0.0 (0.0-0.0)*	0.0 (0.0-0.0)*	n.d.**	n.d.**

*) the lower and upper 95% confidence limits are given in brackets
**) based on the analysis of results, this value was determined as >1000.0 mg/kg dry weight of soil
n.d. impossible to determine for mathematical reasons

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not relevant. No studies submitted.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Not relevant. No studies submitted.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not relevant. No studies submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. The validity criteria were met in accordance with Guideline OECD 216. Differences in the content of nitrate in individual replicates were less than ±15%: – Day 0: 2.56% – Day 7: 1.58% – Day 14: 1.17% – Day 28: 1.49% **Agreed endpoints:** The differences in the nitrate formation rate between the control soil and the one treated with the test material (at the concentrations of 0.9982 and 4.9910 mg/kg of soil) did not exceed 25% on all days of analysis. Taking the obtained results into account, it was assessed that FLD-HER 306 SE at the concentrations of 0.9982 and 4.9910 mg/kg of soil, corresponding to the PEC and 5 x PEC, can be evaluated as having no long-term influence on the nitrogen transformation in soil.

Reference:	KCP 10.5/01
Report	Study of impact on soil microorganisms - nitrogen transformation according OECD 216; Parma P.; 2019; Study Code: 0005/0083/E
Guideline(s):	Yes, OECD 216
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021

2. Vehicle and/or positive control: vehicle: deionized water
positive control: not relevant

3. Test organism

Soil:	not ploughed for the last six months, which were not treated with plant protection products for a minimum of one year before being used for testing, nor fertilized with any fertilizer for at least six months
Source:	Fraunhofer Institute for Molecular Biology and Applied Ecology IME in Germany
Soil preparation:	The test item was applied to the soil using vehicle The vehicle was deionized water, since the test item forms emulsions, solutions, suspensions. An equal amount of water was added to the soil control sample as for the test samples, but without the test item additive.
Test units:	darkened plastic containers with perforated lids

4. Environmental conditions:

Temperature:	the average temperature 19.95°C (minimal temperature 19.30°C, maximal temperature 20.30°C)
Humidity:	the average humidity 59.1% (minimal humidity 56%, maximal humidity 63%)
Photoperiod:	photoperiod 24h darkness

STUDY DESIGN AND METHOD

The aim of the study was to assess the long-term adverse effects of the test item FLD-HER 306 SE, on the activity of soil microorganisms responsible for nitrogen transformation occurring in aerobic surface soils was conducted. The study consisted of comparing the rate of nitrate production in the soil exposed to the test item with the rate of nitrate production in the control soil. The soil for testing was bought in Fraunhofer Institute for Molecular Biology and Applied Ecology IME in Germany. The soil was obtained from agricultural areas not ploughed for the last six months, which were not treated with plant protection products for a minimum of one year before being used for testing, nor fertilized with any fertilizer for at least six months. Soil samples were not collected during or immediately after long periods (over 30 days) of drought or rainfall. Soil samples for testing were transported to the laboratory in dark containers, which guaranteed that the initial soil properties did not change significantly. The soil was modified by the addition of powdered lucerne meal in an amount of 5 g/kg dry soil. The water content in the soil was main-

tained by the addition of deionized water at about 40-60% of its maximum water capacity. The test material in the form of a water solution was introduced to the soil at the concentrations of 0.9982 mg test item/kg soil (1xPEC) and 4.9910 mg test item/kg soil (5xPEC). An equal amount of water was added to the soil control sample as for the test samples. The control soil and the soil treated with the test material were incubated in three replicates. The time of soil incubation was 28 days. On 0, the 7th, the 14th and the 28th day of incubation the samples of both soils were collected and the amount of nitrates was determined.

Test design:	concentrations and control in 3 replicates
Exposure time:	28 days
Tested concentrations, definitive test:	1PEC – 0.9982 mg test item/kg soil, 5PEC - 5-times the maximal single dose recommended by the producer, i.e. 4.9910 mg test item/kg soil
Dates:	start of the study 27.08.2019 start of the experimental part: 07.11.2019 end of the experimental part: 05.12.2019 end of the study: 18.12.2019
Statistic:	ToxRat Professional statistical program

CONCLUSION

The differences in the nitrate formation rate between the control soil and the one treated with the test material (at the concentrations of 0.9982 and 4.9910 mg/kg of soil) did not exceed 25% on all days of analysis. Taking the obtained results into account, it was assessed that FLD-HER 306 SE at the concentrations of 0.9982 and 4.9910 mg/kg of soil, corresponding to the PEC and 5 x PEC, can be evaluated as having no long-term influence on the nitrogen transformation in soil.

Table KCP 10.5.-1: Nitrogen transformation – final results calculated by ToxRat Professional

Concentration [mg/kg of soil]	Average rate of nitrate production [mg nitrate/kg dry soil/day]	Inhibition in relation to control [%]	Statistical significance***)
Control	2.159	not applicable	not applicable
1PEC*)	2.136	1.1	-
5PEC*)	2.366	-9.6	-

***)** (Predicted Environmental Concentration): maximum predicted effective concentration in soil (0.9982 mg of test item/kg of soil

**) (Predicted Environmental Concentration): 5 times the maximum expected effective concentration in soil (4.9910 mg of testitem/ kg soil)

****)** significance calculated by ToxRat Professional using the Student's t test at the significance level of $p \leq 0.05$

- statistically insignificant

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

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

The test meets the validity criteria from the point of view of the Guideline OECD 208:

– seedling emergence in control was at least 70%:

- onion *Allium cepa* 75%
- carrot *Daucus carota* 100%
- cucumber *Cucumis dativus* 100%
- oilseed rape *Brassica napus* 85%
- lettuce *Lactuca sativa* 80%
- perennial ryegrass *Lolium perenne* 95%

– in none of the control replications of any plants species there were any signs of intoxications visible (i.e. chlorosis, necrosis, wilting, leaf/stalk deformation)

– mean survival of plants in control was 100% for every species (required at least 90%)

– environmental conditions and soil were identical for all used in the experiment plants species.

Agreed endpoints:

		Dicotylenodae			
		Fresh weight	**Dry weight**	**Shoot length**	**Em**
Carrot *Daucus carota*	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	
	LOER [L/ha]	n.d.	>0.7	n.d.	
	NOER [L/ha]	n.d.	≥0.7	n.d.	
Cucumber *Cucumis dativus*	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	
	LOER [L/ha]	>0.7	>0.7	>0.7	
	NOER [L/ha]	≥0.7	≥0.7	≥0.7	
Oilseed rape *Brassica napus*	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	(n
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	
	LOER [L/ha]	>0.7	>0.7	>0.7	
	NOER [L/ha]	≥0.7	≥0.7	≥0.7	
Lettuce *Lactuca sativa*	**ER_{25}/LR_{25} [L/ha]**	0.699 (n.d.-n.d.)	0.516 (0.205-1.331)	0.569 (0.383-0.856)	
	ER_{50}/LR_{50} [L/ha]	0.700 (n.d.-n.d.)	0.686 (0.206-2.202)	>0.7	
	LOER [L/ha]	0.7	0.7	0.7	
	NOER [L/ha]	0.28	0.28	0.28	
		Monocotylenodae			
		Fresh weight	**Dry weight**	**Shoot length**	**Em**

	Onion ***Allium cepa***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	
		ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	
		LOER [L/ha]	≤0.0179	≤0.0179	>0.7	
		NOER [L/ha]	<0.0179	<0.0179	≥0.7	
	Perennial ryegrass ***Lolium perenne***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	
		ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	
		LOER [L/ha]	>0.7	>0.7	>0.7	
		NOER [L/ha]	≥0.7	≥0.7	≥0.7	
	n.d. impossible to calculate due to mathematical reasons **) based on the analysis of the results, this value was defined as > 0.7 L/ha					

Reference:	KCP 10.6.2/01
Report	Seedling emergence and seedling growth test according to OECD 208; Parma P.; 2019; Study Code: 0005/0081/E
Guideline(s):	Yes, OECD 208
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	stability test of the test item have not been performed

2. Vehicle and/or positive control: vehicle control: water
positive control: not relevant

3. Test plants: monocotyledonous/ dicotyledonous: onion, carrot, cucumber, oilseed rape, lettuce, perennial ryegrass

Test containers:	plastic pots, with diameter of 11 cm and area of approx. 95 cm^2, with stands and filled with 0.5 kg of soil
4. Environmental conditions:	
Temperature:	the average temperature 22.28°C (minimal temperature 20.30°C, maximal temperature 23.70°C)
Relative humidity:	the mean air relative humidity 53.44% (minimal humidity 50%, maximal humidity 56%)
Photoperiod:	daily cycle of light (16h/8h); the mean light intensity 25993.75 lux (minimal light intensity 24100 lux, maximal light intensity 28300 lux)
CO_2 concentration:	mean CO_2 concentration was 353.31 ppm (minimal CO_2 concentration 321 ppm, maximal CO_2 concentration 383 ppm)

STUDY DESIGN AND METHODS

The aim of the study was to term of the influence of the test material, FLD-HER 306 SE against growth and germination of plants, the number of germinated and the surviving plants, fresh and dry weight, as well as length of the shoots. The study was performed to compare the effect of the test item on the above parameters in relation to control and estimation of the ER_x/LR_x as well as NOER and LOER values. Plants were among those recommended by the OECD 208 Guideline. Also, it was predicted that the plants would meet established in the reference test criterion. Selected plants represent different families and groups – four species from the group of dicotyledonous plants and two from the group of monocotyledonous plants. The seeds were not encapsulated or treated with any pesticides. An experiment with a reference substance boric acid was performed on 27.02.2019 - 25.03.2019. Its purpose was to confirm that the response of the test plants is correct and experimental conditions do not change significantly over time. In the experiment the reference item boric acid there were used a single concentration of 250 mg/kg and a control. The seeds were planted into the soil, where rate of test item was applied. Then, in the period 14 days after emergence of at least 50% of the seed in the control, observations were performed, in order to establish a potential seeds emergence inhibition and shoot growth.

Test design:	control and test item doses in 4 replications, 5 seeds for each replication
Exposure time:	14-21 days since emergence of 50% seeds in control
Tested concentrations, definitive test:	0.7, 0.28, 0.112, 0.0448 and 0.0179 L/ha (200 L_{water}/ha)
Dates:	start of the study 05.08.2019 start of the experimental part: 08.08.2019 end of the experimental part: 12.11.2019 end of the study: 18.12.2019
Statistic:	ToxRat Professional statistical program

RESULTS AND DISCUSSIONS

During the experiment, there were no statistically significant differences in the number of emerged seeds in relation to the control in all plant species. In the course of the study, there were no statistically significant differences in the shoot mortality in relation to the control in all tested plant species. During the experiment there were statistically significant differences compared to control for shoot fresh weight in all tested rates for onion, at the highest rate for lettuce and at the lowest rate for carrot. For other plant species, there were no statistically significant differences for this parameter. In the case of dry weight there

were statistically significant differences compared to control in all tested rates for onion and at the highest tested rate for lettuce. For other plant species, there were no statistically significant differences for this parameter. During the experiment there were statistically significant differences compared to control for shoot length in the highest tested rate for lettuce and in the lowest tested rate for carrot. For other plant species, there were no statistically significant differences for this parameter.

Table KCP 10.6.2-1: Seedling emergence and seedling growth test – final results

Dicotylenodae						
		Fresh weight	**Dry weight**	**Shoot length**	**Emergence**	**Survival**
Carrot ***Daucus carota***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	n.d.	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	n.d.	n.d.**
	LOER [L/ha]	n.d.	>0.7	n.d.	>0.7	n.d.**
	NOER [L/ha]	n.d.	≥0.7	n.d.	≥0.7	n.d.**
Cucumber ***Cucumis dativus***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	n.d.	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	n.d.	n.d.**
	LOER [L/ha]	>0.7	>0.7	>0.7	>0.7	n.d.**
	NOER [L/ha]	≥0.7	≥0.7	≥0.7	≥0.7	n.d.**
Oilseed rape ***Brassica napus***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	0.183 (n.d.-n.d.)	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	n.d.	n.d.**
	LOER [L/ha]	>0.7	>0.7	>0.7	>0.7	n.d.**
	NOER [L/ha]	≥0.7	≥0.7	≥0.7	≥0.7	n.d.**
Lettuce ***Lactuca sativa***	**ER_{25}/LR_{25} [L/ha]**	0.699 (n.d.-n.d.)	0.516 (0.205-1.331)	0.569 (0.383-0.856)	n.d.	n.d.**
	ER_{50}/LR_{50} [L/ha]	0.700 (n.d.-n.d.)	0.686 (0.206-2.202)	>0.7	n.d.	n.d.**
	LOER [L/ha]	0.7	0.7	0.7	>0.7	n.d.**
	NOER [L/ha]	0.28	0.28	0.28	≥0.7	n.d.**
Monocotylenodae						
		Fresh weight	**Dry weight**	**Shoot length**	**Emergence**	**Survival**
Onion ***Allium cepa***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	n.d.	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	n.d.	n.d.**
	LOER [L/ha]	≤0.0179	≤0.0179	>0.7	>0.7	n.d.**
	NOER [L/ha]	<0.0179	<0.0179	≥0.7	≥0.7	n.d.**
Perennial ryegrass ***Lolium perenne***	**ER_{25}/LR_{25} [L/ha]**	n.d.	n.d.	n.d.	n.d.	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.	n.d.	n.d.	n.d.	n.d.**
	LOER [L/ha]	>0.7	>0.7	>0.7	>0.7	n.d.**

	NOER [L/ha]	≥0.7	≥0.7	≥0.7	≥0.7	n.d.**

n.d. impossible to calculate due to mathematical reasons
**) based on the analysis of the results, this value was defined as > 0.7 L/ha

CONCLUSION

Based on the conducted test, observations and results obtained, it was stated that test item – FLD-HER 306 SE, exhibits ecotoxic effects at the tested rates in relation to the control on onion (fresh and dry weight), carrot (shoot length and fresh weight) and lettuce (shoot length, fresh and dry weight). It does not cause ecotoxic effects in the case of cucumber, oilseed rape and perennial ryegrass.

Comments of zRMS:

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

- seedling emergence in control was at least 70%:
  - onion *Allium cepa* 100%
  - carrot *Daucus carota* 100%
  - cucumber *Cucumis dativus* 100%
  - oilseed rape *Brassica napus* 100%
  - lettuce *Lactuca sativa* 100%
  - perennial ryegrass *Lolium perenne* 90%
- in none of the control replications of any plants species there were any signs of intoxications visible (i.e. chlorosis, necrosis, wilting, leaf/stalk deformation) for every species
- mean survival of plants in control was 90% for perennial ryegrass and 100% for the rest of species (required at least 90%)
- environmental conditions and soil were identical for all used in the experiment plants species.

Agreed endpoints:

Dicotyledons					
		Fresh weight	**Dry weight**	**Shoot length**	**Survival**
Oilseed rape *Brassica napus*	**ER_{25}/LR_{25} [L/ha]**	0.205 (0.075–0.578)*	0.088 (0.032–0.245)*	n.d.**	0.597 (0.273–17.463)*
	ER_{50}/LR_{50} [L/ha]	0.524 (0.147-1.801)*	0.255 (0.082-0.837)*	n.d.**	n.d.**
	LOER [L/ha]	0.28	0.112	>0.7	>0.7
	NOER [L/ha]	0.112	0.0448	≥0.7	≥0.7
Carrot *Daucus carota*	**ER_{25}/LR_{25} [L/ha]**	0.320 (0.093-1.133)*	0.128 (0.055-0.306)*	0.485 (0.300-0.795)*	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.**	0.288 (0.110-0.785)*	n.d.**	n.d.**
	LOER [L/ha]	>0.7	0.28	0.28	n.d.**
	NOER [L/ha]	≥0.7	0.112	0.112	n.d.**
Cucumber *Cucumis dativus*	**ER_{25}/LR_{25} [L/ha]**	n.d.**	n.d.**	n.d.**	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.**	n.d.**	n.d.**	n.d.**
	LOER [L/ha]	>0.7	>0.7	>0.7	n.d.**
	NOER	≥0.7	≥0.7	≥0.7	n.d.**

		[L/ha]				
	Lettuce *Lactuca sativa*	**ER_{25}/LR_{25} [L/ha]**	0.084 (0.048-0.149)*	0.056 (0.032-0.099)*	0.160 (0.077-0.342)*	0.546 (nd.-nd.)*
		ER_{50}/LR_{50} [L/ha]	0.156 (0.083-0.304)*	0.110 (0.059-0.210)*	0.582 (0.0212-1.515)*	0.595 (nd.-nd.)*
		LOER [L/ha]	0.112	0.112	nd.	0.7
		NOER [L/ha]	0.0448	0.0448	nd.	0.28
	Monocots					
			Fresh weight	**Dry weight**	**Shoot length**	**Survival**
	Onion *Allium cepa*	**ER_{25}/LR_{25} [L/ha]**	0.147 (0.015-1.540)*	0.097 (0.008-1.233)*	0.310 (0.074-1.334)*	nd**
		ER_{50}/LR_{50} [L/ha]	0.469 (0.029-7.507)*	0.380 (0.021-7.444)*	nd**	nd**
		LOER [L/ha]	>0.7	0.28	0.28	>0.7
		NOER [L/ha]	≥0.7	0.112	0.112	≥0.7
	Perennial ryegrass *Lolium perenne*	**ER_{25}/LR_{25} [L/ha]**	nd.**	0.536 (0.338-0.863)*	nd.**	nd.**
		ER_{50}/LR_{50} [L/ha]	nd.**	nd.**	nd.**	nd.**
		LOER [L/ha]	0.7	0.7	>0.7	>0.7
		NOER [L/ha]	0.28	0.28	≥0.7	≥0.7
	nd. impossible to determine due to mathematical reasons *) the upper and lower 95% confidence intervals are given in parentheses **) based on the analysis of the results, this value was defined as >0.7 L/ha					

Reference:	KCP 10.6.2/02
Report	Vegetative Vigour Test according to OECD 227; Parma P.; 2019; Study Code: 0005/0082/E
Guideline(s):	Yes, OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	FLD-HER 306 SE
Formulation:	SE (florasulam 6.25 g/L + 2,4-D 300 g/L)
Description (physical state):	white liquid
Batch no.:	2/2019
Production date:	04.2019
Expiration date:	04.2021
Stability of test compound:	stability test of the test item have not been performed
2. Vehicle and/or positive control:	vehicle control: water positive control: trichloroacetic acid
3. Test plants:	monocotyledonous/ dicotyledonous: onion, carrot, cucumber, oilseed rape, lettuce, perennial ryegrass
Test containers:	plastic pots, with diameter of 11 cm and area of approx. 95 cm^2, with stands and filled with 0.5 kg of soil
4. Environmental conditions:	
Temperature:	the average temperature 22.64°C (minimal temperature 20.50°C, maximal temperature 25.20°C)
Relative humidity:	the mean air relative humidity 53.35% (minimal humidity 50%, maximal humidity 56%)
Photoperiod:	daily cycle of light (16h/8h); the mean light intensity 25595.65 lux (minimal light intensity 23900 lux, maximal light intensity 28300 lux)
CO_2 concentration:	mean CO_2 concentration was 354.26 ppm (minimal CO_2 concentration 321 ppm, maximal CO_2 concentration 383 ppm)

STUDY DESIGN AND METHODS

Toxicity study of test item, FLD-HER 306 SE against vigour of plants, the number of the surviving plants and length of the shoots, as well as fresh and dry weight was performed. The study was performed to compare the effect of the test item on the above parameters in relation to control and estimation of the ER_x/LR_x as well as NOER and LOER values. Plants were among those recommended by the OECD Guideline 227. Plants would meet established in the reference test criterion. Selected plants represent different families and groups – four species from the group of dicotyledonous plants and two from the group of monocotyledonous plants. The seeds were not encapsulated or treated with any pesticides. An experiment with a reference substance trichloroacetic acid was performed on 27.02.2019 - 09.04.2019. Its purpose was to confirm that the response of the test plants and experimental conditions do not change significantly over time. In the experiment the reference item trichloroacetic acid there were used a single concentration of 8 kg/ha and a control. Experiment consisted of planting seeds into the soil and next when 2-4 true leaves were grown, applied rate of test item by spraying the whole surface of the leaf using a calibrated spray atomizer. From this moment during 21 days, observations were performed, to estimate the survival and the rate of plant growth.

Test design:	control and test item doses in 4 replications, 5 seeds for each replication
Exposure time:	21 days since the application of the test item
Tested concentrations, definitive test:	0.7, 0.28, 0.112, 0.0448 and 0.0179 L/ha (200 L_{water}/ha);
Dates:	start of the study 06.08.2019 start of the experimental part: 09.08.201 end of the experimental part: 07.11.2019; end of the study: 18.12.2019
Statistic:	ToxRat Professional statistical program

RESULTS AND DISCUSSIONS

In the course of the study, there were statistically significant differences in the shoot mortality in relation to the control in lettuce at the rate 0.7 L/ha. In case of other plant species, no statistically significant differences were recorded for this parameter.
In the course of the test statistically significant differences for shoot fresh weight in comparison to control were observed at two highest rates for oilseed rape, three highest rates for lettuce and the highest rate for perennial ryegrass. In case of the other species, no statistically significant differences for this parameter were observed.
In the course of the test statistically significant differences for shoot dry weight in comparison to control were observed at the rates 0.28 and 0.7 L/ha for onion and carrot at the rates 0.112, 0.28 and 0.7 L/ha for oilseed rape and lettuce and at the rate 0.7 L/ha for perennial ryegrass. In case of cucumber, no statistically significant differences for this parameter were observed.
In the course of the test statistically significant differences for shoot length in comparison to control were observed at the rates of 0.28 and 0.7 L/ha for onion and carrot, at the rates of 0.112 and 0.28 L/ha for lettuce. In case of the other species, no statistically significant differences for this parameter were observed.

Table KCP 10.6.2-2: Vegetative Vigour Test – final results

Dicotyledons					
		Fresh weight	**Dry weight**	**Shoot length**	**Survival**
Oilseed rape *Brassica napus*	**ER_{25}/LR_{25} [L/ha]**	0.205 (0.075–0.578)*	0.088 (0.032–0.245)*	n.d.**	0.597 (0.273–17.463)*
	ER_{50}/LR_{50} [L/ha]	0.524 (0.147-1.801)*	0.255 (0.082-0.837)*	n.d.**	n.d.**
	LOER [L/ha]	0.28	0.112	>0.7	>0.7
	NOER [L/ha]	0.112	0.0448	≥0.7	≥0.7
Carrot *Daucus carota*	**ER_{25}/LR_{25} [L/ha]**	0.320 (0.093-1.133)*	0.128 (0.055-0.306)*	0.485 (0.300-0.795)*	n.d.**
	ER_{50}/LR_{50} [L/ha]	n.d.**	0.288 (0.110-0.785)*	n.d.**	n.d.**
	LOER [L/ha]	>0.7	0.28	0.28	n.d.**
	NOER [L/ha]	≥0.7	0.112	0.112	n.d.**
Cucumber *Cucumis dativus*	**ER_{25}/LR_{25} [L/ha]**	n.d.**	n.d.**	n.d.**	n.d.**
	ER_{50}/LR_{50}	n.d.**	n.d.**	n.d.**	n.d.**

	[L/ha]				
	LOER [L/ha]	>0.7	>0.7	>0.7	n.d.**
	NOER [L/ha]	≥0.7	≥0.7	≥0.7	n.d.**
Lettuce *Lactuca sativa*	**ER_{25}/LR_{25} [L/ha]**	0.084 (0.048-0.149)*	0.056 (0.032-0.099)*	0.160 (0.077-0.342)*	0.546 (nd.-nd.)*
	ER_{50}/LR_{50} [L/ha]	0.156 (0.083-0.304)*	0.110 (0.059-0.210)*	0.582 (0.0212-1.515)*	0.595 (nd.-nd.)*
	LOER [L/ha]	0.112	0.112	nd.	0.7
	NOER [L/ha]	0.0448	0.0448	nd.	0.28
Monocots					
		Fresh weight	**Dry weight**	**Shoot length**	**Survival**
Onion *Allium cepa*	**ER_{25}/LR_{25} [L/ha]**	0.147 (0.015-1.540)*	0.097 (0.008-1.233)*	0.310 (0.074-1.334)*	nd**
	ER_{50}/LR_{50} [L/ha]	0.469 (0.029-7.507)*	0.380 (0.021-7.444)*	nd**	nd**
	LOER [L/ha]	>0.7	0.28	0.28	>0.7
	NOER [L/ha]	≥0.7	0.112	0.112	≥0.7
Perennial ryegrass *Lolium perenne*	**ER_{25}/LR_{25} [L/ha]**	nd.**	0.536 (0.338-0.863)*	nd.**	nd.**
	ER_{50}/LR_{50} [L/ha]	nd.**	nd.**	nd.**	nd.**
	LOER [L/ha]	0.7	0.7	>0.7	>0.7
	NOER [L/ha]	0.28	0.28	≥0.7	≥0.7

nd. impossible to determine due to mathematical reasons
*) the upper and lower 95% confidence intervals are given in parentheses
**) based on the analysis of the results, this value was defined as >0.7 L/ha

CONCLUSION

Based on the performed experiment, executed observations and obtained results, it was stated that the tested item FLD-HER 306 SE exhibits significant differences ecotoxic effects at test rates in relation to the control on oilseed rape (fresh and dry weight), carrot and onion (shoot length, dry weight) lettuce (survival, shoot length, fresh and dry weight) and perennial ryegrass (fresh and dry weight). It does not cause ecotoxic effects on cucumber.

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

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.8 KCP 10.8 Monitoring data

Not relevant. No studies submitted.