REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: EF-243

Product name(s): Lontrel 300

Chemical active substance:

Clopyralid-olamine, 395 g/l (300 g ae/l)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Renewal of Authorization under Art.43)

Applicant: Corteva Agriscience

Submission date: 22/12/2021

MS Finalisation date: 05/12/2022

After commenting: 22/02/2023

Version history

When	What
December 2021	Article 43 submission for re-registration of EF-243 following Clopyralid Renewal of approval (Commission Implementing Regulation (EU) 2021/1191)
December 2022	First zRMS evaluation
February 2023	After commenting

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9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicology studies and risk assessment for clopyralid and the plant protection product EF-243 a soluble concentrate (SL) formulation containing 300 g clopyralid ae/L.

Clopyralid is formulated as a soluble concentrate (SL) or soluble granule (SG) formulation, known by the trademark LONTREL. A series of SL formulations are available as dilutions of clopyralid (as monoethanolamine salt) in water. The single SG formulation is a granule formulation of clopyralid (as monoethanolamine salt) containing a small amount of water. GF-2895 is a dilution of clopyralid (as dimethylamine salt) in water. Following dilution with water with-in the spray tank, immediately prior to application, the resulting spray solutions are equivalent for purposes of ecotoxicology. A summary of the different formulations covered in this section is provided below.

Name	**Code**	**Formulation type**	**Clopyralid content**
LONTREL 100	EF-1136 EF-255	soluble concentrate	100 g/L
LONTREL 300	EF-243	Soluble concentrate	300 g/L
LONTREL 72 SG	GF-1966	soluble granule	720 g/kg
	GF-2895	Soluble concentrate	600 g/L

According to Regulation (EU) No 284/2013 formulation testing for some of the areas is required when the toxicity cannot be predicted on the basis of the data for the active substance. As EF-243 only contains clopyralid and small amount of water most of the endpoints used in this assessment are from the active substance.

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: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Poland	Sugar beet, EPPO Code: BEAVC, BEAVA, BEAVD EU MRL Code: 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom	BBCH 12-39 (until July 1st)	a) 1 b) 1	NA	a) 0,3 to 0,4 b) 03 to 0,4	a) AS1: 118,578 to 158,104 (as/ha), 90 to 120 (ae/ha) b) AS1: 118,578 to 158,104 (as/ha), 90 to 120 (ae/ha)	100-400	42 days	One application every two years. Maximum total dose rate must not exceed 120 g ae clopyralid/ha per crop; maximum individual dose: 120 g ae clopyralid/ha. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations.							
2	Poland	Sugar beet, EPPO Code: BEAVC, BEAVA, BEAVD EU MRL Code: 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom, split application	BBCH 12-15 First application at BBCH 12-15. Second application at BBCH 12-15.	a) 2 b) 2	7 day interval	a) 0,2 b) 0,4	a) AS1: 79,052 (as/ha), 60 (ae/ha) b) AS1: 158,104 (as/ha), 120 (ae/ha)	100-400	42 days	Only every three years. Split application: First application at 60 gae clopyralid/ha (0,2L of EF-243/ha) at BBCH 12-15 followed 7-days later by a second application at BBCH 12-15 at 60 gae clopyralid/ha (0,2 L EF-243/ha). Maximum total dose rate must not exceed 120 g ae clopyralid/ha per crop; maximum individual dose: 120 g ae clopyralid/ha. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
3	Czech Republic, Slovakia	Fodder beet, Sugar beet, EPPO Code: BEAVC, BEAVD, BEAVA, EU MRL Code: 0213010, 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom	BBCH 12-39 (until July 1st)	a) 1 b) 1	NA	a) 0,35 b) 0,35	a) AS1: 138,341 (as/ha), 105 (ae/ha) b) AS1: 138,341 (as/ha), 105 (ae/ha)	100-400	42 days	One application every two years. Maximum total dose rate must not exceed 105 g ae clopyralid/ha per crop; maximum individual dose: 105 g ae clopyralid/ha. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													application of clopyralid. For crop rotation management, see label for recommendations.							
4	Czech Republic, Slovakia, Poland	Fodder beet*, Sugar beet, EPPO Code: BEAVC, BEAVD, BEAVA, BEAVC EU MRL Code: 0213010, 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom, split application	BBCH 12-15 First application at BBCH 12-15. Second application at BBCH 12-15.	a) 2 b) 2	7 day interval	a) 0,175 b) 0,35	a) AS1: 69,17 (as/ha), 52,5 (ae/ha) b) AS1: 138,341 (as/ha), 105 (ae/ha)	100-400	42 days	*Fodder beet not supported in Poland Only every three years. Split application: First application at 52,5 gae clopyralid/ha (0,175L of EF-243/ha) at BBCH 12-15 followed 7-days later by a second application at BBCH 12-15 at 52,5 gae clopyralid/ha (0,175 L EF-243/ha). Maximum total dose rate must not exceed 105 g ae clopyralid/ha per crop; maximum individual dose: 105 g ae clopyralid/ha. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
5	Czech Republic, Slovakia, Poland	Fodder beet*, Sugar beet, EPPO Code: BEAVC, BEAVD, BEAVA, BEAVC EU MRL Code: 0213010, 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom, split application	BBCH 15-31 First application at BBCH 15. Second application at BBCH 31.	a) 2 b) 2	10-day interval	a) 0,175 b) 0,35	a) AS1: 69,17 (as/ha), 52,5 (ae/ha) b) AS1: 138,341 (as/ha), 105 (ae/ha)	100-400	42 days	*Fodder beet not supported in Poland Every two years. Split application: first application at 52,5 gae clopyralid/ha (0,175L EF243/ha) at BBCH 15 followed 10 days later by a second application (at BBCH 31) at 52,5 gae clopyralid/ha (0,175 L EF-243/ha). Maximum total dose rate must not exceed 105 g ae clopyralid/ha per crop; maximum individual dose: 105 g ae clopyralid/ha. For residue management in crop rotation: no mitigation measures							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
6	Poland	Sugar beet, EPPO Code: BEAVC, BEAVA, BEAVD EU MRL Code: 0213010, 0900010, 0213010 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast, Foliar Tractor mounted boom, split application	BBCH 15-31 First application at BBCH 15. Second application at BBCH 31.	a) 2 b) 2	10-day interval	a) 0,2 b) 0,4	a) AS1: 79,05 (as/ha), 60 (ae/ha) b) AS1: 158,1 (as/ha), 120(ae/ha)	100-400	42 days	Every two years. Split application: first application at 60 gae clopyralid/ha (0,2 L EF243/ha) at BBCH 15 followed 10 days later by a second application (at BBCH 31) at 60 gae clopyralid/ha (0,2 L EF-243/ha). Maximum total dose rate must not exceed 120 g ae clopyralid/ha per crop; maximum individual dose: 120 g ae clopyralid/ha. For residue							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
7	Poland	Winter Oilseed rape EPPO Code: BRSNW EU MRL Code: 0401060 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Centaurea cyanus, Matricaria spp)	Broadcast, Foliar Tractor mounted boom	BBCH 30-51	a) 1 b) 1	NA	a) 0,4 b) 0,4	a) AS1: 158,104 (g as/ha), 120 (g ae/ha) b) AS1: 158,104 (g as/ha), 120 (g ae/ha)	100-400	Not applicable*	For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
8	Slovakia, Czech Rep.	Winter Oilseed rape EPPO Code: BRSNW EU MRL Code: 0401060 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Centaurea cyanus, Matricaria spp)	Broadcast, Foliar Tractor mounted boom	BBCH 30-51	a) 1 b) 1	NA	a) 0,35 b) 0,35	a) AS1: 138,341 (g as/ha), 105 (g ae/ha) b) AS1: 138,341 (g as/ha), 105 (g ae/ha)	100-400	Not applicable*	For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
9	Slovakia	Gladiolus EPPO Code: 1GLAG EU MRL Code: NA	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast foliar Tractor mounted boom	BBCH 12-19 (spring/summer)	a) 1 b) 1	NA	a) 0,4 b) 0,4	a) AS1: 158,104 (as/ha), 120 (ae/ha) b) AS1: 158,104 (as/ha), 120 (ae/ha)	100-400	Not applicable	One application every 2 years.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10	Czech Republic, Slovakia	Spring Barley Spring Wheat Spring Oat Spring Rye Spring Triticale EPPO Code: HORVS TRZAS AVESP SECCS TTLSO EU MRL Code: 0500010 0500090 0500050 0500070 0500990 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Centaurea cyanus, Matricaria spp)	Broadcast foliar Tractor mounted boom	BBCH 30-39	a) 1 b) 1	NA	a) 0,3 b) 0,3	a) AS1: 118,578 (as/ha), 90 (ae/ha) b) AS1: 118,578 (as/ha), 90 (ae/ha)	100-400	Not applicable*	*BBCH For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
11	Czech Republic, Slovakia	Winter Barley Winter Wheat Winter Oat Winter Rye Winter Triticale EPPO Code: HORVW TRZAW AVESW SECCW TTLWI EU MRL Code: 0500010 0500090 0500050 0500070 0500990 Raw Human consumption Processed goods Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Centaurea cyanus, Matricaria spp)	Foliar broadcast Tractor mounted boom	BBCH 30-39	a) 1 b) 1	NA	a) 0,3 b) 0,3	a) AS1: 118,578 (as/ha), 90 (ae/ha) b) AS1: 118,578 (as/ha), 90 (ae/ha)	100-400	Not applicable*	*BBCH For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
12	Czech Republic, Slovakia	Grass for seeds (more than one-year old) EPPO Code: YGRAS EU MRL Code: NA	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense)	Broadcast foliar Tractor mounted boom	March 01 to July 15, one application every year.	a) 1 b) 1	NA	a) 0,4 b) 0,4	a) AS1: 158,104 (g as/ha), 120 (g ae/ha) b) AS1: 158,104 g (as/ha), 120 (g ae/ha)	100-400	7-days	One application every year. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
13	Czech Republic, Slovakia	Grass for seeds (less than one-year old) EPPO Code: YGRAS EU MRL Code: NA	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense)	Broadcast foliar Tractor mounted boom	From BBCH 14 Slovakia: 01 March to 01 July Czeck Rep: 01 April to 01 July	a) 1 b) 1	NA	a) 0,4 b) 0,4	a) AS1: 158,104 (g as/ha), 120 (g ae/ha) b) AS1: 158,104 g (as/ha), 120 (g ae/ha)	100-400	7-days	One application every 3 years. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
14	Czech Republic, Slovakia	Lawn (established grass, more than one-year old) EPPO Code: NNNZW EU MRL Code: NA	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense)	Broadcast foliar Tractor mounted boom	March 01 to July 01, one application every year.	a) 1 b) 1	NA	a) 0,67 b) 0,67	a) AS1: 264,8 g (as/ha), 200 (g ae/ha) b) AS1: 264,8 (g as/ha), 200 (g ae/ha)	200-400	Not applicable	One application every year.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
15	Slovakia	Maize (grain, forage) EPPO Code: ZEAMX EU MRL Code: 0500030 Processed goods Human consumption (seeds) Cattle consumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast foliar Tractor mounted boom	BBCH 10-19	a) 1 b) 1	NA	a) 0,34 b) 0,34	a) AS1: 134,38 (as/ha), 102 (ae/ha) b) AS1: 134,38 (as/ha), 102 (ae/ha)	100-400	60 days for forage, 90 days for grain	One application every year. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations.							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
16	Slovakia	Maize (grain, forage) EPPO Code: ZEAMX EU MRL Code: 0500030 Processed goods Human consumption (seeds) Cattle cosumption	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast foliar Tractor mounted boom	BBCH 30-32	a) 1 b) 1	NA	a) 0,34 b) 0,34	a) AS1: 134,38 (as/ha), 102 (ae/ha) b) AS1: 134,38 (as/ha), 102 (ae/ha)	100-400	60 days for forage, 90 days for grain	One application every 2 years. For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
17	Czech Republic, Slovakia, Poland	Onion for Seeds EPPO Code: ALLCE EU MRL Code: 0220020 Raw Human consumption Processed goods	F	Broad-leaved weeds (BBBBB) (including but not only Cirsium arvense, Matricaria spp.)	Broadcast Foliar Tractor mounted boom	BBCH 11-16	a) 1 b) 1	NA	a) 0,4 b) 0,4	a) AS1: 158,104 (as/ha), 120 (ae/ha) b) AS1: 158,104 (as/ha), 120 (ae/ha)	100-400	42-days	For residue management in crop rotation: no mitigation measures are required for Leafy and Brassica vegetables or for Oilseeds. For all other food and feed commodities except sugar canes, a 30-day PBI is supported. It is recommended that sugar canes not be planted for 125 days after application of clopyralid. For crop rotation management, see label for recommendations..							
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
Minor uses according to Article 51 (field uses)																				
Minor uses according to Article 51 (interzonal uses)																				

* 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

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)

Regulatory testing has been conducted with clopyralid in accordance with EU requirements. New data has generated for EF-243. The risk assessment will be based on the active substance endpoints. Acceptable acute and long-term risk to birds and mammals at screening step is concluded based on the intended uses without the need for risk mitigation measures.
No data on reptiles and terrestrial amphibians are available for clopyralid. No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, acceptable acute and long-term risks were concluded for birds and mammals (TER_A>10; TER_{LT} > 5) under the very conservative assumptions of the screening level approach with a high margin of safety. As such no adverse effects or risks are expected for reptiles and amphibians exposed to clopyralid following applications of EF-243.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Regulatory testing has been conducted clopyralid metabolites and clopyralid solo-formulations in accordance with EU requirements. The acute and chronic risk assessment for aquatic organisms indicated an acceptable risk to aquatic organisms from all the intended uses of EF-243 without the need for mitigation measures.

9.1.1.3 Effects on bees (KCP 10.3.1)

Regulatory testing has been conducted with clopyralid in accordance with EU requirements. An acceptable acute and chronic risk to bees/bumblebees is concluded from the proposed uses of EF-243 without the need for risk mitigation measures.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Regulatory testing has been conducted with a clopyralid solo-formulation in accordance with EU requirements. An acceptable risk to non-target arthropods is concluded from the proposed uses of EF-243 without the need for risk mitigation measures

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

Regulatory testing has been conducted with clopyralid and the product in accordance with EU requirements. An acceptable risk to soil macro and micro-organisms is concluded from the proposed uses of EF-243 without the need of any risk mitigation.

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

Regulatory testing has been conducted with a clopyralid solo-formulation in accordance with EU requirements. An acceptable risk to non-target plants is expected from the proposed uses A-E and G (max application rate 120 g ae/ha) of EF-243 without the need of any risk mitigation measurement.
For Group F (established grassland >1 year; max application rate 200 g ae/ha) an acceptable risk to terrestrial non-target plants can be anticipated following uses of EF-243 with the following mitigation measurements:

- 1 m distance to the treated field when 50% drift reducing nozzles are used during terrestrial applications or
- 5 m distance to the treated field without drift reducing nozzles

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

No effects on other terrestrial organisms are anticipated after applications of EF-243.

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 EF-243

Grouping for birds and mammals			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
A	001-006 Fodder beet, Sugar beet, Red Beet	Crop group: sugar beet (max application rate 120 g ae/ha)	***Birds:*** Although the application rate of Group E (established grassland) is the highest for all the supported uses the screening shortcut value is lower than for the other uses. The "indicator species for screening" is the same for all the other groups and therefore the highest apllication rate from groups A, B and C (i.e. 120 g a.e./ha) has been selected for the risk assessment. ***Mammals:*** Although the "indicator species for screening" is the same for all the intended uses the shortcut values for grassland/maize are higher so Group E will cover the other groups with rate resulting in the highest daily dietary dose (DDD): 200 g ae/ha
B	007-008 winter oilseed rape	Crop group: oilseed rape (max application rate 120 g ae/ha)	
C	009 gladiolus 017 onion for seeds	Croup group: bulbs and onion like crops (max application rate 120 g ae/ha)	
D	010-011 spring and winter cereals	Crop group: cereals (max application rate 90 g ae/ha)	
E	012-013 grass for seed (more and less than a year old) 014 lawn (more than a year old)	Croup group: grassland (max application rate 200 g ae/ha)	
F	015-016 maize (grain a forage)	Croup group: maize (max application rate 102 g ae/ha)	
Grouping for aquatic organism			
Group	**Intended uses**	**Relevant use parameters for**	**Relevant parameter or value**

		grouping	**for sorting**
A	001-006 Fodder beet, Sugar beet, Red Beet	Crop group: sugar beet (max application rate 120 g ae/ha)	Grouping based on FOCUS crops scenarios Highest PECsw for all the application rates and BBCH considered
B	007-008 winter oilseed rape	Crop group: oilseed rape (max application rate 120 g ae/ha)	
C	009 gladiolus 017 onion for seeds	Croup group: Vegetable, bulb (max application rate 120 g ae/ha)	
D	010 Spring cereals	Crop group: spring cereals (risk envelope max application rate 120 g ae/ha)	
E	0011 Winter cereals	Crop group: Winter cereals (risk envelope max application rate 120 g ae/ha)	
F	015-016 maize (grain a forage)	Crop group: Maize (risk envelope max application rate 120 g ae/ha)	
G	012 grass for seed (more than a year old) 014 lawn (more than a year old)	Croup group: grass/alfalfa (max application rate 200 g ae/ha)	
H	013 grass for seed (less than a year old)	Croup group: grass/alfalfa (max application rate 120 g ae/ha)	
Grouping for bees, NTAs and NTTPs			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
A	001-006 Fodder beet, Sugar beet, Red Beet	Crop group: sugar beet (max application rate 120 g ae/ha)	***Bees and NTAs:*** Maximum application rate (i.e 200 g ae/ha) from Group F ***NTTPs***: due to the difference in risk mitigations needed the risk assessment has been conducted for Group F (max application rate 200 g ae/ha) and Groups A-E and G (max application rate 120 g ae/ha)
B	007-008 winter oilseed rape	Crop group: oilseed rape (max application rate 120 g ae/ha)	
C	009 gladiolus 017 onion for seeds	Croup group: bulbs and onion like crops (max application rate 120 g ae/ha)	
D	010-011 spring and winter cereals	Crop group: cereals (max application rate 90 g ae/ha)	
E	012-013 grass for seed (more and less than a year old)	Croup group: grassland (max application rate 120 g ae/ha)	
F	014 lawn (more than a year old)	Croup group: grassland (max application rate 200 g ae/ha)	
G	015-016 maize (grain a forage)	Croup group: maize (max application rate 102 g ae/ha)	
Grouping for soil organism and soil microbial activity			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
A	001-006 Fodder beet, Sugar beet, Red Beet	Crop group: sugar beet (max application rate 120 g/ha; 20%	Highest PECsoil based on application rate and crop

		crop interception)	interception. Group C has the highest PECsoil due to the 10% crop interception.
B	007-008 winter oilseed rape	Crop group: winter oilseed rape (max application rate 120 g/ha; 80% crop interception)	
C	009 gladiolus 017 onion for seeds	Crop group: onions (risk envelope max application rate 120 g/ha; 10% crop interception)	
D	010-011 spring and winter cereals	Crop group: cereals (risk envelope max application rate 120 g/ha; 80% crop interception)	
E	012 grass for seed (more than a year old) 014 lawn (more than a year old)	Crop group: grass/alfalfa (>1 year) (200 g a.e./ha, 90% crop interception)	
F	014 grass for seed (less than a year old)	Crop group: grass/alfalfa (<1 year) (120 g a.e./ha, 40% crop interception)	
G	015-016 maize (grain a forage)	Crop group: maize (max application rate 100 g/ha; 25% crop interception)	

9.1.3 Consideration of metabolites

The occurrence and risk from potentially ecotoxicologically relevant metabolites have been considered, detailed discussion was provided in the EFSA Conclusion for clopyralid and in dRR Part B8. There are no relevant metabolites to which non-target organisms could be exposed.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of EF-243 were not evaluated as part of the latest EU assessment of clopyralid. New data has generated for EF-243. New data submitted with this application is listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**.The risk assessment will be based on the active substance endpoints.

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
Anas platyrhynchos (mallard duck)	clopyralid	Oral 1 d Acute	LD_{50} = 1465 mg/kg bw	EFSA Conclusion ([redacted]

Species	Substance	Exposure System	Results	Reference
				[redacted]
Bobwhite quail	EF-243	Oral 1 d Acute	LD_{50} > 2000 mg EF-243/kg bw	[redacted]
Anas platyrhynchos (mallard duck)	clopyralid	Dietary Reproductive toxicity	NOEL = 118 mg/kg bw/d (the highest concentration tested.)	EFSA Conclusion ([redacted]

EFSA Conclusion: EFSA Journal 2018;16(6):5389

9.2.1.1 Justification for new endpoints

Not applicable.

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. Although the application rate of Group E (established grassland) is the highest for all the supported uses the screening shortcut value is lower than for the other uses. The "indicator species for screening" is the same for all the other groups and therefore the highest application rate from groups A, B and C (i.e. 120 g a.e./ha) has been selected for the risk assessment. As the "indicator species for screening" is the same for all the intended uses the rate resulting in the highest daily dietary dose (DDD): 200 g ae/ha (Group E grassland) also covers the risk for birds from all other intended uses (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: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of EF-243 Groups A, B and C; max application rate 120 g a.e./ha)

Intended use	~~Grassland~~ Bulbs and onion like crops, cereals, maize, oilseed rape, sugar beet				
Active substance/product	Clopyralid (as EF-243)				
Application rate (g/ha)	1 × 120				
Acute toxicity (mg/kg bw)	1465				
TER criterion	10				
Crop scenario	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90}**	**TER_a**

Growth stage				**(mg/kg bw/d)**	
N/A	Small omnivorous bird	158.8	1	19.6	76.9
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
N/A	Small omnivorous bird	64.8	1 x 0.53	4.12	28.6

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

Added just for clarity by evaluator:

Intended use	Grassland				
Active substance/product	Clopyralid (as EF-243)				
Application rate (g/ha)	1 × 200				
Acute toxicity (mg/kg bw)	1465				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
N/A	Small omnivorous bird	30.5	1	6.10	240.2
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
N/A	Small omnivorous bird	16.2	1 x 0.53	1.72	68.7

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

9.2.2.2 Higher-tier risk assessment

Not applicable as safe risk has been concluded at screening step.

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 EF-243 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc of 1.41, clopyralid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group E also covers the risk for birds from all other intended uses in groups A-D and F (see 9.1.2).

Effective application rate (g/ha)	=	200		
Acute toxicity (mg/kg bw)	=	1465	quotient	= 0.136
Reprod. toxicity (mg/kg bw/d)	=	118	quotient	= 1.69

Since the ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) don't exceed the critical value of 50 a quantitative risk assessment (calculation of TER values) is not necessary.

9.2.2.4 Effects of secondary poisoning

The Log P_{ow} of clopyralid amounts to -2.63 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

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

An acceptable acute and long-term risk to birds is expected from the proposed uses of EF-243 without the need of any refinement.

Review comments:

The risk assessment to birds was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).

The results of the 'screening phase' acute and long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substance following use of Lontrel 300 SL (EF-243) in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of Lontrel 300 SL according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

A risk assessment for effects due to secondary poisoning is not required because of the low Log Pow of clopyralid (-2.63).

No risk mitigation measures are required.

Conclusion

According to the performed risk assessment there is no potential of risk to birds resulting from exposure to active substance following use of Lontrel 300 SL (EF-243) in compliance with proposed GAP.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of EF-243 were not evaluated as part of the EU assessment of clopyralid. However, the provision of further data on the EF-243 is not considered essential, because the toxicity can be predicted on the basis of the data for the active substance due to the fact that EF-243 is an aqueous soluble concentrate of clopyralid with no additional co-formulants.

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
Rat	Clopyralid	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw	EFSA Conclusion ([redacted] see section B6)
Rat	Clopyralid	2-year chronic study	NOAEL = 50 mg/kg bw/d (Reduction in body weight)	EFSA Conclusion ([redacted] see section B6)

EFSA Conclusion: EFSA Journal 2018;16(6):5389

9.3.1.1 Justification for new endpoints

Not applicable

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. Although the "indicator species for screening" is the same for all the intended uses the shortcut values for grassland/maize are higher so Group E (grassland, max application rate 200 g ae/ha) also covers the risk for mammals from all other intended uses (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: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of EF-243 in grassland (Group E, max application rate 200 g a.e./ha)

Intended use	Grassland				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 × 200				
Acute toxicity (mg/kg bw)	>5000				
TER criterion	10				
Crop scenario **Growth stage**	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

N/A	Small herbivorous mammal	136.4	1	27.28	183.3
Reprod. toxicity (mg/kg bw/d) **TER criterion**	50 5				
Crop scenario **Growth stage**	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
N/A	Small herbivorous mammal	72.3	1 x 0.53	7.66	6.52

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

Not applicable as acceptable risk has been concluded at screening step.

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 1.41, clopyralid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group E also covers the risk for mammals from all other intended uses in groups A-D and F (see 9.1.2).

Effective application rate (g/ha)	=	200		
Acute toxicity (mg/kg bw)	=	>5000	quotient =	<0.04
Reprod. toxicity (mg/kg bw/d)	=	50	quotient =	4

Since the ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) don't exceed the critical value of 50 a quantitative risk assessment (calculation of TER values) is not necessary.

9.3.2.4 Effects of secondary poisoning

The Log P_{ow} of clopyralid amounts to -2.63 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

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

An acceptable acute and long-term risk to mammals is expected from the intended uses of EF-243 without the need of any refinement.

Review comments:

The risk assessment to mammals was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).
The results of the 'screening phase' acute and long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for wild mammals resulting from acute and long-term exposure to active substances following use of Lontrel 300 SL (EF-243) in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of Lontrel 300 SL (EF-243) according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

A risk assessment for effects due to secondary poisoning is not required because of the low Log Pow of clopyralid (-2.63).

No risk mitigation measures are required.

Conclusion
According to the performed risk assessment there is no potential of risk to wild mammals resulting from exposure to active substance following use of Lontrel 300 SL (EF-243) in compliance with proposed GAP.

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

According to the data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013

and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. However, there is no guidance or validated regulatory protocol yet available, neither on the type of the necessary regulatory testing nor how to conduct a risk assessment for amphibians and reptiles. Accordingly, specific toxicity tests for amphibian and reptile species are not requested and therefore no data on reptiles and terrestrial amphibians is available for clopyralid. In the EU there is no guidance or validated regulatory protocols yet available either on the type of regulatory testing necessary or how to conduct a risk assessment for amphibian and reptiles.
According to the new aquatic guidance document (EFSA, 2013) amphibians should be included in the aquatic and terrestrial risk assessment. In the absence of GLP studies, the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment, several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2009, 2012; Weltje et al., 2013). Based on these extensive data reviews, it can be concluded that the acute and chronic risk to aquatic life stages of amphibians is covered by the currently requested and conducted risk assessment for aquatic organisms (see KCP 10.2 / Part B, Section 9; 9.5).
With regard to the terrestrial vertebrate risk assessment, in the absence of a specific framework, the data and risk assessment for birds and mammals are considered an adequate surrogate for other terrestrial vertebrates. In the few cases where terrestrial stages of amphibians were tested in this kind of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (Tables 12 and 13 in Fryday and Thompson, 2012). It can be concluded that the acute and chronic risk to terrestrial life stages of amphibians is covered by the currently requested and conducted risk assessment for terrestrial vertebrates (see KCP 10.1/ Part B, Section 9; 9.2 & 9.3).
In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherms (i.e. do not maintain a constant body temperature) and as a result, feeding activity will peak on warm days and will be zero during hibernation or on cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday and Thompson 2009). There is no indication from ‘read across’ that reptiles either could be particularly sensitive or would not be covered by the available vertebrate data and risk assessments.

References

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.
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Fryday S and Thompson H (2009a): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).
Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK
Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with clopyralid. Full details of these

studies are provided in the respective EU DAR and related documents.

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 – clopyralid

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	clopyralid	96 h, s	LC_{50} =>99.9 mg a.s./L $_{mm}$	EFSA Conclusion (Marino, T.A. *et al.*/ 2000/ DAS Study ID 001024)
Pimephales promelas	clopyralid	34 d (ELS), f	NOEC = 10.8 mg a.s./L $_{mm}$	EFSA Conclusion (Marino, T. A. *et al*/ 2000/ DAS Study ID 001017)
Daphnia magna	clopyralid	48 h, s	EC_{50} = >99.0 mg a.s./L $_{mm}$	EFSA Conclusion (Marino, T. A. *et al.*/2000/DAS Study ID 001025)
Daphnia magna	clopyralid	21 d, ss	NOEC = 17 mg a.s./L $_{mm}$	EFSA Conclusion (Douglas, M. T *et al.*/ 1992/ DAS Study ID DWC 615/911087)
Chironomus riparius	clopyralid	28 d, spiked water	NOEC = 50 mg a.s./L $_{mm}$	EFSA Conclusion (Barrett, K/2001/ DAS Study ID GHE-T-1122)
Pseudokirchneriella subcapitata	clopyralid	72 h, s	E_rC_{50} = 30 mg a.s./L $_{mm}$ E_bC_{50} = 30.9 mg a.s./L $_{mm}$	EFSA Conclusion (Kirk, H. D. *et al.*/ 2000/ DAS Study ID 001040)
Navicula pelliculosa	clopyralid	72 h, s	E_rC_{50} = 31.3 mg a.s./L $_{mm}$ E_yC_{50} = 31.5 mg a.s./L $_{mm}$	EFSA Conclusion (Aufderheide, J./2015/DAS Study ID 140515)
Lemna gibba	clopyralid	14 d, ss	EC_{50} ((plants/fronds) = 89 mg a.s./L $_{mm}$	EFSA Conclusion (Cowgill, U.M. *et al*/1990/ DAS Study ID ES-2243)
Myriophyllum spicatum	clopyralid	14 d, s	E_rC_{50} = >3 mg a.s./L $_{nom}$ E_yC_{50} (shoot length) = 1.225 mg a.s./L $_{nom}$	EFSA Conclusion (Banman, C. S. & Moore, S./2015/DAS Study ID 140735)
Higher-tier studies (micro- or mesocosm studies)				

Not relevant

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
EFSA Conclusion: EFSA Journal 2018;16(6):5389

Effects on aquatic organisms of EF-243 were not evaluated as part of the last EU assessment of clopyralid. Data on EF-255, EF-243, GF-2895 and GF-1966 is available. EF-255/EF-243 are a dilution of clopyralid (as monoethanolamine salt) in water. GF-1966 is a granule formulation of clopyralid (as monoethanolamine salt) containing a small amount of water. GF-2895 is a dilution of clopyralid (as dimethylamine salt) in water. EF-1136 a soluble concentrate (SL) formulation containing 100 g clopyralid ae/L Following dilution with water within the spray tank, immediately prior to application, the resulting spray solutions are equivalent for purposes of ecotoxicology. New data submitted with this application is listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – EF-243

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Lontrel 100 (EF-255)	96 h, s	LC_{50} = 53 mg a.e./L $_{nom}$	EFSA Scientific Report ([redacted]/1989/DAS Study ID IRI 140485 & IRI 140731)
Oncorhynchus mykiss	Lontrel 300 (EF-243)	96 h, s	EC_{50} = 297 mg EF-243/L $_{nom}$ Based on clopyralid content EC_{50} = 78 mg a.e/L $_{nom}$	[redacted] Study ID 200842[redacted]21
Daphnia magna	Lontrel 100 (EF-255)	48 h, s	EC_{50} = 130 mg a.e/L $_{nom}$	EFSA Scientific Report (Caley, C.Y. et al/1989/DAS Study ID IRI 140464 & IRI 140731)
Daphnia magna	Lontrel 300 (EF-243)	48 h, s	EC_{50} > 381 mg EF-243/L $_{nom}$ Based on clopyralid content EC_{50} > 100 mg a.e/L $_{nom}$	Ross, T. L., Zhao, E., Zhang, L., Schneider, S.Z./2020/DAS Study ID 200842
Daphnia magna	Lontrel 100 (EF-255)	20 d, ss	NOEC = 7 mg a.e./L mm	EFSA Scientific Report (Caley, C.Y. et al/1990/DAS Study ID IRI 140553)
Selenastrum capricornutum	Lontrel 100 (EF-255)	72 h, s	E_rC_{50} = 77.4 mg a.e./L $_{nom}$ E_bC_{50} = 47.6 mg a.e./L $_{nom}$	EFSA Scientific Report (Caley, C.Y. et

				al/1990/DAS Study ID IRI 140490 & IRI 140731)
Selenastrum capricornutum	Lontrel 300 (EF-243)	72 h, s	E_rC_{50} = 77.4 mg EF-243/L $_{nom}$ E_yC_{50} = 47.6 mg EF-243/L $_{nom}$ Based on clopyralid content E_rC_{50} = 25.9 mg a.e./L $_{nom}$ E_yC_{50} = 8.7 mg a.e./L $_{nom}$	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L., Fierman, L.A./2020/ DAS Study ID 200843
Selenastrum capricornutum	GF-2895	72 h, s	E_rC_{50} > 100 mg GF-2895/L $_{nom}$ E_yC_{50} > 100 mg GF-2895/L $_{nom}$ Based on clopyralid content E_rC_{50} >48.4 mg a.e./L $_{nom}$ E_yC_{50} >48.4 mg a.e./L $_{nom}$	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L./2020/DAS Study ID 191747
Myriophyllum spicatum	GF-1966	14 d, s	E_rC_{50} (dry weight) = 4.039 mg GF-1966/L $_{nom}$ E_yC_{50} (dry weight) = 0.715 mg GF-1966/L $_{nom}$ Based on clopyralid content E_rC_{50} (dry weight) = 2.9 mg ae/L $_{nom}$ E_yC_{50} (dry weight) = 0.54 mg ae/L $_{nom}$	Banman, C. S. and S. Moore /2015 /DAS Study ID 150051
Myriophyllum spicatum	GF-2895	14 d, s	E_rC_{50} (fresh weight) = 73.1 mg GF-2895/L $_{nom}$ E_yC_{50} (fresh weight) = 56.3 mg GF-2895/L $_{nom}$ Based on clopyralid content E_rC_{50} (dry weight) = 36.55 mg ae/L $_{nom}$ E_yC_{50} (dry weight) = 28.15 mg ae/L $_{nom}$	Gonsior G./2018/DAS Study ID 170354
Higher-tier studies (micro- or mesocosm studies)				
Not relevant				

EFSA Scientific Report (2005) 50: 1–65
s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

As the endpoints for the formulated product for fish, chronic *Daphnia*, green algae and *Myriophyllum* (based on clopyralid content) are lower than the endpoints for the active substance the worst-case endpoints are used to ensure the risk assessment is protective of the environment.

9.5.1.1 Justification for new endpoints

Not relevant

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products

for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum PEC_{sw} for each of the groups cover from all other intended uses in each group (see 9.1.2).

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 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in sugar beet (Group A, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	5.41	0.0102	0.0050	0.0055	0.0077	0.0011	0.0021	0.0187
S-Europe	9.72	0.0183	0.0090	0.0098	0.0139	0.0019	0.0038	0.0335
Step 3								
D3/ditch	0.643	0.0012	0.0006	0.00064	0.0009	0.0001	0.0002	0.0022
D4/pond	0.045	0.0001	0.00004	0.00005	0.00006	0.00001	0.00002	0.0002
D4/stream	0.525	0.0010	0.0005	0.00053	0.0008	0.0001	0.0002	0.0018
R1/pond	0.025	0.0000	0.00002	0.00003	0.00004	0.00001	0.00001	0.0001
R1/stream	0.466	0.0009	0.0004	0.00047	0.0007	0.0001	0.0002	0.0016
R3/stream	1.98	0.0037	0.00183	0.00200	0.00283	0.00040	0.00076	0.0068

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 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in winter oilseed rape (Group B, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	5.14	0.0097	0.0048	0.0052	0.0073	0.0010	0.0020	0.0177
S-Europe	4.33	0.0082	0.0040	0.0044	0.0062	0.0009	0.0017	0.0149
Step 3								
D3/ditch	0.76	0.0014	0.0007	0.00077	0.0011	0.0002	0.0003	0.0026
D4/pond	0.028	0.0001	0.00003	0.00003	0.00004	0.00001	0.00001	0.0001
D4/stream	0.604	0.0011	0.0006	0.00061	0.0009	0.0001	0.0002	0.0021
D5/pond	0.026	0.0000	0.00002	0.00003	0.00004	0.00001	0.00001	0.0001
D5/stream	0.606	0.0011	0.0006	0.0006	0.0009	0.00012	0.0002	0.0021
R1/pond	0.037	0.00007	0.00003	0.00004	0.00005	0.00001	0.00001	0.0001
R1/stream	1.76	0.0033	0.0016	0.0018	0.0025	0.0004	0.0007	0.0061
R3/stream	1.13	0.0021	0.0010	0.0011	0.0016	0.0002	0.0004	0.0039

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 clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in vegetable, bulb (Group C, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	5.95	0.0112	0.0055	0.0060	0.0085	0.0012	0.0023	0.0205
S-Europe	10.8	0.0204	0.0100	0.0109	0.0154	0.0022	0.0042	0.0372
Step 3								
D3/ditch	0.776	0.0015	0.0007	0.00078	0.0011	0.0002	0.0003	0.0027
D4/pond	0.111	0.0002	0.00010	0.00011	0.00016	0.00002	0.00004	0.0004
D4/stream	0.591	0.0011	0.0005	0.00060	0.0008	0.0001	0.0002	0.0020
R1/pond	0.015	0.0000	0.00001	0.00002	0.00002	0.00000	0.00001	0.0001
R1/stream	1.22	0.0023	0.0011	0.0012	0.0017	0.00024	0.0005	0.0042
R3/stream	0.704	0.0013	0.0007	0.0007	0.0010	0.0001	0.0003	0.0024
R4/stream	5.61	0.0106	0.0052	0.0057	0.0080	0.0011	0.0022	0.0193

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in spring cereals (Group D, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	5.41	0.0102	0.0050	0.0055	0.0077	0.0011	0.0021	0.0187
S-Europe	9.72	0.0183	0.0090	0.0098	0.0139	0.0019	0.0038	0.0335
Step 3								
D3/ditch	0.767	0.0014	0.0007	0.00077	0.0011	0.0002	0.0003	0.0026
D4/pond	0.037	0.0001	0.00003	0.00004	0.00005	0.00001	0.00001	0.0001
D4/stream	0.627	0.0012	0.0006	0.00063	0.0009	0.0001	0.0002	0.0022
D5/pond	0.026	0.0000	0.00002	0.00003	0.00004	0.00001	0.00001	0.0001
D5/stream	0.639	0.0012	0.0006	0.0006	0.0009	0.00013	0.0002	0.0022
R4/stream	3.49	0.00658	0.00323	0.00353	0.00499	0.00070	0.00135	0.0120

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 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in winter cereals (Group E, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	11.9	0.0225	0.0110	0.0120	0.0170	0.0024	0.0040	0.0410
S-Europe	9.72	0.0183	0.0090	0.0098	0.0139	0.0019	0.0032	0.0335
Step 3								
D3/ditch	0.765	0.0014	0.0007	0.00077	0.0011	0.0002	0.0003	0.0026
D4/pond	0.028	0.0001	0.00003	0.00003	0.00004	0.00001	0.00001	0.0001
D4/stream	0.564	0.0011	0.0005	0.0006	0.0008	0.00011	0.0002	0.0019
D5/pond	0.026	0.00005	0.00002	0.00003	0.00004	0.00001	0.00001	0.0001
D5/stream	0.607	0.0011	0.0006	0.0006	0.0009	0.0001	0.0002	0.0021
R1/pond	0.029	0.0001	0.00003	0.00003	0.00004	0.0000	0.0000	0.0001
R1/stream	1.6	0.0030	0.0015	0.0016	0.0023	0.0003	0.0006	0.0055
R3/stream	0.704	0.0013	0.0007	0.0007	0.0010	0.0001	0.0003	0.0024
R4/stream	0.503	0.0009	0.0005	0.0005	0.0007	0.0001	0.0002	0.0017

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 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in maize (Group F, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	41	0.0774	0.0380	0.0414	0.0586	0.0082	0.0158	0.1414
Step 2								
N-Europe	5.14	0.0097	0.0048	0.0052	0.0073	0.0010	0.0020	0.0177
S-Europe	9.18	0.0173	0.0085	0.0093	0.0131	0.0018	0.0035	0.0317
Step 3								
D3/ditch	0.64	0.0012	0.0006	0.0006	0.0009	0.0001	0.0002	0.0022
D4/pond	0.029	0.0001	0.0000	0.00003	0.00004	0.00001	0.00001	0.0001
D4/stream	0.541	0.0010	0.0005	0.0005	0.0008	0.00011	0.0002	0.0019
D5/pond	0.027	0.0001	0.0000	0.0000	0.0000	0.00001	0.0000	0.0001
D5/stream	0.538	0.0010	0.00050	0.00054	0.00077	0.00011	0.00021	0.0019
R1/pond	0.033	0.0001	0.00003	0.000033	0.000047	0.000007	0.000013	0.0001
R1/stream	1.07	0.0020	0.00099	0.00108	0.00153	0.00021	0.00041	0.0037
R3/stream	3.93	0.0074	0.00364	0.00397	0.00561	0.00079	0.00152	0.0136
R4/stream	4.94	0.0093	0.00457	0.00499	0.00706	0.00099	0.00191	0.0170

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 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in grass > a year old (Group G, max application rate 200 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	68.4	0.1291	0.0633	0.0691	0.0977	0.0137	0.0264	0.2359
Step 2								
N-Europe	7.22	0.0136	0.0067	0.0073	0.0103	0.0014	0.0028	0.0249
S-Europe	12.6	0.0238	0.0117	0.0127	0.0180	0.0025	0.0049	0.0434
Step 3								
D3/ditch	1.61	0.0030	0.0015	0.0016	0.0023	0.0003	0.0006	0.0056
D4/pond	0.133	0.0003	0.0001	0.00013	0.00019	0.00003	0.00005	0.0005
D4/stream	1.1	0.0021	0.0010	0.0011	0.0016	0.00022	0.0004	0.0038
D5/pond	0.136	0.0003	0.0001	0.0001	0.0002	0.00003	0.0001	0.0005
D5/stream	1.18	0.0022	0.00109	0.00119	0.00169	0.00024	0.00046	0.0041
R3/stream	3.42	0.0065	0.00317	0.003455	0.004886	0.000684	0.001320	0.0118

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 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EF-243 in grass < a year old (Group H, max application rate 120 g a.e./ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. pro-longed**	**Sed. dwell. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P.promelas***	***D. magna***	***D. magna***	***C. riparius***	***P. subcapitata***	***M. spicatum***
Endpoint (µg/L)		LC_{50} 53,000	NOEC 10,800	EC_{50} >99,000	NOEC 7,000	NOEC 50,000	E_rC_{50} 25,900	E_rC_{50} 2,900
AF		100	10	100	10	10	10	10
RAC (µg/L)		530	1,080	>990	700	5,000	2,590	290
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)***	**PEC/RAC Ratio**						
Step 1	68.4	0.1291	0.0633	0.0691	0.0977	0.0137	0.0264	0.2359
Step 2								
N-Europe	7.22	0.0136	0.0067	0.0073	0.0103	0.0014	0.0028	0.0249
S-Europe	12.6	0.0238	0.0117	0.0127	0.0180	0.0025	0.0049	0.0434
Step 3								
D3/ditch	0.815	0.0015	0.0008	0.0008	0.0012	0.0002	0.0003	0.0028
D4/pond	0.035	0.0001	0.00003	0.00004	0.00005	0.00001	0.00001	0.0001
D4/stream	0.658	0.0012	0.0006	0.0007	0.0009	0.00013	0.0003	0.0023
D5/pond	0.032	0.00006	0.00003	0.0000	0.00005	0.00001	0.00001	0.0001
D5/stream	0.71	0.0013	0.0007	0.0007	0.0010	0.0001	0.0003	0.0024
R3/stream	1.17	0.0022	0.00108	0.0012	0.00167	0.0002	0.0005	0.0040

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 of EF-243, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by an E_rC_{50} for Myriophyllum spicatum of 2900 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-3 scenarios. Therefore, no further assessment is necessary.

9.5.3 Overall conclusions

An acceptable risk for aquatic organisms is concluded for all the intended uses of EF-243 without the need of any mitigation.

Review comments:

The risk assessment for aquatic organisms was performed in accordance with the recommendation of Guidance Document on Aquatic Ecotoxicology, as provided by the Commission Services (SANCO/3268/2001 rev.4 (final), 17 October 2002).

This risk envelope approach is acceptable. The predicted environmental concentrations in surface water (PECsw) of the active substance from Part B Section 8 were taken into account for the risk assessment.

For the risk assessment the EU agreed endpoints for active substance and endpoints for formulation EF-243 were presented. Additionally, endpoints for formulations EF-255 (EFSA Scientific Report (2005)50, 1-65), GF-2895 and GF-1966 were reported.
The formulation endpoints (expressed as clopyralid content) for acute fish, chronic daphnia, algae and aquatic plants are lower than the EU agreed endpoints for active substance. For the risk assessment the lowest endpoints were used.

All PEC/RAC ratios are below the trigger value of 1 in all FOCUS Step 1 scenarios.

No risk mitigation measures are required.

Conclusion
According to the performed risk assessment there is no potential of risk for aquatic organisms resulting from acute and long-term exposure to active substance following use of EF-243 (Lontrel 300) in compliance with proposed GAP.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with clopyralid Full details of these studies are provided in the respective EU DAR and related documents. New data submitted with this application is listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**.

Effects on bees of EF-243 were not evaluated as part of the last EU assessment of clopyralid. However, the provision of further data on the EF-243 is not considered essential, because the toxicity can be predicted on the basis of the data for the active substance due to the fact that EF-243 is an aqueous soluble concentrate of clopyralid with no additional co-formulants.

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
Apis mellifera	clopyralid	Oral	LD_{50} = >100 µg a.s./bee	EFSA Conclusion (Wainwright, M/2001/DAS report no. GHE-T-1091)
Apis mellifera	clopyralid	Contact	LD_{50} = >98.1 µg a.s./bee	EFSA Conclusion (Wainwright, M/2001/DAS report no. GHE-T-1091)
Apis mellifera	clopyralid	10-d feeding test adult	NOED =71.2 µg a.s./bee/day LDD_{50} = >71.2 µg a.s./bee/day	EFSA Conclusion (Leonard, J and Moore,S/2017/DAS report no. 170098)
Bombus terrestris	Clopyralid	Oral	LD_{50} = >203 µg a.s./bee	Tänzler, V., Kowalczyk, F./2019/DAS Study ID 190300
Bombus terrestris	Clopyralid	Contact	LD_{50} = >200 µg a.s./bee	Tänzler, V., Kowalczyk, F./2019/DAS Study ID 190300
Apis mellifera	clopyralid	Repeat exposure larvae	NOED =12.5 µg a.s./larva	EFSA Conclusion (Leonard, J and Moore,S/2017/DAS report no. 170099)
Apis mellifera	Lontrel 100 (EF-1136)	Oral	LD_{50} = > 200 µg a.s./bee	EFSA Scientific Report (Wainwright, M/2001/DAS report no. DOS 166/004732)
Apis mellifera	Lontrel 100 (EF-1136)	Contact	LD_{50} = >98.1 µg as/bee	EFSA Scientific Report (Wainwright, M/2001/DAS report no. DOS 166/004732)
Higher-tier studies (tunnel test, field studies)				
Not needed				

EFSA Conclusion: EFSA Journal 2018;16(6):5389

EFSA Scientific Report (2005) 50: 1–65

9.6.1.1 Justification for new endpoints

A study assessing the acute oral and contact toxicity of clopyralid to bumblebees was recently completed and is submitted for completeness. New data submitted with this application is listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**.

9.6.2 Risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum application rate for Group E (i.e. 200 g ae/ha) has been used also covers the risk for non-target arthropods from all other intended uses in groups A-E and G (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of EF-243 in Group E (grassland, max application rate 200 g a.e/ha)

Intended use	Grassland		
Active substance	Clopyralid		
Application rate (g/ha)	1 × 200		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
~~Oral toxicity~~ Contact toxicity	>98.1	200	< 2.03
~~Contact toxicity~~ Oral toxicity	100		< 2

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

The applicant recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013 hasn't been noted and it is currently under revision., the risk assessment below has been conducted following the EPPO 2010[1] scheme which provides a comparable level of protection to the EFSA approach and is based on the current scientific state of the art for bee pollinator risk assessment. However, in order to address the National Requirements for some CZ MS the risk assessment according to the draft EFSA Guidance document has been also conducted.

Risk assessment according to the EPPO modified scheme

[1] EPPO (2010a). Side-effects on honey bees. Bulletin OEPP/EPPO Bulletin 40: 313-319.
EPPO (2010b). Environmental risk assessment scheme for plant protection products. Bulletin OEPP/EPPO Bulletin 40: 323-331.

Worst-case data from Rortais et al., 2005[2] as proposed in the EPPO scheme have been used to estimate the consumption by **honeybee larvae**. Based on the data in this publication, a worker larva consumes 59.4 mg sugar in 5 days. Assuming a 30% sugar content of nectar, the resulting worst-case consumption for a worker larva is: 59.4/0.30 = *198 mg nectar in 5 days* (larval development). In addition, a worker larva is considered to consume *2 mg pollen during its development phase* (EFSA 2013). Thus, considering the mean RUD values for nectar (i.e. 2.9 mg/kg) and pollen (i.e. 6.1 mg/kg) from foliar sprays in EFSA 2013 (Appendix F), exposure can be estimated for the whole larval development period of 5 days. The final estimated exposure levels deriving from nectar and pollen consumption can be compared to the available larval NOEL value for clopyralid. The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. Results are presented in the following table:

Table 9.6-3: Assessment of the risk for bee larvae due to the use of EF-253 in Group E (grassland, max application rate 200 g a.e/ha)

Intended use		Grassland		
Active substance		Clopyralid		
Application rate (kg/ha)		1 × 0.200		
NOEL (µg/bee/developmental period)		12.5		
Food item	**Consumption (kg/bee/dev. period)**	**RUD (µg/kg/kg/ha)**	**Dietary dose (µg/bee/dev. period)**	**TER criterion: TER ≥1**
Nectar	198×10^{-6}	2.9×10^{3}	0.11484	107
Pollen	2×10^{-6}	6.1×10^{3}	0.00244	
		Total	0.11728	

The risk assessment for chronic exposure of **adult honeybees** is based upon the method of EPPO 2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the endpoint so avoids the issues associated with the generation of LDD_{50} values for substances of low toxicity and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals) and derives a TER value. Worst-case data from Rortais et al., 2005 indicates a sugar need of 128 mg/bee/day for a bee feeding exclusively from nectar containing 30% sugar. This results in a worst-case consumption for an adult honeybee is: 128/0.30 = *427 mg nectar/day*. Considering the mean RUD value for nectar from foliar sprays (i.e. 2.9 mg/kg) in EFSA 2013 (Appendix F), the daily dietary exposure for adult honeybees can be estimated and it can be compared to the available chronic adult NOEDD value for clopyralid. The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. Results are presented in the following table

[2] Rortais A, Arnold G, Halm M-P, Touffet-Briens F (2005) Modes of honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36: 71–83

Table 9.6-4: Assessment of the risk for adult bee due to the use of EF-253 in Group E (grassland, max application rate 200 g a.e/ha)

Intended use **Active substance** **Application rate (kg/ha)**	Grassland Clopyralid 1 × 0.200			
NOEDD (µg/bee/day)	71.2			
Food item	**Consumption (kg/bee/day)**	**RUD (µg/kg/kg/ha)**	**Dietary dose (µg/bee/day)**	**TER criterion: TER ≥1**
Nectar	427×10^{-6}	2.9×10^{3}	0.24747	288

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. Therefore, an acceptable risk to chronic adult and bee larval development is concluded.

Risk assessment according to the draft EFSA bee guidance document (EFSA, 2013)

Table 9.6-5: Screening assessment for the risk for bees due to the use of EF-243 in Group E (grassland, max application rate 200 g a.e/ha)

Active substance **Application rate (g/ha)**	Clopyralid 1 × 200		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**HQ criterion: HQ ≤ 42**
Honeybee acute adult contact toxicity	~~>100~~ >98.1	200	< 2
Test design	**LD_{50} (lab.) (µg/bumblebee)**	**Single application rate (g/ha)**	**HQ criterion: HQ ≤ 7**
Bumblebee acute adult contact toxicity	>200	200	< 1
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**ETR criterion: ETR ≤ 0.2**
Honeybee acute adult oral toxicity	>100 ~~>98.1~~	200	< 0.02
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**ETR criterion: ETR ≤ 0.036**
Bumblebee acute adult oral toxicity	>203	200	< 0.01
Test design	**LDD_{50} (lab.) (µg/bee/day)**	**Single application rate (g/ha)**	**ETR criterion: ETR ≤ 0.03**
Honeybee adult chronic toxicity	>71.2	200	< 0.021
Test design	**NOEC (lab.) (µg/larvae)**	**Single application rate (g/ha)**	**ETR criterion: ETR ≤ 0.2**
Honeybee larvae chronic toxicity	12.5	200	0.07

HQ: Hazard quotients for contact exposure. HQ values shown in bold breach the relevant trigger.
ETR: Exposure toxicity ratio. ETR values shown in bold breach the relevant trigger

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

Not relevant.

9.6.3 Effects on bumble bees

A study assessing the acute oral and contact toxicity of clopyralid to bumblebees was recently completed and is submitted for completeness. The study is listed in Appendix 1 and summarised in Appendix 2 (under acute honeybee)

9.6.4 Effects on solitary bees

No data available. There are no testing requirements for any bee other than the honey bee within the current implemented Regulation (EC) No. 1107/2009. Furthermore, there is currently no harmonized and ring tested test guideline available in Europe to assess the acute toxicity to solitary bees. There have been attempts within the ICPPR non-Apis group to develop a method with *Osmia* spp. but there is insufficient progress within the European bee testing community to provide some experimental evidence on the acute toxicity to solitary bees.

9.6.5 Overall conclusions

An acceptable risk to bees is expected from the proposed uses of EF-243 without the need of any risk mitigation.

Review 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 specific requirements of the Regulation (EU) 546/2011 regarding effects on bee brood development and possible chronic effects on adults were included by the Applicant.

Provided the risk assessment according to the new bee guidance "EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp.) and solitary bees", EFSA Journal 2013; 11(7):3295. For chronic oral exposure of adult bees and honeybee larvae an acceptable risk was indicated in the screening step. Also results of chronic oral exposure of adult bees and honeybee larvae risk assessment according to the modified EPPO 2010 approach as suggested by ECPA (2017) indicated acceptable risk for honeybee larvae and adult bees for the highest proposed application rate.

The acute and long term risk to honey bees (adult and larvae) and to bumble bees is acceptable when following the proposed GAP of Lontrel 300 SL (EF-243).

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

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

Effects on non-target arthropods of EF-243 were not evaluated as part of the EU assessment of clopyralid. Data is available for EF-1136. Following dilution with water within the spray tank, immediately prior to application, all formulations are equivalent. Therefore, all will have equivalent toxicological properties to arthropods and thus additional testing with EF-243 is not necessary for the purposes of risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	Lontrel 100 (EF-1136)	Laboratory test glass plates (2D)	LR_{50} = >200 g a.s./ha	EFSA Conclusion (Sankanu A./ 2000/DAS report no. GHE-P-8725)
Aphidius rhopalosiphi (adults)	Lontrel 100 (EF-1136)	Laboratory test glass plates (2D)	LR_{50} > 200 g a.s./ha	EFSA Conclusion (Sankanu A./ 2000/DAS report no. GHE-P-8416)
Aphidius rhopalosiphi (adults)	Lontrel 100 (EF-1136)	Laboratory test glass plates (2D)	LR_{50} > 300 g a.s./ha	EFSA Conclusion (Halsall, N./2005/ DAS report no. 050171)
Field or semi-field tests				
Not relevant				

EFSA Conclusion: EFSA Journal 2018;16(6):5389

9.7.1.1 Justification for new endpoints

Not relevant

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 maximum application rate for Group E (i.e. 200 g ae/ha) has been used also covers the risk for non-target arthropods from all other intended uses in groups A-E and G (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 EF-243 Group F (max application rate 200 g a.e/ha)

Intended use	Grassland		
Active substance/product	Clopyralid		
Application rate (g/ha)	1 × 200		
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	>200	200	<1
Aphidius rhopalosiphi	>200		<1

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

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

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum application rate for Group F (i.e. 200 g ae/ha) has been used also covers the risk for non-target arthropods from all other intended uses in groups A-F and G (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of EF-243 in Group F (max application rate 200 g a.e/ha)

Intended use	Grassland				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 × 200				
MAF	1				
vdf	5 (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	>200	2.77 % (1 m)	11.08	10	<0.06
Aphidius rhopalosiphi	>200				<0.06

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

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

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

An acceptable risk for non-target arthropods is concluded for all the intended uses of EF-243 without the need of any mitigation.

Review comments:

The endpoints as proposed by the notifier are considered acceptable and are used in the risk assessment.

Risk for other arthropods species was assessed 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 ESCORT 2.

Risk assessment demonstrates an acceptable in-field and off-field risk to non-target arthropods from the proposed uses of Lontrel 300 SL (EF-243) with no need for risk mitigation measures.

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

9.8.1 Toxicity data

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

Effects on earthworms of EF-243 were not evaluated as part of the EU assessment of clopyralid. The DT_{90f} of clopyralid has a mean value of 38 days. Furthermore, the HQs for arthropods were less than 2. Due to the lack of effects and clopyralid not being persistent in soils, further data on other non-target soil organisms (meso- and macrofauna) are deemed unnecessary. However, for completeness data on other non-target soil organism (springtail and soil mite) has been generated for GF-1966. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**.
Following dilution with water within the spray tank, immediately prior to application, all LONTREL formulations are equivalent. Therefore, all will have equivalent toxicological properties to arthropods. Therefore, additional testing with EF-243 is not necessary for the purposes of risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Clopyralid as Lontrel	Mixed into substrate	NOEC = ~~≥ 2~~ 1.97 mg	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
	100 (EF-1136)	56 d, chronic 10 % peat content	a.s./kg dw	(Hayward, J. C./2001/DAS Study ID GHE-T-1135)
Folsomia candida	Clopyralid (as GF-1966)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC= 556 mg GF-1966/kg soil dw (400 mg a.e./kg soil dw)	Pavić, B./2020/DAS Study ID 201708
Hypoaspis aculeifer	Clopyralid (as GF-1966)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC= 556 mg GF-1966/kg soil dw (400 mg a.e./kg soil dw)	Pavić, B./2020/DAS Study ID 201709
Litter bag test				
The DT_{90f} values for clopyralid are < 365 days. Consequently, studies to determine effects on organic matter breakdown are not required.				
Field studies				
Not needed				

EFSA Conclusion: EFSA Journal 2018;16(6):5389

9.8.1.1 Justification for new endpoints

Not relevant.

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. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for clopyralid.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group C (onions; max application rate 120 g ae/ha 10% crop interception) also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in groups A-B and D to H (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 EF-243 in onions (Group C)

Intended use	Onions		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**

Clopyralid	~~≥2~~ 1.97	0.144	~~≥13.88~~ 13.68
Chronic effects on other soil macro- and mesofauna; springtail			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Clopyralid (as GF-1966)	400	0.144	2,777
Chronic effects on other soil macro- and mesofauna: soil mite			
Clopyralid (as GF-1966)	400	0.144	2,777

TER values shown in **bold** fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

An acceptable risk to earthworms and soil macro-organisms is expected from the proposed uses of EF-243 for all the intended uses without the need of any risk mitigation.

Review comments:

The predicted environmental concentrations (PECsoil) values were calculated considering the GAP of EF-243 (Lontrel 300) (see Part B Section 8). The highest PECsoil for onions, as a worst-case, was taken into account for the risk assessment because it covers the risk for all intended uses. This risk envelope approach is acceptable.

The risk assessment for earthworms was based on the EU agreed endpoint (EFSA Journal, 2018). For the risk assessment for other non-target soil organisms the endpoints from studies with formulation GF-1966 (expressed as clopyralid content) were used.

Earthworms
The long-term TER value for the clopyralid is above the trigger value of 5.

Other soil macro-organisms
The long-term TER value for the clopyralid is above the trigger value of 5.

Conclusion:
According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substances following use of EF-243 (Lontrel 300) in compliance with proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of EF-243 were not evaluated as part of the EU assessment of clopyralid. Data is available for EF-1136. Following dilution with water within the spray tank, immediately prior to application, all formulations are equivalent. Therefore, all will have equivalent toxicological properties to soil microorganisms and thus additional testing with EF-243 is not necessary for the purposes of risk assessment

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	clopyralid	56 d, aerobic standard soil according to OECD 216 and 217	Nitrate formation rate 209 mg/kg soil dw +2.88%	EFSA Conclusion (Schöbinger, U./2013/DAS report no. 130283)
C-mineralisation	clopyralid	56 d, aerobic standard soil according to OECD 216 and 217	CO_2 formation 209 mg/kg soil dw -18.2 %	EFSA Conclusion (Schöbinger, U./2013/DAS report no. 130283)
N-mineralisation	Lontrel 100 (EF-1136)	28 d, aerobic sandy soil	Nitrate formation rate 1500 ga.s./kg soil dw +18 %	EU DAR clopyralid (Hayward, J.C. & Morgan, A.J./2003/DAS report no. 031001)
C-mineralisation	Lontrel 100 (EF-1136)	28 d, aerobic sandy soil	CO_2 formation 1500 ga.s./kg soil dw -7.8 %	EU DAR clopyralid (Hayward, J.C. & Morgan, A.J./2003/DAS report no. 031001)

EFSA Conclusion: EFSA Journal 2018;16(6):5389

9.9.1.1 Justification for new endpoints

Not relevant

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 C (onions; max application rate 120 g ae/ha 10% crop interception) also covers the risk for soil microorganisms from all other intended uses in groups A-B and D to H (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of formulation in onions (Group C)

Intended use	Onions		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Clopyralid	209 (at 56 d)	0.144	yes
C-mineralisation			
Product/active substance	**Max. conc. With effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Clopyralid	209 (at 56d)	0.144	yes

9.9.3 Overall conclusions

An acceptable risk to soil micro-organisms is expected from the proposed uses of EF-243 without the need of any risk mitigation.

Review comments:

The risk assessment was performed in accordance with the: Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC" (Sanco/10329/2002 rev 2 final 17 October 2002r.) on the basis of the worst-case application scenario.

The predicted environmental concentrations (PECsoil) values were calculated considering the GAP of EF-243 (Lontrel 300) (see Part B Section 8). The highest PECsoil for onions, as a worst-case, was taken into account for the risk assessment, because it covers the risk for all intended uses. This risk envelope approach is acceptable.

For the assessment of risk to micro-organisms the UE agreed endpoint was used.

Conclusion:
Since no effects (> 25%) were seen at application rates far higher than the values of PECsoil for active substance, it can be concluded that application of EF-243 (Lontrel 300), according to the GAP, will not cause any detrimental effect to soil micro-organisms

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

9.10.1 Toxicity data

Effects on non-target terrestrial plants of EF-243 were not evaluated as part of the EU assessment of clopyralid. New data for GF-1966 is submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** summarised in **Błąd! Nie można odnaleźć źródła odwołania.**. The new studies for GF-1966 follow the OECD plant density recommendations and include the ER_{50} based on visual injury. Following dilution with water within the spray tank, immediately prior to application, all LONTREL formulations are equivalent. Therefore, additional testing with EF-243 is not necessary for the purposes of risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Soybean *(Glycine max)*$_d$	Clopyralid (as GF-1966)	28 d Seedling emergence	ER_{50} shoot dry weight = 25.64 g a.e./ha	Stead, A./2019/DAS Study ID 190288
Tomato (*Lycopersicon esculentum*)$_d$	Clopyralid (as GF-1966)	21 d Vegetative vigour	ER_{50} visual injury = 21.74 g a.e./ha	Davies, C./2019/DAS Study ID 190287
*Glycine max**	Clopyralid	21 d Seedling emergence	$ER_{50\ shoot\ weight}$ = 21.47 g a.s/ha	EFSA Conclusion 2018;16(7):5389
~~*Lactuca sativa**~~	~~Reference product GF-1374~~	~~21 d Seedling emergence~~	~~$ER_{50\ shoot\ weight}$ = 0.46 L product/ha~~	~~EFSA Conclusion 2018;16(7):5389~~
*Lactuca sativa**	Clopyralid	21 d Vegetative vigour	ER50 shoot weight = 33.78 g ai/ha	EFSA Conclusion 2018;16(7):5389

m: monocotyledonous; d: dicotyledonous
*EU agreed endpoints

9.10.1.1 Justification for new endpoints

Not relevant.

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 maximum application rates (i.e. 200 and 120 g ae/ha) also covers the risk for non-target terrestrial plants from all other intended application rates.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of EF-243 in Group F (max application rate 200 g ae/ha)

Intended use	Group F (established grassland >1 year old)
Active substance/product	Clopyralid
Application rate (g/ha)	1× 200 g a.s./ha (0.67 L product/ha)
MAF	N/A

Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Soybean *(Glycine max)* **Seedling emergence**	25.64 g a.e./ha	2.77% (1m)	5.54	**4.63**
*Glycine max** **Seedling emergence**	21.47 g a.s/ha	2.77%	5.54	**3.88**
~~*Lactuca sativa** **Seedling emergence~~	~~0.46 L product/ha~~	~~2.77%~~	~~0.01856~~	~~24.78~~
Tomato (*Lycopersicon esculentum*) **Vegetative vigour**	21.74 g a.e./ha	2.77% (1m)	5.54	**3.92**
*Lactuca sativa** **Vegetative vigour**	33.78 g ai/ha	2.77%	5.54	6.10

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Risk assessment based on the EU agreed endpoints

Table 9.10-3: Assessment of the risk for non-target plants due to the use of EF-243 (max application rate 120 g ae/ha)

Intended use	Groups A-E and G
Active substance/product	Clopyralid
Application rate (g/ha)	1× 120 g a.s./ha (0.4 L product/ha)
MAF	N/A

Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Soybean *(Glycine max)* **Seedling emergence**	25.64 g a.e./ha	2.77% (1m)	3.324	7.71
Glycine max * **Seedling emergence**	21.47 g a.s/ha	2.77%	3.324	6.46

~~*Lactuca sativa* * **Seedling emergence**~~	~~0.46 L product/ha~~	~~2.77%~~	~~0.01108~~	~~41.52~~
Tomato (*Lycopersicon esculentum*) **Vegetative vigour**	21.74 g a.e./ha	2.77% (1m)	3.324	6.54
*Lactuca sativa** **Vegetative vigour**	33.78 g ai/ha	2.77	3.324	10.16

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Risk assessment based on the EU agreed endpoints

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-4: Risk assessment for non-target terrestrial plants due to the use of EF-243 in established grassland (Group F, max application rate 200 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use **Active substance/product** **Application rate (g/ha)** **MAF**		Group F (established grassland >1 year old) Clopyralid 1 × 200 N/A			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	5.54	2.77	1.385	0.554
5	0.57	1.14	0.57	0.285	0.114
Toxicity value Vegetative vigour Tomato (*Lycopersicon esculentum*) ER_{50} = 21.74 g/ha		**TER** **criterion: TER ≥ 5**			
1		**3.92**	7.85	15.70	39.24
5		19.07	38.14	76.28	190.70
Toxicity value **Seedling emergence** *Glycine max**					

ER_{50} = 21.47 g a.s/ha				
1	**3.88**	7.75	-	-
5	19.07	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.
*Risk assessment based on the worst-case endpoint

9.10.3 Overall conclusions

An acceptable risk to non-target plants is expected from the proposed uses A-E and G (max application rate 120 g ae/ha) of EF-243 without the need of any risk mitigation measurement.
For Group F (established grassland >1 year; max application rate 200 g ae/ha) an acceptable risk to terrestrial non-target plants can be anticipated following uses of EF-243 with the following mitigation measurements:

- 1 m distance to the treated field when 50% drift reducing nozzles are used during terrestrial applications or
- 5 m distance to the treated field without drift reducing nozzles

Review comments:

The risk assessment was performed in accordance with the: Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC" (Sanco/10329/2002 rev 2 final 17 October 2002r.) on the basis of the worst-case application scenario.

For the risk assessment the EU agreed endpoints and formulation endpoints were used.
The TER is above the trigger value of 5 for the proposed uses A-E and G (max application rate 120 g ae/ha) without the need of any risk mitigation measurement.

In order to reduce the off-field exposure, risk mitigation measures was implemented based on the lowest EU agreed endpoint ER50 = 21.47 g a.s/ha and PER off -field for max. application rate = 1× 200 g a.s./ha (0.67 L product/ha).
The TER is above the trigger value of 5 when 5 m buffer zone or 50 % drift reducing nozzles are applied for the proposed uses F (max application rate 200 g ae/ha).

Conclusion:
No unacceptable risk to non-target terrestrial plants is expected following the application of EF-243 (Lontrel 300)

- for groups A-E and G (max application rate 120 g ae/ha) without the need of any risk mitigation measurement,
- for group F (max application rate 200 g ae/ha) with 5 m buffer zone or 50 % drift reducing nozzles are applied.

According to the CZ comments the endpoint for Lactuca sativa ER50= 0.46 L product GF-1374/ha expressed as product/ha is not possible used for risk assessment for product EF-243. Therefore the risk assessment based on this endpoint was omitted.
However, since this endpoint is not a driver in the risk assessment, the conclusions remain un-changed.

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

No effects on other terrestrial organisms are anticipated if the previously proposed risk mitigations are implemented during applications of EF-243 in all the intended uses.

9.12 Monitoring data (KCP 10.8)

Monitoring studies are not available for clopyralid and EF-1136 and are not considered necessary in light of the acceptable risk concluded for all non-target organisms from uses of EF-243 in all the intended uses at rates up to 200 g clopyralid ae/ha.

9.13 Classification and Labelling

A 1.1 CLP Classification

Hazard symbol(s)

GHS09

Signal word Warning

Hazard statement(s)

Chronic aquatic Cat 1	H410	Very toxic to aquatic life with long lasting effects.

Precautionary statement(s)

P391	Collect spillage.
P501	Dispose of contents/container in accordance with applicable regulations.

EU specific statement(s)

EUH401	To avoid risks to human health and the environment, comply with the instructions for use.

See Part C for full explanation.

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**	**Verte-brate study Y/N**	**Owner**
KCP 10.1.1	[redacted]	2020	EF-243: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure [redacted] GLP Unpublished	Y	Corteva Agriscience
KCP 10.2	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L., Fierman, L.A.	2020	EF-243: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*) DAS Study ID 200843 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L	2020	GF-2895: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*) DAS Study ID 191747 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Banman, C. S. and S. Moore	2015	GF-1966: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*. DAS Study ID 150051	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			SynTech Research Laboratory Services GLP Unpublished		
KCP 10.2	Gonsior G.	2018	GF-2895: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System DAS Study ID 170354 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Corteva Agriscience
KCP 10.2	[redacted]	2020	EF-243: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) [redacted] GLP Unpublished	Y	Corteva Agriscience
KCP 10.2	Ross, T. L., Zhao, E., Zhang, L., Schneider, S.Z.;	2020	EF-243: A 48-Hour Static Acute Toxicity Test With the Cladoceran (*Daphnia magna*) DAS Study ID 200842 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCA 8.3.1	Tänzler, V., Kowalczyk, F.	2019	Clopyralid: Effects (Acute Contact and Oral) on Bumblebees (*Bombus terrestris* L.) in the Laboratory DAS Study ID 190300 ibacon GmbH GLP Unpublished	N	Corteva Agriscience
KCP 10/4	Pavić, B.	2020	GF-1966: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil DAS Study ID 201708 ibacon GmbH GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 10/4	Pavić, B.	2020	GF-1966: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil DAS Study ID 201709 ibacon GmbH GLP Unpublished	N	Corteva Agriscience
KCP 10.6	Stead, A.	2019	GF-1966: Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants DAS Study ID 190288 Stockbridge Technology Centre Ltd GLP Unpublished	N	Corteva Agriscience
KCP 10.6	Davies, C.	2019	GF-1966: Vegetative Vigour Test Terrestrial Non Target Plants DAS Study ID 190287 Stockbridge Technology Centre Ltd GLP Unpublished	N	Corteva Agriscience

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

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 10.1.1	[redacted]	1980	Acute Oral LD_{50} – Mallard Duck – [redacted]	Y	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			Wildlife Internation Ltd. GLP Unpublished		
KCP 10.1.1	[redacted]	1985	Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (*Anas platyrhynchos*) - Final Report. [redacted] GLP Unpublished	Y	Corteva Agriscience
KCP 10.2	Aufderheide, J.	2014	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa* DAS Study ID 140515 ABC Laboratories, Inc. GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Banman, C. S., Moore, S	2015	Clopyralid: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum* DAS Study ID 140735 SynTech Research Laboratory Services LLC GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Barrett, K	2001	Clopyralid Technical Toxicity to the Sediment Dwelling Phase of the Midge *Chironomus riparius* DAS Study ID GHE-T-1122 Huntingdon Research Centre Ltd. GLP Unpublished	N	Corteva Agriscience
KCP 10.2	[redacted]	1989	Lontrel 100: Determination of acute toxicity (LC_{50}) to rainbow trout (96h, static). [redacted]	Y	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
	Wright, J.G.		GLP Unpublished		
KCP 10.2	Caley, C.Y., Cameron, B.D., Chapleo, S. & Wright, J.G.	1989	Lontrel 100: Determination of acute toxicity (LC_{50}) to Daphnia (48h, static). DAS Study ID IRI 140464 & IRI 140731 Inveresk Research International GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Caley, C.Y., Cameron, B.D., Chapleo, S. & Wright, J.G.	1990	Lontrel 100: Daphnia reproduction test (20 day, semi-static) DAS Study ID IRI 140553 Inveresk Research International GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Caley, C.Y., Cameron, B.D. & Chapleo, S	1989	Lontrel 100: Alga, growth inhibition test (72h EC_{50}). DAS Study ID IRI 140490 & IRI 140731 Inveresk Research International GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Cowgill, U. M. ; Milazzo, D. P. ; Potter, R. B.	1990	The Fourteen Day Toxicity of Lontrel T to *Lemna gibba* L G-3 (Duckweed) DAS Study ID ES-2243 Toxicology & Environmental Research and Consulting Laboratory (TERC) GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Kirk, H. D.; Gilles, M. M.; McClymont, E. L. ; McFadden, L.G.,	2000	Clopyralid: Growth Inhibition Test with the Freshwater Green Alga, *Selenastrum capricornutum* Printz DAS Study ID 001040 Toxicology & Environmental Research and Consulting Laboratory (TERC) GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 10.2	[redacted]	2000	Clopyralid: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Walbaum [redacted] GLP Unpublished	Y	Corteva Agriscience
KCP 10.2	[redacted]	2000	Clopyralid: Toxicity to the Early Life Stages of the Fathead Minnow, *Pimephales Promelas* Rafinesque. [redacted] GLP Unpublished	Y	Corteva Agriscience
KCP 10.2	Marino, T. A. ; McClymont, E. L. ; Staley, J. L.,	2000	Clopyralid: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus DAS Study ID 001025 Toxicology & Environmental Research and Consulting Laboratory (TERC) GLP Unpublished	N	Corteva Agriscience
KCP 10.2	Douglas, M. T. ; Bell, G. ; Macdonald, I. A.	1992	An Assessment of the Effects of Lontrel T on the Reproduction of *Daphnia magna* DAS Study ID DWC 615/911087 Huntingdon Research Centre Ltd. GLP Unpublished	N	Corteva Agriscience
KCP 10.3.1	Leonard, J. and Moore, S.	2017	Clopyralid: A laboratory study to determine the chronic oral toxicity to the adult worker honey bee *Apis mellifera* L. (Hymenoptera: Apidae) DAS Study ID 170098 SynTech Research, LLC GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 10.3.1	Leonard, J. and Moore, S.	2017	Clopyralid: A repeated-exposure laboratory toxicity study in larvae, pupae and emergent adults of the honey bee *Apis mellifera* Linnaeus. (Hymenoptera: Apidae) DAS Study ID 170099 SynTech Research, LLC GLP Unpublished	N	Corteva Agriscience
KCP 10.3.1	Wainwright, M.	2001	Clopyralid Technical Acute Toxicity To Honey Bees DAS Study ID GHE-T-1091 Huntingdon Life Sciences Ltd GLP Unpublished	N	Corteva Agriscience
KCP 10.3.1	Wainwright, M.	2001	EF-1136: Acute toxicity to honey bees (*Apis mellifera*). DAS Study ID DOS 166/004732 Huntingdon Life Sciences Ltd GLP Unpublished	N	Corteva Agriscience
KCP 10.3.2	Halsall, N.	2005	A laboratory rate response test to determine the effects of EF-1136 on the parasitic wasp, *Aphidius rhopalosiphi* DAS Study ID 050171 Insect Investigations Services GLP Unpublished	N	Corteva Agriscience
KCP 10.3.2	Sankanu A.	2000	A laboratory study to evaluate the effects of clopyralid (EF-1136, an SL formulation containing 100 g/L clopyralid) on *Typhlodromus pyri* (Acari: Phytoseiidae). DAS Study ID GHE-P-8416 Ecotox Limited GLP	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			Unpublished		
KCP 10.3.2	Sankanu A.	2000	laboratory study to evaluate the effects of clopyralid (EF-1136, an SL formulation containing 100 g/L clopyralid) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). DAS Study ID GHE-P-8725 Ecotox Limited GLP Unpublished	N	Corteva Agriscience
KCP 10.4	Hayward, J. C.	2001	The Effects of EF-1136 on Reproduction and Growth in the Earthworm *Eisenia fetida* DAS Study ID GHE-T-1135 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 10.5	Hayward, J.C. & Morgan, A.J.	2003	EF-1136: Effects on Soil Microflora Activity. DAS Study ID 031001 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 10.5	Schöbinger, U.	2003	Clopyralid: Effects on the Activity of the Soil Microflora under Laboratory Conditions (Nitrogen and Carbon Transformation) DAS Study ID 130283 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Corteva Agriscience
KCP 10.6	Rockliff, C.	2013	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (based on OECD Guideline 208) – China, 2013 DAS Study ID 130095 Stockbridge Technology Centre Ltd	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			GLP Unpublished		
KCP 10.6	Rockliff, C.	2013	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Vegetative Vigour Test Terrestrial Non Target Plants (based on OECD Guideline 227) – China 2013 DAS Study ID 130094 Stockbridge Technology Centre Ltd GLP Unpublished	N	Corteva Agriscience

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**	**Verte-brate study Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.1 Acute oral toxicity bobwhite EF-243

Comments of zRMS:	The study was conducted to OECD guidelines 223 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.1.1.1
Report	[redacted] An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure ; [redacted] ; Unpublished
Guideline(s):	Yes (OECD TGD 223)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	EF-243
Purity:	34.5 wt% (394 g/L) clopyralid-olamine [26.2 wt% (300 g ae/L) clopyralid]
Description (physical state):	Liquid (dilutable concentrate)
Lot/batch no.:	F006H1K005(TSN400033)

Test System

Organism (*Species*):	Northern Bobwhite (*Colinus virginianus*)
Study type:	Acute oral
Study duration:	14 days
Parameters measured:	Mortality, body weight, feed consumption
Observation intervals:	Multiple observations on Day 0 and twice daily observations on remaining days.
Age range of birds at test initiation:	47 weeks
Weight range of birds at study initiation:	195-231 grams
Test concentrations:	0 and 2000 mg/kg body weight
No. of feed withholding days before dosing:	17.3 hours
Method of test item administration:	Oral gavage
Diet:	Eurofins-Easton Basal Diet
Number of birds per dose group:	5
Number of birds per control group:	5
Housing:	GQF Manufacturing Co. Model No. 0315
Environmental conditions:	Temperature: Mean 21.2 °C (range 20.3-22.8 °C) Photoperiod: 8 h light: 16 h dark Humidity: Mean 45% (range 23-62%)

Methodology

This test was conducted according to the sequential design OECD test guideline 223. A limit test was conducted with a dosage of 2000 mg/kg.

Birds were acclimated to the facility for 27 weeks and to the caging for 6 weeks prior to initiation of the test. The birds were fasted for approximately 17.3 hours prior to dosing. At experimental start, a single dose of the test substance was orally administered directly into the crop or proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of EF-243 per kilogram of body weight (mg/kg). The control birds received a volume of reverse osmosis deionized water equal to the volume of test substance used to dose the treatment birds. All birds were dosed at a volume of 1.75 mL/kg of body weight.

Following dosing, the birds were observed continuously for at least two hours, with particular attention being paid for signs of regurgitation. Following this period, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behavior. Body weights were measured individually on the day of dosing (Day 0) and on Days 3, 7, and 14 of each stage. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to 1, Day 1 to 2, and Day 2 to 3. Average daily feed consumption was then determined from Days 3 to 7, and Days 7 to 14.

RESULTS AND DISCUSSION

There were no mortalities, no effects on bodyweight and no effects on feed consumption in the control group or at the 2000 mg/kg dosage level. No regurgitation was noted among the control birds or

among any of the treatment birds. All control and treatment birds were normal in appearance and behavior throughout the test. The acute oral LD_{50} value for northern bobwhite exposed to EF-243 as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest level tested.

Table 5: Effect of EF-243 on mortality of Northern Bobwhite

Treatment (mg/kg bw)	No. of birds	Cumulative mortality		
		At day 7	At day 14	Total (%)
Negative control	5	0	0	0
2000	5	0	0	0
LD_{50}	> 2000 mg /kg			
95% C.I.	not determined			
NOEL	2000 mg/kg			

Table 6: Effect of EF-243 on body weight and feed consumption of Northern Bobwhite

Treatment (mg/kg bw)		Observation								
		Mean body weight (g)				Feed consumption (g/bird/day)				
	Day(s)	0	3	7	14	0-1	1-2	2-3	3-7	7-14
Negative control	Mean	210	212	210	213	17	18	14	19	17
	SD	5	6	6	7	5	3	4	9	4
2000	Mean	214	217	218	219	16	15	14	18	13
	SD	5	6	5	6	3	3	3	6	4

CONCLUSION

The acute oral LD_{50} value for northern bobwhite exposed to EF-243 as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest level tested. The no-mortality and no-observed-effect level (NOEL) was 2000 mg/kg body weight.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Bobwhite quail	*Colinus virginianus*	EF-243	14 day	LD_{50}	>2000	mg/kg bw

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No new or additional studies have been submitted

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No new or additional studies have been submitted

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No new or additional studies have been submitted

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

No new or additional studies have been 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 Green algae study with EF-243

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. The pH in the negative control increased by 2 units over the course of the study, exceeding the OECD 201 recommended maximum of 1.5 units. This deviation was not considered to be detrimental to the results of the study or their interpretation. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/1
Report	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L., Fierman, L.A.; 2020; EF-243: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*); Eurofins EAG Agroscience, LLC, Easton, Maryland, USA; Lab Study No. 379P-176; DAS Study No. 200843 ; Unpublished
Guideline(s):	Yes (OECD TGD 201)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	EF-243
Purity:	34.5% clopyralid-olamine (26.2 wt% a.e. clopyralid)
Description (physical state):	Liquid (SC)
Lot/batch no.:	F006H1K005 (TSN400033)

Test System

Organism (*Species*):	Freshwater Alga (*Raphidocelis subcapitata*)
Study type:	Laboratory study assessing algal growth
Study design:	Static
Test concentrations:	Nominal: 0 (control) 6.25, 12.5, 25, 50 and 100 mg EF-243/L, equivalent to 1.64, 3.28, 6.55, 13.1, and 26.2 mg a.e./L Mean measured: <LOD (control), 6.56, 13.2, 26.5, 52.7, and 106 mg EF-243/L, equivalent to 1.72, 3.47, 6.94, 13.8, and 27.8 mg clopyralid a.e./L
Duration:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 7.4 to 9.4 Test solution temperature (range): 24.7 to 25.0°C Temperature (range): 24 ± 2ºC (SD) Photoperiod: Continuous Light intensity (range): 5,550 to 6,430
Observation intervals:	0, 24, 48, 72 hours
Age of inoculum:	4 days
Acclimation period/conditions:	Algal cells used in this test were obtained from Eurofins cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. Algal cells for this study were taken from a culture that had been transferred to fresh medium four days prior to test initiation.
Initial cell density:	10,000 cells/mL

Growth medium:	Name: Freshwater AAP medium pH at test initiation: 7.5 pH at test termination: 9.5 Constant stirring?: Continuously shaken on a mechanical shaker at 100 rpm.
Method of test item added to the test medium:	A primary stock solution, which also served as the highest test concentration, was prepared at a nominal concentration of 100 mg/L by dissolving 0.1000 g of EF-243 in 1000 mL of freshwater AAP medium. The primary stock was mixed by inversion at least twenty times, sonicated approximately 15 minutes, and was stirred while subsequent dilutions were prepared. After mixing, the primary stock appeared clear and colorless with no visible surface slicks or particulates. Test solutions were prepared at nominal concentrations of 6.25, 12.5, 25, and 50 mg/L by diluting aliquots of the 100 mg/L stock with freshwater AAP medium. The negative control solution consisted of freshwater AAP medium without test substance added. All test solutions appeared clear and colorless, with no visible surface slicks or particulates.
No. of control replicates:	6
No. of test concentration replicates:	3
Analytical verification:	Method: measuring concentrations of clopyralid using HPLC-MS/MS Samples taken : 0 and 72 hrs Limit of Detection: 0.0495 mg a.e./L clopyralid (equivalent to 0.189 mg EF-243/L) Limit of Quantitation: 0.165 mg a.e./L (equivalent to 0.630 mg EF-243/L) Recoveries from QC fortifications: 101 to 105 (range)
Reference substance:	Zinc chloride (conducted as a separate non-GLP study)

Methodology

Test chambers were sterile, 250-mL glass Erlenmeyer flasks plugged with sterile foam stoppers and contained 100 mL of test or control medium. At test initiation, the test solutions were inoculated with *Raphidocelis subcapitata* to achieve a theoretical initial cell density of 10,000 cells/mL. Test chambers were held in an environmental chamber at a temperature of 24 ± 2ºC and under continuous cool-white fluorescent light. Test solution pH was measured at test initiation and test termination. Test solution samples were collected at approximately 24, 48, and 72hours from each replicate of the treatment and control groups for the determination of algal cell densities. Cell counts were performed using an electronic particle counter (Beckman Coulter Z2 Series). Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of clopyralid. The samples were diluted with freshwater AAP medium, as necessary, into the range of the calibration curve and analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS). ECx estimates were calculated using non-linear regression and NOEC values were determined based on the results of statistical comparisons between treatment and control data (Dunnett's test, $\alpha = 0.05$).

RESULTS AND DISCUSSION

EF-243 was not detected in samples collected from the negative control at any sampling interval. Measured concentrations of clopyralid in samples collected from the EF-243 treatment groups at test initiation (0 hour) ranged from 105 to 107% of nominal. Measured concentrations of clopyralid in samples collected from the EF-243 treatment groups at test termination (72 hours) ranged from 104 to

105% of nominal. Therefore, the biological results were reported based on nominal test concentrations.

After 72 hours of exposure, inhibition of cell density in the nominal 6.25, 12.5, 25, 50 and 100 mg EF-243/L treatment groups was 6, 9, 35, 73, and 95%, respectively, relative to the negative control. After 72 hours of exposure, inhibition of growth rate in the nominal 6.25, 12.5, 25, 50 and 100 mg EF-243/L treatment groups was 1, 2, 7, 22, and 51%, respectively, relative to the negative control. Inhibition of yield in the nominal 6.25, 12.5, 25, 50 and 100 mg EF-243/L treatment groups was 6, 9, 35, 73, and 95%, respectively, relative to the negative control. Mean cell density, mean growth rate, and mean yield were significantly reduced in the nominal 25, 50, and 100 mg EF-243/L treatment groups when compared to the negative control. The 72-hour NOEC was determined to be 12.5 mg EF-243/L, based on statistically significant reductions in yield and growth rate. Based on nominal EF-243 concentrations, the 72-hour E_rC_{10}, E_rC_{20} and E_rC_{50} values were determined to be 31.2, 46.4 and 98.8 mg EF-243/L, respectively; and the 72-hour E_yC_{10}, E_yC_{20} and E_yC_{50} values were determined to be 14.1, 18.9 and 33.2 mg EF-243/L, respectively.

All validity criteria for the study were met: 1) Control cell density must increase by a factor of at least 16 within the 72-hour test period (actual factor was 364); 2) The mean coefficient of variation for section-by-section specific growth rates in the control must be ≤ 35% (it was 15.6%); and 3) The coefficient of variation of average specific growth rates in the control during the whole test period must be ≤ 7% (it was 1.68%).

Table 7: Mean cell density

Nominal EF-243 Concentration (mg/L)	Mean cell density (cells/mL)	% inhibition
	72 h	72 h
Negative Control	3,637,908	--
6.25	3,427,287	6
12.5	3,315,461	9
25	2,378,500*	35
50	983,479*	73
100	182,018*	95

* Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the negative control mean.

Table 2: Mean growth rate and yield

Nominal EF-243 Concentration (mg/L)	Mean growth rate (cells/ml/h)	% inhibition	Mean yield (cells/ml)	% inhibition
	0-72 h	72 h	72 h	72 h
Negative Control	0.0818	--	3,627,908	--
6.25	0.0810	1	3,417,287	6
12.5	0.0806	2	3,305,461	9
25	0.0759*	7	2,368,500*	35
50	0.0636*	22	973,479*	73
100	0.0402*	51	172,018*	95

* Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the negative control mean.

Table 3: Effects of EF-243 on algal growth based on nominal concentrations

Hour	EC Type	EC Value [mg EF-243/L]	95% Confidence Limits [mg EF-243/L]	NOEC [mg EF-243/L]
72	E_rC_{10}	31.2	28.0 to 34.8	12.5
	E_rC_{20}	46.4	43.1 to 50.0	
	E_rC_{50}	98.8	94.9 to >100	
	E_yC_{10}	14.1	11.2 to 17.8	12.5
	E_yC_{20}	18.9	15.7 to 22.9	
	E_yC_{50}	33.2	29.6 to 37.3	

CONCLUSION

The freshwater alga, *Raphidocelis subcapitata*, was exposed to a geometric series of five treatment levels of EF-243 ranging from 6.25 to 100 mg EF-243/L. All the study validity criteria were met. Based on nominal EF-243 concentrations: the 72-hour NOEC value for growth and yield was determined to be 12.5 mg EF-243/L; the 72-hour E_rC_{10}, E_rC_{20} and E_rC_{50} values were determined to be 31.2, 46.4 and 98.8 mg EF-243/L, respectively; and the 72-hour E_yC_{10}, E_yC_{20} and E_yC_{50} values were determined to be 14.1, 18.9 and 33.2 mg EF-243/L, respectively. The 72-hour E_rC_{50} and E_yC_{50} values were equivalent to 25.9 and 8.7 mg a.e./L, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Freshwater alga	*Raphidocelis subcapitata*	EF-243	72-hr	E_yC_{50}	33.2	mg/L
Freshwater alga	*Raphidocelis subcapitata*	EF-243	72-hr	E_rC_{50}	98.8	mg/L

A 2.2.1.2 Green algae study with GF-2895

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/2
Report	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L.; 2020; GF-2895: A 72-Hour Toxicity Test with the Freshwater Alga (Raphidocelis subcapitata); Eurofins EAG Agroscience, LLC, Easton, MD, USA; Lab Study No.

	379P-168; DAS Study No. 191747; Unpublished
Guideline(s):	Yes (OECD TGD 201)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-2895
Purity:	48.4% w/w clopyralid acid equivalent (602 g/L)
Description (physical state):	Liquid (end use product)
Lot/batch no.:	F811H1K011 [TSN316252]

Test System

Organism (*Species*):	Freshwater alga (*Raphidocelis subcapitata*)
Study type:	Laboratory study assessing algal growth
Study design:	Static
Test concentrations:	Nominal: 6.3, 13, 25, 50, and 100 mg GF-2895/L Nominal active: 3.05, 6.29, 12.1, 24.2, 48.4 mg a.e./L
Duration:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 7.2 to 9.4 Temperature (range): 24.0 to 24.2 °C Photoperiod: continuous cool-white fluorescent light Light intensity (range): 5,590 to 5,880
Observation intervals:	0, 24, 48, 72 hours
Age of inoculum:	3 days
Acclimation period/conditions:	Cultured under similar conditions as the definitive test
Initial cell density:	10,000 cells/mL
Growth medium:	Name: freshwater AAP medium pH at test initiation: 7.3 pH at test termination: 8.4 Constant stirring?: test vessels shaken at 100 rpm
Method of test item added to the test medium:	A primary stock solution, that also served as the highest test concentration, was prepared at a nominal concentration of 100 mg/L by dissolving 0.1000 g of GF-2895 in 1000 mL of freshwater AAP medium. The primary stock was mixed by inversion at least twenty times and was stirred while subsequent dilutions were prepared. After mixing, the primary stock appeared clear and colorless with no visible surface slicks. A few particulates were observed in the primary stock. Test solutions were prepared at nominal concentrations of 6.3, 13, 25, and 50 mg/L by diluting aliquots of the 100 mg/L stock with freshwater AAP medium. The negative control solution consisted of freshwater AAP medium without test substance added. All test solutions appeared clear and colorless, with no visible surface slicks, however, a few particulates were observed in all treatment levels.

No. of control replicates:	6
No. of test concentration replicates:	3
Analytical verification:	Method: measuring concentrations of clopyralid acid using HPLC-UV Samples taken : 0 and 72 hrs Limit of Detection: 0.930 mg GF-2895/L (0.450 mg a.e./L) Limit of Quantitation: 3.10 mg GF-2895/L (1.50 mg a.e./L) Recoveries from QC fortifications: 100 to 103%
Reference substance:	Clopyralid analytical standard; lot YC2-106153-68

Methodology

Test chambers were sterile, 250-mL glass Erlenmeyer flasks plugged with sterile foam stoppers, and contained 100 mL of test or control medium. At test initiation, the test solutions were inoculated with *Raphidocelis subcapitata* to achieve a theoretical initial cell density of 10,000 cells/mL. Test chambers were held in an environmental chamber at a temperature of 24 ± 2ºC and under continuous cool-white fluorescent light. Test solution pH was measured at test initiation and test termination. Test solution samples were collected at approximately 24, 48, and 72 hours from each replicate of the treatment and control groups for the determination of algal cell densities. Cell counts were performed using an electronic particle counter (Coulter Electronics, Inc.). Cell densities were used to calculate growth rates and yields which were subsequently used to calculate percent inhibition values relative to the negative control over the 72-hour exposure period. ErC_{50} and EyC_{50} values (i.e., the theoretical concentrations that would produce a 50% reduction in growth rate and yield, respectively) were calculated, when possible, at 72 hours of exposure. No-observed-effect-concentrations (NOEC) were determined at 72 hours through statistical evaluation of the growth rate and yield data, as well as examination of the concentration-response pattern. Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of clopyalid acid by LC-UV.

RESULTS AND DISCUSSION

Measured concentrations of non-centrifuged samples of GF-2895 ranged from 101 to 105% of nominal. Measured concentrations of Day 0 centrifuged samples of GF-2895 ranged from approximately 102 to 106% of nominal. The results of the centrifuged samples indicated that the particulates observed were not the active substance, clopyralid. On day 3 measured concentrations ranged from 101 to 103% of the nominal in the GF-2895 treatment groups. The results of the study were based on nominal GF-2895 concentrations as well as nominal active ingredient (clopyralid) concentrations. After 72 hours of exposure, inhibition of cell density in the nominal 6.3, 13, 25, 50 and 100 mg/L treatment groups was 4, 3, -2, 16, and 24%, respectively, relative to the negative control. After 72 hours of exposure, inhibition of growth rate in the nominal 6.3, 13, 25, 50 and 100 mg/L treatment groups was 1, 0, 0, 3, and 5%, respectively, relative to the negative control. Inhibition of yield in the nominal 6.3, 13, 25, 50 and 100 mg/L treatment groups was 4, 3, -2, 16, and 24%, respectively, relative to the negative control. Based on nominal GF-2895 concentrations, the 72-hour E_rC_{50}, EC_{50}, and E_yC_{50} values were determined to be >100 mg/L, for all endpoints. Mean growth rate was significantly reduced (Dunnett's Test; $p < 0.05$) in the nominal 50 and 100 mg/L treatment groups when compared to the negative control. Mean yield was significantly reduced (Dunnett's Test; $p < 0.05$) in the nominal 100 mg/L treatment group when compared to the negative control. The 72-hour NOEC was determined to be 25 mg/L, based on statistically significant reductions in growth rate.

Table 8: Mean cell density

Nominal GF-2895 Concentration (mg/L)	Mean cell density (cells/mL)	% inhibition
	72 h	72 h
Negative Control	2,796,667	--
6.3	2,696,667	4
13	2,713,333	3
25	2,846,667	-2
50	2,350,000	16
100	2,131,667*	24

* Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the negative control mean.

Table 9: Mean growth rate and yield

Nominal GF-2895 Concentration (mg/L)	Mean growth rate ($hour^{-1}$)	% inhibition	Mean yield (cells/mL)	% inhibition
	0-72 h	72 h	72 h	72 h
Negative Control	0.0782	--	2,786,667	--
6.3	0.0777	1	2,686,667	4
13	0.0778	0	2,703,333	3
25	0.0785	0	2,836,667	-2
50	0.0757*	3	2,340,000	16
100	0.0745*	5	2,121,667*	24

* Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the negative control mean.

Table 10: Effects of GF-2895 on algal growth based on nominal concentrations

Hour	EC Type	EC Value [mg GF-2895/L]	95% Confidence Limits [mg GF-2895/L]	NOEC [mg GF-2895/L]
72	E_rC_{10}	>100	n/a	25
	E_rC_{20}	>100	n/a	
	E_rC_{50}	>100	n/a	
	E_yC_{10}	46	20 to 106 [a]	50
	E_yC_{20}	82	52 to 128[a]	
	E_yC_{50}	>100	n/a	

[a] Extrapolated value. Upper confidence bound exceeds the maximum concentration tested.

CONCLUSION

The freshwater alga, *Raphidocelis subcapitata*, was exposed to a geometric series of five treatment levels of GF-2895 ranging from 6.3 to 100 mg/L (3.05 to 48.4 mg a.e./L), based on nominal, total formulation concentrations. All validity criteria were achieved for growth in the negative control replicates. Toxicity of GF-2895 to *R. subcapitata* was assessed based on effects on growth rate, cell density, and yield. The 72-hour NOEC value was determined to be 25 mg/L (12.1 mg a.e./L) and was based on statistically significant reductions in growth rate. Based on nominal GF-2895 concentrations, the 72-hour E_rC_{50}, EC_{50}, and E_yC_{50} values were all determined to be > 100 mg/L (>48.4 mg a.e./L).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Freshwater green algae	*Raphidocelis subcapitata*	GF-2895	72-hr	E_rC_{50}	>100	mg/L

A 2.2.1.3 Aquatic macrophyte study with GF-1966

Comments of zRMS:	The study was performed according to OECD TG 239 and principles of GLP. The validity criteria are met. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/3
Report	Banman, C. S. and S. Moore (2015): GF-1966: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*. SynTech Research Laboratory Services Study ID: 014SRLS15C01; DAS Study No. 150051; 01 July 2015; Unpublished
Guideline(s):	Yes (OECD TGD 239)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

ISO Common name:	
Test item (chemical/other name):	GF-1966 (clopyralid)
Purity:	97.5%
Description (physical state):	Liquid
Lot/batch no.:	1K211618A5
CAS no.:	1702-17-6

Test System

Organism (*Species*):	Aquatic plant, *Myriophyllum spicatum*
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (range): 8.3 to 9.8 Test solution temperature (range): 19.9 to 20.6°C Oxygen saturation (range): 9.3 to 13.2 mg/L
Environmental conditions:	Photoperiod: 16 hours light / 8 hours dark Light intensity (range): 11,260 to 11,910 lux Temperature (range): 19.9 to 20.6°C
Observation intervals:	Daily

Test concentrations:	Nominal: Control, 14.9, 47.7, 153, 488, 1563 and 5000 µg formulation/L Mean calculated concentrations: Control (<LOQ), 10.1, 29.8, 108, 311 and 935 µg a.i./L
Acclimation period/conditions:	16 hours light: 8 hours dark. 20.0 ± 5.0 °C.
Growth medium:	Name: Hard Processed Water (blended spring and R.O. water)
Method of test item added to the test medium:	Water stock prepared and stirred into treatment vessels
No. of control replicates:	10
No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	4 plants, thinned to 3 plants at the start of the exposure period
Analytical verification:	Method: measuring concentrations of clopyralid using LC-MS/MS Samples taken : 0 and 14days Limit of Detection: Not applicable Limit of Quantitation: 2.0 µg/L Recoveries from QC fortifications: 96 to 97%
Test substance renewal days:	None

Methodology

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions. Samples were analyzed for concentration of clopyralid. Shoots within a replicate were planted in sediment within a 300-mL borosilicate glass crystallization dish housed in a 2-L glass beaker. Parameters measured included growth rate and yield (NOEC, LOEC and EC_{50}) of total shoot lengths, total plant wet weight and total plant dry weight.

Results and discussions

Mean measured recoveries from day 0 and 14 ranged from 96 to 102% of the nominal concentrations. Samples were analyzed for clopyralid. The toxicity values were calculated based on nominal concentrations in units of µg formulation/L. Shoots and roots of all plants in the control vessels all treatment levels were observed to be normal throughout the study. The lowest ErC_{50} for growth rate in the 14-day exposure of the rooted aquatic macrophyte Myriophyllum spicatum to GF-1966 was obtained for dry weight. . The statistical NOE_rC, LOE_rC and E_rC_{50} for this endpoint were <14.9, 14.9 and 4039 µg formulation/L, respectively.

Table 11: Mean total shoot length including side shoots (cm)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	10.0	46.4	36.4	NA	0.1092	NA
14.9		36.1	26.1	28.2*	0.0914	16.3*
47.7		34.5	24.5	32.8*	0.0879	19.5*
153		32.2	22.2	39.0*	0.0833	23.7*
488		35.5	25.5	38.2*	0.0835	23.6*
1563		30.2	20.1	44.6*	0.0782	28.4*
5000		25.7	15.7	56.7*	0.0673	38.4*

* significantly different reduction compared to the pooled control
1) based on 15 additional plants, representative of those used in the test

Table 12: Mean total plant fresh weight (g)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.4302	1.7881	1.3579	NA	0.1015	NA
14.9		1.4131	0.9829	27.6*	0.0847	16.6*
47.7		1.4164	0.9862	27.4*	0.0848	16.5*
153		1.4321	1.0019	26.2*	0.0857	15.6*
488		1.3299	0.8997	33.7*	0.0802	21.0*
1563		1.3237	0.8935	34.2*	0.0793	21.9*
5000		1.0460	0.6158	54.7*	0.0632	37.8*

* significantly different reduction compared to the pooled control
1) based on 15 additional plants, representative of those used in the test

Table 13: Mean total plant dry weight (g)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.0487	0.1773	0.1287	NA	0.0920	NA
14.9		0.1349	0.0863	32.9*	0.0727	21.0*
47.7		0.1281	0.0795	38.2*	0.0691	24.9*
153		0.1174	0.0688	46.5*	0.0619	32.7*
488		0.1183	0.0696	45.9*	0.0619	32.7*
1563		0.1030	0.0543	57.8*	0.521	43.4*
5000		0.0919	0.0432	66.4*	0.447	51.5*

* significantly different reduction compared to the pooled control
1) based on 15 additional plants, representative of those used in the test

The calculated EC_{50} values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below.

Table 14: Summary of biological results (based on nominal concentrations - µg /L)

Parameter (µg/L)	Total shoot length		Total plant fresh weight		Total plant dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC_{50}	>5000	2620	>5000	3838	4039	715
95% Conf. Limits	NA	136 to 4268	NA	331 to NA	NA	NA to 2823
14-day NOEC	<14.9	<14.9	<14.9	<14.9	<14.9	<14.9
14-day LOEC	14.9	14.9	14.9	14.9	14.9	14.9

NA = could not be calculated

Conclusion

The lowest ErC_{50} for growth rate in the 14 day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to GF-1966 was obtained for dry weight. The statistical NOErC, LOErC and ErC_{50} for this endpoint were <14.9, 14.9 and 4039 µg formulation/L, respectively.

The lowest EyC_{50} for yield in the 14 day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to GF-1966 was obtained for dry weight. The statistical NOEyC, LOEyC and EyC_{50} for this endpoint were <14.9, 14.9 and 715 µg formulation/L, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Aquatic macrophyte	*Myriophyllum spicatum*	GF-1966	14 day	E_rC_{50}	4039	µg/L
Aquatic macrophyte	*Myriophyllum spicatum*	GF-1966	14 day	E_yC_{50}	715	µg/L

A 2.2.1.4 Aquatic macrophyte study with GF-2895

Comments of zRMS:	The study was performed according to OECD TG 239 and principles of GLP. The validity criteria are met. In deviation to the guideline recommendation which only evaluates the shoot biomass, the total plant biomass comprising roots and shoots was assessed. The same deviation was noted in the study with the new representative formulation GF1374 for clopyralid (dRAR Vol. 3 CP, June 2018, 10.2.1.3/6). Based on the RMS opinion this deviation was accepted since the same methodology was used in study with active substance and the endpoints are comparable. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/4
Report	Gonsior, G.; 2018; GF-2895: Growth Inhibition of Myriophyllum spicatum in a Water/Sediment System; Eurofins Agroscience Services Eco-Chem GmbH; Lab Study No. S17-01552; DAS Study No. 170354; Unpublished
Guideline(s):	Yes (OECD TGD 239)
Deviations:	None
GLP:	Yes

Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	GF-2895
Purity:	concentration of clopyralid-dimethylammonium salt in GF-2895: 61.7 % w/s (analysed)
Description (physical state):	liquid/ yellow
Lot/batch no.:	ENBK-147165-002 (TSN312900)

Test System

Organism (*Species*):	Aquatic plant, *Myriophyllum spicatum* L
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (mean + SD): 8.19 ± 0.67 Test solution temperature (mean + SD): 20.6 ± 0.3 °C Oxygen saturation (mean + SD): 119 ± 26 %
Environmental conditions:	Photoperiod: 16-h day-length Light intensity (range): 120 – 160 $\mu Em^{-2}s^{-1}$
Observation intervals:	0, 7 and 14 days
Test concentrations:	Nominal: 0.954, 3.05, 9.77, 31.3 and 100 mg test item /L
Acclimation period/conditions:	>14 days
Growth medium:	Smart and Barko medium
Method of test item added to the test medium:	Spiked water
No. of control replicates:	10
No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	1
Analytical verification:	Method: measuring concentrations of clopyralid using HPLC-MS/MS Samples taken : 0 and 14 days Limit of Detection: The limit of detection (LOD) was defined as 30 % of the limit of quantification Limit of Quantitation: 0.0450 mg/L of clopyralid. An LOQ of 0.100 mg/kg of clopyralid was confirmed in sediment. Recoveries from QC fortifications: (70-110 % mean recovery, ≤ 20 % RSD)
Test substance renewal days:	-

Methodology

Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-culture. On the day of application of the test item, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0.954, 3.05, 9.77, 31.3 and 100 mg GF-2895/L. Ten replicates were used for the control and five for each test item group. On day 0 fifteen additional plants, representative of those used in the test, were selected from

the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours. The weight of the dry plant samples was recorded. On day 14 plants were harvested from each treatment group for assessment of total plant fresh weight, total plant dry weight, shoot length and number and length of side shoots. Data were used to calculate the $EC_{10, 20, 50}$ values, and NOEC/LOEC values where possible for: growth rate and yield for total shoot length; growth rate and yield for total plant fresh weight; and growth rate and yield for total plant dry weight. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

RESULTS AND DISCUSSION

The measured concentration of the test item based on the clopyralid content in the test vessels at test start ranged between 108 and 115% of nominal in the overlaying water. The mean measured content for all concentrations at test start was 111 % of nominal for clopyralid. After 14 days clopyralid concentrations in the water were found at all concentration levels with recoveries between 106 and 112% of nominal. In the sediment, concentrations of clopyralid were found at 3.05, 9.77, 31.3 and 100 mg/L with recoveries between 4 and 6% of nominal. As the content of clopyralid for all concentration levels was > 80% and < 120% of nominal during the test, all toxicological endpoints were evaluated using nominal concentrations of the test item (GF-2895).

The mean control growth rate based on shoot length, fresh weight and dry weight was 0.1738, 0.1266 and 0.1049 /day respectively, which is equivalent to a mean doubling time of 4.0, 5.5 and 6.6 days respectively. The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight 4.6 %, 8.9 % and 11.6 % respectively.

The mean control yield (and C.V.) based on shoot length was 46.0 cm (C.V. = 12.7 %), for fresh weight yield was 0.9749 g (C.V. = 18.0 %), and for dry weight yield was 0.0756 g (C.V. = 23.3 %). Since the CV for fresh weight and shoot length yield was below 35 % and a doubling of shoot biomass and length was reached within the test duration the mean control growth rates and variability were considered acceptable.

Table 15: Mean total shoot length including side shoots (cm)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	4.4	50.4	46.0	-	0.1738	-
0.954	4.4	58.0	53.6	-16.5	0.1837	-5.7
3.05	4.4	54.2	49.8	-8.3	0.1779	-2.4
9.77	4.4	47.4	43.0	6.5	0.1674	3.7
31.3	4.4	44.1	39.7	13.7	0.1639	5.7
100	4.4	19.8	15.4*	66.5*	0.1052*	39.5*

* significantly different reduction compared to the control

1) based on 15 additional plants, representative of those used in the test

Table 16: Mean total plant fresh weight (g)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.1970	1.1719	0.9749	-	0.1266	-
0.954	0.1970	1.2840	1.0870	-11.5	0.1327	-4.8
3.05	0.1970	1.2610	1.0640	-9.1	0.1321	-4.3
9.77	0.1970	1.1735	0.9765	-0.2	0.1257	0.7
31.3	0.1970	1.0298	0.8328	14.6	0.1169	7.7
100	0.1970	0.3433	0.1463*	85.0*	0.0385*	69.6*

* significantly different reduction compared to the control
1) based on 15 additional plants, representative of those used in the test

Table 17: Mean total plant dry weight (g)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.0222	0.0978	0.0756	-	0.1049	-
0.954	0.0222	0.0997	0.0775	-2.5	0.1064	-1.4
3.05	0.0222	0.0994	0.0772	-2.1	0.1063	-1.3
9.77	0.0222	0.0937	0.0715	5.4	0.1013	3.4
31.3	0.0222	0.0844	0.0622	17.7	0.0946	9.8
100	0.0222	0.0450	0.0228*	69.8*	0.0488*	53.5*

* significantly different reduction compared to the control
1) based on 15 additional plants, representative of those used in the test

The calculated EC_{50} values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below.

Table 18: Summary of biological results (based on nominal or measured concentrations)

Parameter (mg/L)	Total shoot length		Fresh Weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC_{50}	> 100	71.6	73.1	56.3	97.6	64.5
95% Conf. Limits	n.d.	59.0 - 90.8	64.0 - 84.7	50.0 - 63.5	78.4 - 131	54.0 - 79.6
14-day NOEC	31.3	31.3	31.3	31.3	31.3	31.3
14-day LOEC	100	100	100	100	100	100

CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to GF-2895 for 14 days, the E_rC_{50} and E_yC_{50} values based on total shoot length were > 100 mg/L and 71.6 mg/L (nominal) respectively. The NOEC for growth rate and yield was 31.3 mg/L (nominal).

The E_rC_{50} and E_yC_{50} values based on biomass (fresh weight) were 73.1 mg/L and 56.3 mg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 31.3 mg/L (nominal).

The E_rC_{50} and E_yC_{50} values based on biomass (dry weight) were 97.6 mg/L and 64.5 mg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 31.3 mg/L (nominal).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Aquatic macrophyte	*Myriophyllum spicatum*	GF-2895	14 day	ErC_{50}(nominal)	73.1	mg/L
Aquatic macrophyte	*Myriophyllum spicatum*	GF-2895	14 day	EyC_{50}(nominal)	56.3	mg/L

A 2.2.1.5 Acute fish study EF-243

Comments of zRMS:	The study was performed according to OECD TG 203 and principles of GLP. The validity criteria are met. Two deviations in the pH measurement were noted. These deviations had no adverse impact upon the results or interpretation of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/5
Report	[redacted] A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*); [redacted]; Unpublished
Guideline(s):	Yes (OECD TGD 203)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	No

Test Item(s)

Test item (Common name):	EF-243
Purity:	34.5 wt%, 394 g/L clopyralid-olamine (26.2 wt%, 300 g a.e./L)
Description (physical state):	Solid
Lot/batch no.:	F006H1K005 (TSN400033)

Test System

Organism (*Species*):	Rainbow Trout (*Oncorhynchus mykiss*)
Study type:	Acute
Study design:	96-h static
Test concentrations:	Nominal: 0 (control), 23.8, 47.6, 95.3, 191 and 381 mg EF-243/L (equivalent to 6.25, 12.5, 25, 50, and 100 mg ae/L clopyralid, respectively) Mean measured: <LOD (control), 23.9, 48.0, 93.4, 187 and 381 mg EF-243/L (equivalent to 6.27, 12.6, 24.5, 49.1, and 100 mg ae/L clopyralid, respectively)
Parameters measured:	Mortality
Observation intervals:	5, 24, 48, 72, and 96 hours

Age, weight and length of fish at test termination:	Juveniles Mean wet weight: 0.37 g (range 0.24 to 0.54 g) Mean length: 3.9 cm (Range 3.6 to 4.2 cm)
Analytical confirmation of test concentrations:	On days: 0 (initiation) and 4 (termination)
No. of holding days before dosing:	At least 9 days
Number of fish per dose group:	Seven
Number of fish per control group:	Seven
Feeding regime:	None
Environmental conditions:	Loading rate: 0.087 g/L Temperature: 11.8 to 12.0°C Photoperiod: 16 hours light and 8 hours dark Dissolved oxygen concentration: ≥ 9.0 mg/L (≥83% air saturation) pH: 8.5– 8.8 Total hardness: 144 mg/L as $CaCO_3$

Methodology

The study was conducted as a concentration response test under static conditions with 5 test item concentrations and one control with untreated test medium. The test chambers, 38-L stainless steel aquaria containing approximately 30 L test solution, were maintained at 12 ± 2ºC in a temperature-controlled water bath. Fluorescent lighting was maintained on a 16-hour light and 8-hour dark photoperiod with 30-minute simulated dawn and dusk periods. Individual test solutions were prepared directly in each test chamber. The test substance was sonicated in dilution water for 15 minutes prior to addition to the test chambers. The test chambers were then stirred with top-down mixers for 15 minutes. No aeration was provided to any test chamber during the test. One replicate test chamber was used for each the control and all test treatments, with 7 fish per replicate. Observations for mortality and sub-lethal responses were made at approximately 5, 24, 48, 72, and 96 hours. Temperature, D.O., and pH were measured in test chamber at approximately 24, 48, 72, and 96 hours. The fish from the control replicate were measured at test termination.
Analytical confirmation of test concentrations was performed on days 0 (initiation) and 4 (termination) by analysis of clopyralid by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).
The mortality data were analyzed using the computer program of C. E. Stephan. Nonlinear interpolation was used to calculate the 48, 72 and 96-hour LC_{50} values and binominal probability was used to calculate the 95% confidence intervals. Since there was <50% mortality at 24 hours, the 24-hour LC_{50} value was estimated to be greater than the highest concentration tested. Due to the method used to calculate the 96-hour LC_{50} value, the slope of the concentration-response curve could not be calculated. The no-mortality concentration and 100% mortality concentration were determined by visual interpretation of the mortality data.

RESULTS AND DISCUSSION

Based on the analysis of clopyralid, the active ingredient in EF-243, the mean concentrations in the test substance treatment solutions during the 96-hour exposure were 23.9, 48.0, 93.4, 187 and 381 mg EF-243/L which represented recoveries of 97.9 to 101% of the nominal concentrations. The biological response results were reported based upon nominal EF-243 concentrations.

After the 96-hour exposure period, all the fish in the negative control group and the 23.8, 47.6, 95.3, and 191 mg EF-243/L treatment groups appeared normal with no signs of toxicity observed. Percent

mortality in the 381 mg EF-243/L treatment group was 86% at test termination. The single surviving fish in this treatment group was observed lying on the bottom of the test chamber at termination.

All study validity criteria were met: 1) control mortality at the end of the test should be ≤1 fish (there was no control mortality); and 2) dissolved oxygen concentration should be ≥60% in all test vessels throughout the exposure (it was ≥83%).

Table 19: Effect of EF-243 on mortality of rainbow trout

Treatment (mg EF-243/L)		No. of fish	Cumulative mortality (%)			
Nominal	Mean measured		24-hr	48-hr	72-hr	96-hr
Negative control	<LOD	7	0	0	0	0
23.8	23.9	7	0	0	0	0
47.6	48.0	7	0	0	0	0
95.3	93.4	7	0	0	0	0
191	187	7	0	0	0	0
381	381	7	14	71	86	86
LC_{50} [a]		297 mg EF-243/L (78 mg a.e./L clopyralid)				
95% C.I. [a]		>191 mg EF-243/L (>50 mg a.e./L clopyralid)				

[a] Based on nominal concentration

Table 20: Sub-lethal effects of EF-243 in rainbow trout

Treatment (mg EF-243/L)		Observation period							
Nominal	Mean measured	Observation 1: Lethargy (% affected)				Observation 2: Lying on bottom (% affected)			
		24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
Negative control	<LOD	0	0	0	0	0	0	0	0
23.8	23.9	0	0	0	0	0	0	0	0
47.6	48.0	0	0	0	0	0	0	0	0
95.3	93.4	0	0	0	0	0	0	0	0
191	187	0	0	0	0	0	0	0	0
381	381	0	0	0	0	86	29	14	14

CONCLUSION

Rainbow trout (*Oncorhynchus mykiss*) were exposed for 96 hours under static conditions to five nominal concentrations of EF-243 ranging from 23.8 to 381 mg EF-243/L. The highest nominal concentration causing no mortality at test end was 191 mg EF-243/L (equivalent to 50 mg ae/L clopyralid), and the lowest nominal concentration causing 100% mortality at test end was >381 mg EF-243/L (equivalent to >100 mg ae/L clopyralid). Based on the nominal test concentrations, the 96-hour LC_{50} value was 297 mg EF-243/L (equivalent to 78 mg a.e./L clopyralid).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	*Oncorhynchus mykiss*	EF-243	96-hr	LC_{50}	297	mg/L

A 2.2.1.6 Acute Daphnia study EF-243

Comments of zRMS:	The study was performed according to OECD TG 202 and principles of GLP. The validity criteria are met.. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2/6
Report	Ross, T. L., Zhao, E., Zhang, L., Schneider, S.Z.; 2020; EF-243: A 48-Hour Static Acute Toxicity Test With the Cladoceran (*Daphnia magna*); Eurofins EAG Agroscience LLC, Easton, Maryland, USA; Lab Study No. 379A-334; DAS Study No. 200842; Unpublished
Guideline(s):	Yes (OECD TGD 202)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	EF-243
Purity:	34.5 wt%, 394 g/L clopyralid-olamine (26.2 wt%, 300 g a.e./L)
Description (physical state):	Liquid
Lot/batch no.:	F006H1K005 (TSN400033)

Test System

Organism (*Species*):	Water flea (*Daphnia magna*)
Study type:	Acute
Study design:	Static
Test concentrations:	Nominal: 0 (control), 23.8, 47.6, 95.3, 191 and 381 mg EF-243/L (equivalent to 6.25, 12.5, 25, 50, and 100 mg ae/L clopyralid, respectively) Mean measured: <LOD (control), 23.2, 46.5, 94.5, 192, 401 mg EF-243/L (equivalent to 6.09, 12.2, 24.8, 50.4, and 105 mg ae/L clopyralid, respectively)
Parameters measured:	Immobility
Observation intervals:	3.5, 24 and 48 hours (± 1 hour) after test initiation
Age of test organisms at test initiation:	<24 hours
Analytical confirmation of test concentrations:	On days: 0 (initiation) and 2 (termination)
No. of holding days before dosing:	14
Number of daphnia per dose group:	20
Number of daphnia per control group:	20
Environmental conditions:	Loading rate: ≥ 40 mL/daphnid Temperature: 19.8 – 20.6°C Photoperiod: 16 hours light and 8 hours dark Dissolved oxygen concentration: ≥8.3 mg/L (≥91% air saturation)

	pH: 8.1-8.5
Reference substances:	Potassium Chloride (conducted as a separate non- GLP study)

Methodology

The study was conducted as a concentration response test under static conditions with 5 test item concentrations and one control with untreated test medium. The test chambers, 250 ml glass beakers were maintained at 20 ± 1ºC in a temperature-controlled environmental chamber. Four replicate test chambers were maintained in each treatment and control group, with five daphnids in each test chamber, for a total of 20 daphnids per concentration. Fluorescent lighting was maintained on a 16-hour light and 8-hour dark photoperiod with 30-minute simulated dawn and dusk periods. The test solutions were sonicated for 15 minutes and then stirred for 15 minutes on magnetic stir plates. No aeration was provided to any test chamber during the test.

Analytical confirmation of test concentrations was performed on days 0 (initiation) and 2 (termination) by analysis of clopyralid by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

The absence of any significant immobility in any of the treatment groups during the test precluded the statistical calculation of EC_{50} values at 24 and 48 hours. Therefore, the EC_{50} values were empirically estimated to be greater than the highest concentration tested. The NOEC, the highest nominal concentration causing no immobility at test end, and the lowest nominal concentration causing 100% immobility at test end were estimated by visual interpretation of the immobility and observation data.

RESULTS AND DISCUSSION

Based on the analysis of clopyralid, the active ingredient in EF-243, the mean concentrations in the test substance treatment solutions during the 96-hour exposure were 23.2, 46.5, 94.5, 192, and 401 mg EF-243/L, which represented recoveries of 97 to 105% of the nominal concentrations. The biological response results were reported based upon nominal EF-243 concentrations.

After the 48-hour exposure period, all the daphnids in the negative control group and all treatment groups appeared normal with no signs of toxicity, except for one immobile daphnid in the 47.6 mg EF-243/L treatment group and one lethargic daphnid in the 191 mg EF-243/L treatment group. Percent immobility for the 23.8, 47.6, 95.3, 191 and 381 mg EF-243/L treatment groups was 0, 5, 0, 0 and 0%, respectively.

All study validity criteria were met: 1) immobility and/or signs of disease or stress (e.g., abnormal behavior) in the daphnids in the control group(s) will not exceed 10% by the end of the test (there was no control mortality); and 2) dissolved oxygen concentration will be ≥3 mg/L throughout the test (it was ≥8.3 mg/L).

Table 21: Effect of EF-243 on immobilisation

Treatment (mg EF-243/L)	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
Negative control	0	0	0	0
23.8	0	0	0	0
47.6	0	0	1	5
95.3	0	0	0	0
191	0	0	0	0
381	0	0	0	0
NOEC[a]	381 mg EF-243/L (100 mg ae/L)		381 mg EF-243/L (100 mg ae/L)	
EC_{50}[a]	>381 mg EF-243/L (>100 mg ae/L)		>381 mg EF-243/L (>100 mg ae/L)	

[a] Based on nominal concentration

Table 22: Sub-lethal effects of EF-243

Treatment (mg EF-243/L)		Observation period	
Nominal	Mean Measured	Lethargy (% affected)	
		24-hr	48-hr
Negative control	<LOD	0	0
23.8	23.2	0	0
47.6	46.5	0	0
95.3	94.5	0	0
191	192	0	5
381	401	0	0

CONCLUSION

The cladoceran, *Daphnia magna*, was exposed for 48 hours under static conditions to five nominal concentrations of EF-243 ranging from 23.8 to 381 mg EF-243/L. Based on nominal test concentrations, the 48-hour EC_{50} value was >381 mg EF-243/L (equivalent to >100 mg ae/L clopyralid). The highest nominal concentration causing no immobility at test end was 381 mg EF-243/L and the lowest nominal concentration causing 100% immobility at test end was >381 mg EF-243/L.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	*Daphnia magna*	EF-243	48-hr	EC_{50}	>381	mg EF-243/L

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

No new or additional studies have been submitted

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No new or additional studies have been 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

A 2.3.1.1.1.1 Clopyralid: Effects (Acute Contact and Oral) on Bumblebees (*Bombus terrestris* L.) in the Laboratory

Comments of zRMS:	The study was conducted to OECD 246 and 247 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCA 8.3.1.1/1
Report	Tänzler, V., Kowalczyk, F.; 2019; Clopyralid: Effects (Acute Contact and Oral) on Bumblebees (Bombus terrestris L.) in the Laboratory; ibacon GmbH, 51381 Leverkusen, Germany; Lab Study No. 141721105; DAS Study No. 190300 ; 30 October 2019; Unpublished
Guideline(s):	OECD 246 and 247 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable

MATERIALS AND METHODS

Test Item(s)

Test item (common name):	Clopyralid
Purity:	95.9 %
Description (physical state):	White to tan (according to MSDS)
Lot/batch no.:	910905 5P (TSN100167)

Test System

Organism (*Species*):	Bumble bee (*Bombus terrestris*)

Study type:	48-hour acute contact and oral toxicity test	
Study design:	Assessment of survival and sublethal effects. 50 (control and solvent control), 50 (test item) and 30 (reference item) replicates per treatment. 1 individual per replicate.	
Age of test organism at initiation:	Adult worker bumble bees	
Test doses (µg/bumble bee):	Contact: 0 (control), 0 (solvent control), and 200 µg a.s./bumble bee. Oral (nominal): 0 (control), 0 (solvent control), and 200 µg a.s./bumble bee. Oral (actual consumed): 0 (control), 0 (solvent control), and 203.1 µg a.s./bumble bee.	
Information on bee colony:	The bumblebees used in the test were from three healthy and queen-right colonies, obtained from a commercial bumblebee breeding company (Koppert Deutschland GmbH, Zeppelinstr. 32, 47638 Straelen, Germany). The bumblebees were maintained in a clean cylindrical, latticed plastic cage.	
Environmental conditions:	Temperature:	Contact: 24.8 to 25.0°C Oral: 25 ± 2 °C
	Relative Humidity:	Contact: 39.9 to 51.9°C Oral: 60 % ± 20 %
	Photoperiod: 24-hour darkness (except room lighting during treatment and observations). Feeding: 50% w/v sucrose solution *ad libitum* given directly after treatment via feeding syringes.	
Reference toxicant:	Dimethoate	

Methodology

Under laboratory conditions 50 worker bumblebees (*Bombus terrestris* L.) were exposed to clopyralid in acetone by contact application (contact limit test). A droplet of 2 µL contained the target dose level of 200 µg a.s./bumblebee and was applied on the dorsal thorax of the bumblebees. Additional bumblebees were exposed to a water control (tap water containing 0.1 % v/v Triton X-100), solvent control (acetone) and reference item (10 µg dimethoate/bumblebee) treatment. An untreated solution of 50 % w/v sugar in water was provided as sustenance for the bumblebees throughout the bioassay. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after treatment.

By oral application (oral limit test) the bumblebees were exposed to clopyralid via feeding syringes placed in each cage. These syringes contained *approx.* 40 µL diluted test item in 50 % w/v sucrose solution containing 5 % w/w acetone. They were weighed before and after introduction into the cages in order to determine the exact consumption. The calculation of the target dose was based on 100 µL food uptake. The nominal target dose level of 200 µg a.s./bumblebee would have been obtained if exactly 40 mg/bumblebee of the treated food had been ingested. Empty syringes were removed, weighed and replaced by syringes containing fresh, untreated food (50 % w/v sucrose solution). After a maximum of 4 hours also the syringes containing remaining food were removed, weighed and replaced by syringes

containing fresh, untreated food (50 % w/v sucrose solution). Individual bumblebees which did not take up at least 80% of the mean food uptake per treatment group were excluded from the evaluation. Additional bumblebees were exposed to a water control (50 % w/v sucrose solution), solvent control (50 % w/v sucrose solution containing 5 % w/w acetone) and reference item (3.6 µg dimethoate/bumblebee) treatment. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after treatment.

As the test item treatment groups in the contact and oral test did not show mortalities above 50.0 % at test end, the LD_{50} could not be statistically calculated. The contact and oral NOED of the test item was estimated using the multiple sequential Fisher Test after Bonferroni-Holm (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

Analysis of the contact treatment solution yielded recovery of 122% of nominal. Analysis of the oral treatment solution yielded recoveries of 102% (stock solution) and 104% (feeding solution) of nominal.

The measured food uptake in the treatment groups ranged between 38 and 111 mg. For this reason, individual bumblebees which did not take up at least 80 % of the mean food uptake per treatment group were excluded from the evaluation of mortality and behavioural abnormalities, as well as from the calculation of the dose in the test item treatment group, this corresponded to actual oral dose of 203.1 µg a.s./bumblebee. For the 203.1 µg a.s./bumblebee test item treatment group 47 bumblebees were considered for the evaluation. For the water control (50 % w/v sucrose solution), solvent control (50 % w/v sucrose solution containing 5 % w/w acetone) and the reference item treatment groups 50, 50 and 25 bumblebees were considered for the evaluation

No mortality or sublethal effects were observed in the contact or oral tests at any time during the 48-hour study period, in any of the control, solvent control or test item treatments.

Study Validity

To demonstrate the validity of the study, the following conditions were fulfilled:

OECD Criteria	Required	Observed
Mean control mortality at the end of the test (Contact test)	≤10%	0%
Mean control mortality at the end of the test (Oral test)	≤10%	0%
Response to the reference toxicant (Contact test): Mean control mortality at the end of the test	≥50%	83.3%
Response to the reference toxicant (Oral test): Mean control mortality at the end of the test	≥50%	100%

Table 23: Analytical verification of treatment solution

Treatment µg a.s./bumble bee	% of nominal clopyralid
Control	<LOD
200 (contact)	122
Stock (oral)	102
200 (oral)	104

Table 24: Mortality (Contact test)

Treatment µg a.s./ bumble bee	Cumulative mortality			
	24-hour		48-hour	
	Mean No. dead	Mean %	Mean No. dead	Mean %
Control	0	0	0	0
Solvent control	0	0	0	0
200	0	0	0	0

Table 25: Mortality (Oral test)

Treatment µg GF-a.s./bumble bee	Actual dose con-sumed µg a.s./bumble bee	Cumulative mortality			
		24-hour		48-hour	
		Mean No. dead	Mean %	Mean No. dead	Mean %
Control		0	0	0	0
Solvent control		0	0	0	0
200	203.1	0	0	0	

Table 26: Sublethal effects (Contact test)

Treatment µg GF-a.s./bumble bee	Cumulative sublethal effects			
	24-hour		48-hour	
	Effects (n)	%	Effects (n)	%
Control	AN	0%	AN	0%
Solvent control	AN	0%	AN	0%
200	AN	0%	AN	0%

AN: All appeared normal

Table 27: Sublethal effects (Oral test)

Treatment µg GF-a.s./bumble bee	Actual dose consumed µg a.s./bumble bee	Cumulative sublethal effects			
		24-hour		48-hour	
		Effects (n)	%	Effects (n)	%
Control		AN	0%	AN	0%
Solvent control		AN	0%	AN	0%
200	203.1	AN	0%	AN	0%

AN: All appeared normal

Table 28: Effects of clopyralid on the bumble bee, Bombus terrestris

Endpoint type		Endpoint value µg a.s./bumble bee	95% confidence limits µg a.s./bumble bee
24-h contact	LD_{50}	>200	N/A
	NOED	≥200	N/A
	LOED	n.d.	N/A
48-h contact	LD_{50}	>200	N/A
	NOED	≥200	N/A
	LOED	n.d.	N/A
24-h oral	LD_{50}	>203.1	N/A
	NOED	≥203.1	N/A
	LOED	n.d.	N/A
48-h oral	LD_{50}	>203.1	N/A
	NOED	≥203.1	N/A
	LOED	n.d.	N/A

N/A: Not applicable; n.d.: not determined

CONCLUSION

For the contact test, the 24-hour and 48-hour LD_{50} values were >200 µg a.s./bumble bee; and the 24-hour and 48-hour NOED values were ≥ 200.0 µg a.s./bumble bee.

For the oral test, the 24-hour and 48-hour LD_{50} values were >203.1 µg a.s./bumble bee; and the 24-hour and 48-hour NOED values were ≥ 203.1 µg a.s./bumble bee.

Common name	Species	Test item	Exposure system	Time scale	Endpoint	Toxicity value	Units of test item
Bumble bee	*Bombus terrestris*	clopyralid	Contact	48-hour	LD_{50}	>200	µg/bee
Bumble bee	*Bombus terrestris*	clopyralid	Oral	48-hour	LD_{50}	>203.1	µg/bee

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

No new or additional studies have been submitted

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new or additional studies have been submitted

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new or additional studies have been submitted

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new or additional studies have been submitted

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

No new or additional studies have been submitted

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

No new or additional studies have been submitted

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target

arthropods

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No new or additional studies have been 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

A 2.4.2.1.1 GF-1966: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil

Comments of zRMS:	The study was performed according to OECD TG 232 and principles of GLP. The validity criteria are met.. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 8.4.2.1/1
Report	Pavić, B.; 2020; GF-1966: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil; ibacon GmbH, Rossdorf, Ger-many; Lab Study No. 154961016; DAS Study No. 201708; 14 December 2020; Unpublished
Guideline(s):	OECD 232, ISO 11267
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name): GF-1966

Purity:	clopyralid-olamine: 94.7 wt% (71.9 a.e. wt%)
Description (physical state):	Solid off-white granules
Lot/batch no.:	D062EAKA04 (TSN309356)

Test System

Organism (*Species*):	Collembola (*Folsomia candida)*
Study type:	28 day reproduction study
Study design:	Assessment of survival and reproduction. 4 replicates, consisting of 10 organisms in each vessel per test concentration; 8 control replicates.
Test concentrations:	Untreated Control, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg GF-1966/kg soil dry weight
Soil parameters:	Soil type: Artificial soil according to OECD 232 pH at initiation: 6.3 to 6.4 pH at termination: 6.0 to 6.3 Water content at initiation: 19.9% to 20.2% (52.4% to 53.2% of WHCmax) Water content at termination: 17.2% to 19.4% (45.2% to 51.0% of WHCmax) WHCmax: 38%
Environmental conditions:	Temperature: 18°C to 22°C Lighting: 16 h light : 8 h dark (400 to 800 lux) Feeding: *ca*. 2 mg dry yeast for each test vessel at the beginning of the test and on day 14.
Reference substance:	Boric acid (conducted as a separate GLP study)

Methodology

28 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the Collembola were introduced on top of the soil; 8 concentrations; 4 replicates/concentration (8 replicates for the untreated control) with 10 Collembola each. Feeding of Collembola with ca. 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioral effects and reproduction after 28 days.

Mortality data were analysed for significance by Chi² 2x2 Table Test (α= 0.05, one-sided greater). The LC_{50} at day 28 was not determined by statistical analysis as no mortality above 50% was observed. Reproduction data were tested for normal distribution and homogeneity of variance (α = 0.01) using the Shapiro-Wilk´s test and the Levene´s test, respectively. Reproduction data were normally distributed and homogeneous, and did follow a monotonicity trend (contrast trend), therefore were analysed by Williams t-test (multiple comparison, α=0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction could not be reliably calculated by statistical methods due to a lack of dose-response relationship. The EC_{50} was visually extrapolated from the data. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

RESULTS AND DISCUSSION

After 28 days of exposure, a mortality of up to 8% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 5% of the Collembolans died. The reproduction of *Folsomia candida* was not statistically significantly different compared to the control up to and including the test concentration of 556 mg test item/kg soil dry weight. At the test concentration of 1000 mg test item/soil dry weight reproduction was statistically significantly different compared to the control. No behavioural effects were observed in any treatment group.

All validity criteria for the study were met: 1) control mortality should be ≤20% (it was 5%); 2) control reproduction should be ≥100 juveniles per container (it was 726 to 1518 juveniles); and 3) control reproduction coefficient of variation should be ≤30% (it was 24.3%).

Table 29: Effects of GF-1966 on Folsomia candida survival and reproduction

Test concentrations (mg/kg sdw)	Mean mortality of adults (%)	Mean no. of juveniles	% Change in no. of juveniles compared to control[1]
Control	5	1267	-
16.3	3	1369	+8.0
29.4	0	1100	-13.2
52.9	8	1119	-11.7
95.3	0	1264	0
171	5	1229	-3.0
309	8	1191	-6.0
556	0	1086	-14.3
1000	3	764	-39.8*

[1] Positive values indicate increased reproduction, and negative values decreased, compared to the control
* Statistically significantly different from the control

CONCLUSION

GF-1966 caused no statistically significant effects on mortality of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 1000 mg GF-1966/kg soil dry weight (719 mg a.e./kg sdw). The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >1000 mg GF-1966/kg soil dry weight (>719 mg a.e./kg sdw). The LC_{50} was estimated to be >1000 mg GF-1966/kg soil dry weight (>719 mg a.e./kg sdw).

The NOEC of GF-1966 for reproduction of *Folsomia candida* was determined to be 556 mg GF-1966/kg soil dry weight (400 mg a.e./kg sdw). The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight (719 mg a.e./kg sdw). The EC_{50} was estimated to be >1000 mg GF-1966/kg soil dry weight (>719 mg a.e./kg sdw).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Collembola	*Folsomia candida*	GF-1966	28 day	NOEC	556	mg/kg sdw

A 2.4.2.1.2 GF-1966: Effects on Reproduction of the Predatory Mite Hypoaspis

aculeifer in Artificial Soil

Comments of zRMS:	The study was performed according to OECD TG 226 and principles of GLP. The validity criteria are met. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 8.4.2.1/2
Report	Pavić, B.; 2020; GF-1966: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil; ibacon GmbH, Rossdorf, Germany; Lab Study No. 154961089; DAS Study No. 201709; 14 December 2020; Unpublished
Guideline(s):	OECD 226, ISO 11267
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	GF-1966
Purity:	clopyralid-olamine: 94.7 wt% (71.9 a.e.wt%)
Description (physical state):	Solid off-white granules
Lot/batch no.:	D062EAKA04 (TSN309356)

Test System

Organism (*Species*):	Predatory soil mite (*Hypoaspis aculeifer)*
Study type:	Reproduction study
Study design:	Assessment of survival and reproduction. 4 replicates, consisting of 10 organisms in each vessel per test concentration; 8 control replicates.
Test concentrations:	Untreated Control, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg GF-1966/kg soil dry weight
Soil parameters:	Soil type: Artificial soil according to OECD 226. pH at initiation: 6.3 to 6.4 pH at termination: 5.4 to 5.6 Water content at initiation: 19.9% to 20.2% (52.4% to 53.2% of WHCmax) Water content at termination: 18.5% to 19.3% (48.6% to 50.7% of WHCmax) WHCmax: 38%
Environmental conditions:	Temperature: 18°C to 22°C Light: 16 h light : 8 h dark (400 to 800 lux) Feeding: Cheese mites (*Tyrophagus putrescentiae* cultured by ibacon), one spatula at experimental start and on day 2, 4, 7, 9 and 11.
Reference substance:	Dimethoate (conducted as a separate GLP study)

Methodology

14-day exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; 8 concentrations; 4 replicates/concentration and 8 replicates for the untreated control, with 10 female predatory mites each. Feeding of the soil mites with cheese mites (*Tyrophagus putrescentiae) ad libitum* at test start and on days 2, 4, 7, 9 and 11. Assessment of adult mortality and reproduction after 14 d (counted after extraction on day 16 after application).

Mortality data were analysed for significance by Chi² 2x2 Table Test (multiple comparison, with Bonferroni Correction, α= 0.05, one-sided greater). The LC_{50} at day 14 was not determined by statistical analysis as no mortality above 50% was observed. Reproduction data were tested for normal distribution and homogeneity of variance (α = 0.01) using the Shapiro-Wilk´s test and the Levene´s test, respectively. Reproduction data were normally distributed and homogeneous, and did follow a monotonicity trend (contrast trend), therefore were analysed by Williams t-test (multiple comparison, α=0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. Due to the lack of a concentration-response relationship no reliable EC_x-calculation was possible. Therefore, no EC_{10}/EC_{20}-value can be reported. The EC_{50} was estimated. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

RESULTS AND DISCUSSION

A mortality of up to 10% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 9% of the adult mites died. The reproduction of the predatory mites exposed to GF-1966 was not statistically significantly different compared to the control up to and including the test concentration of 556 mg/kg soil dry weight. At the test concentration of 1000 mg/kg soil dry weight reproduction was statistically significantly different compared to the control. No differences in morphology or any abnormalities were observed in any of the treatment groups.

All validity criteria for the study were met: 1) control mortality should be ≤20% (it was 9%); 2) control reproduction should be ≥ 50 juveniles per container (it was 163 to 227 juveniles); and 3) control reproduction coefficient of variation should be ≤30% (it was 10.2%).

Table 30: Effects of GF-1966 on Hypoaspis aculeifer survival and reproduction

Test concentrations (mg/kg sdw)	Mean mortality of adults (%)	Mean no. of juveniles	% change in no. of juveniles compared to control[1]
Control	9	197	-
16.3	8	182	-7.8
29.4	5	206	+4.0
52.9	8	155	-21.6
95.3	3	214	+9.0
171	3	185	-6.1
309	5	171	-13.2
556	5	203	+3.0
1000	10	146	-26.1*

[1]Positive values indicate increased reproduction, and negative values decreased, compared to the control
* Statistically significantly different from the control

CONCLUSION

GF-1966 caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 1000 mg GF-1966/kg soil (719 mg a.e./kg sdw). The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >1000 mg GF-1966/kg soil (>719 mg a.e./kg sdw). The LC_{50} was estimated to be >1000 mg GF-1966/kg soil (>719 mg a.e./kg sdw).

The NOEC of GF-1966 for reproduction of *Hypoaspis aculeifer* was determined to be 556 mg GF-1966/kg soil dry weight (400 mg a.e./kg sdw). The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight (719 mg a.e./kg sdw). The EC_{50} was estimated to be >1000 mg GF-1966/kg soil (>719 mg a.e./kg sdw).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Predatory soil mite	*Hypoaspis aculeifer*	GF-1966	14 day	NOEC	556	mg/kg sdw

KCP 10.4.2.2 Higher tier testing

No new or additional studies have been submitted

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

No new or additional studies have been submitted

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

No new or additional studies have been submitted

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 GF-1966: Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants

Comments of zRMS:	The study was performed according to OECD TG 208 and principles of GLP. The validity criteria are met. Few deviations to the guideline were noted: 1. The temperature in the glasshouse went above 32°C due to hot, sunny weather on two days (32.2°C and 32.8°C respectively). According to the author of the study: "*This was not to the detriment of the plants as photographs of the untreated plants taken at harvest show. This deviation had not impact on the validity of the study*." 2. The Study Plan states that daytime relative humidity in the glasshouse should be 70% (± 25%). Throughout the field phase of this study, minimum relative humidity fell below 45% (70% - 25%) and on one occasion rose above 95% (70% + 25%). According to the author of the study: "*Relative humidity is a measure of the moisture level in the air in the glasshouse. Moisture is dependent on the number of plants in the glasshouse and the amount of watering they receive which is a situation that can vary over a period of time. As the plants are watered by putting water into saucers, the relative humidity can also be affected by when they are watered and can potentially decrease before being re-watered. Consequently, on some occasions, relative humidity can be slightly below or slightly above 70% (±25%). However, for this study this was not to the detriment of the plants as photographs of the untreated plants taken at harvest show*." 3. In the Study Plan on page 10, an inscription error was made. It was corrected. 4. The Study Plan states that statistical regression reports containing the calculated ER_{25} ER_{50} values based on shoot fresh weight reduction and shoot dry weight reduction and reports containing the calculated NOER values based on shoot fresh weight reduction and shoot dry weight reduction will be produced. The Sponsor requested that ER_{50} values were calculated on visual injury at harvest and these were carried out. These deviations had no impact on the validity of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/1
Report	Stead, A; 2019; Summary of GF-1966: Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants; Stockbridge Technology Centre, Cawood, Selby, North Yorkshire, UK, YO8 3TZ; Lab Study No. STC/19/E1262; DAS Study No. 190288 ; 11 October 2019; Unpublished
Guideline(s):	OECD TGD 208
Deviations:	No

GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	GF-1966
Purity:	Clopyralid 719 g a.e./kg
Description (physical state):	Water soluble granule (SG)
Lot/batch no.:	D062EAKA04 (TSN309356)

Test System

Monocotyledonous species:	*Lolium perenne* (ryegrass), *Avena sativa* (oats), *Allium cepa* (onion)
Dicotyledonous species:	*Brassica napus* (oilseed rape), *Glycine max* (soybean), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato), *Cucumis sativus* (cucumber)
Study type:	Greenhouse study assessing Seedling Emergence and Seedling Growth
Parameters measured:	Emergence counts: 14 and 21 or 22 days after 50% emergence on the untreated water only controls Number of dead plants:14 and 21 or 22 days after 50% emergence on the untreated water only controls Shoot fresh weight: 21 or 22 days after 50% emergence on the untreated water only controls Shoot dry weight: 21 or 22 days after 50% emergence on the untreated water only controls Phytotoxicity rating system, if used: at 14 and 21 or 22 days (harvest) after 50% emergence. Visual injury scale: 0% = no visual injury, 1% -39% = slight visual injury, 40% - 69% = moderate visual injury, 70% - 99% = severe visual injury, 100% = all plants dead and/or not emerged
Growth conditions:	Temperature (range): 16.7 to 32.8°C Photoperiod: natural day-length plus supplementary lighting with 5000 lux to extend day-length to 16 hours Light intensity (range): 435 to 1652 µmol/m^2/second Relative humidity: 13 to 99% Water regime and schedules: Following treatment pots placed in plastic saucers and lightly watered overhead with a hosepipe and rose. All subsequent water was applied to the saucers. Plants were inspected daily and watered according to crop requirements. Water source/type: mains water

	Pest control method /fertilisation, if used: NPK powdered feed applied as required
Growth medium:	Soil type: Sandy loam Details of nutrient medium, if used: All species: Sand 77.60%, Silt 14.33%, Clay 8.07% Organic matter 1.2% pH: 7.4
Test concentrations:	Ryegrass, oats, onion, oilseed rape, sugar beet, cucumber: 18.75, 37.5, 75, 150 and 300 g clopyralid a.e./ha Soybean, carrot, lettuce, tomato: 4.69, 9.38, 18.75, 37.5, 75, 150 and 300 g clopyralid a.e./ha Mean calculated concentrations: N/A
Analytical verification:	Highest Treatment (300 g clopyralid a.e./ha) Clopyralid: recovery of 100% of nominal
Test material application:	Method: Pre-emergence application using gas pressurised Oxford Precision Sprayer with a 2m boom fitted with 4 flat fan tip 110° standard nozzles (ISO: 01-F110) mounted on a battery powered track sprayer. Application interval: N/A Reference chemical (if used): N/A
Seeds:	Source: commercial seed lots Method of seeding: by hand Prior seed treatment/sterilisation: none Number of seeds per replicate pot: Ryegrass, oats, onion: 5 Oilseed rape, carrot, lettuce: 3 Soybean, sugar beet, tomato, cucumber: 2 Growth stage at application: N/A (seeds)
Number of control replicates:	Ryegrass, oats, onion: 4 Oilseed rape, carrot, lettuce: 7 Soybean, sugar beet, tomato, cucumber: 10
Number of test concentration replicates:	Ryegrass, oats, onion: 4 Oilseed rape, carrot, lettuce: 7 Soybean, sugar beet, tomato, cucumber: 10

Methodology

Seeds were sown on the day before treatment application. All treatment applications were made using a track sprayer calibrated to deliver 200 L water/ha (± 10%) starting with the water only control. The test item was added to water to give the highest treatment rate. All subsequent applications were diluted in sequence to produce the lower rates. After treatment application the pots were removed to a glasshouse and laid out in randomised blocks. All pots were placed in saucers and water was applied directly onto the soil surface after treatment. All subsequent water was placed in the saucers. Plants were assessed for visual injury and plant death. Shoot fresh weights and shoot dry weights were recorded at harvest (21 or 22 days after 50% emergence of the untreated control). The ER_{25} and ER_{50} values for each species were calculated using shoot fresh weight and shoot dry weight expressed as a percentage of the untreated control and ER_{50} values for each species calculated on visual injury at harvest and were capped at 100% using JMP v.8 statistical package.
NOER values for each species were calculated using the CETIS v.1.9 package.

RESULTS AND DISCUSSION

See tabulated results.

Table 31: Observations of plant mortality: % Emergence, % survival, shoot fresh/dry weight (g)and % visual injury at harvest: Monocotyledonous species

	Lolium perenne (ryegrass)					*Avena sativa* (oats)					*Allium cepa* (onion)				
Treatment: GF-1966 g clopyralid a.e./ha	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest
Control	100	100	1.189	0.200	0	100	100	5.209	0.813	0	100	100	2.542	0.197	0
18.75	100	100	1.190	0.198	0	100	100	5.105	0.800	0	100	100	2.581	0.202	0
37.5	100	100	1.324	0.224	0	100	100	5.726	0.911	0	100	100	2.284	0.178	0
75	100	100	1.256	0.211	0	100	100	5.391	0.841	0	100	100	1.676	0.141	4
150	100	100	1.167	0.195	1	100	100	4.556	0.701	0	95	100	2.198	0.173	10
300	100	100	1.205	0.197	5	100	100	5.335	0.867	0	100	95	1.832	0.143	13

Table 32: Observations of plant mortality: % Emergence, % survival shoot fresh/dry weight (g) and % visual injury at harvest: Dicotyledonous species

	Brassica napus (oilseed rape)					*Beta vulgaris* (sugar beet)					*Cucumis sativus* (cucumber)				
Treatment: GF-1966 g clopyralid a.e./ha	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest
Control	95	100	13.585	2.116	0	100	100	6.499	0.652	0	100	100	20.129	2.500	0
18.75	100	100	13.372	2.053	0	100	100	6.036	0.586	0	100	100	18.266	2.362	0
37.5	100	100	14.854	2.189	0	100	100	6.162	0.637	0	100	100	19.934	2.335	2
75	100	100	12.597	1.943	0	100	100	7.853	0.764	1	100	100	19.374	2.304	12
150	100	100	15.487	2.241	0	100	100	6.784	0.662	2	100	100	22.934	2.246	33
300	100	100	14.730	2.246	0	100	100	7.733	0.726	12	100	100	21.936	1.936	35

	Glycine max (soybean)					*Daucus carota* (carrot)					*Lactuca sativa* (lettuce)				
Treatment: GF-1966 g clopyralid a.e./ha	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at harvest
Control	100	100	6.763	1.486	0	100	100	2.240	0.328	0	100	100	8.500	0.793	0
4.69	100	100	6.161	1.335	3	100	100	2.077	0.303	0	100	100	7.596	0.773	0
9.38	100	100	6.677	1.248	11	100	100	2.212	0.315	0	100	100	8.166	0.840	0
18.75	90	100	4.662	0.795	28	100	100	2.370	0.347	0	100	100	8.391	0.782	0
37.5	100	85	3.797	0.626	53	86	100	1.746	0.244	2	100	100	6.755	0.670	2
75	100	80	2.260	0.326	78	100	100	1.349	0.188	24	100	100	6.846	0.603	19
150	95	32	0.653	0.085	91	95	95	1.164	0.161	36	86	89	4.129	0.345	48
300	80	0	0.000	0.000	100	100	90	0.759	0.100	67	19	25	0.068	0.010	99

	Lycopersicon esculentum (tomato)				
Treatment: GF-1966 g clopyralid a.e./ha	Emergence	Survival	Shoot fresh weight	Shoot dry weight	Visual injury at har-vest
Control	100	100	9.221	1.205	0
4.69	100	100	10.014	1.159	15
9.38	100	100	10.941	1.470	0
18.75	100	100	10.167	1.363	1
37.5	100	100	9.152	1.123	4
75	100	100	10.636	1.002	34
150	95	74	4.514	0.375	64
300	100	70	2.330	0.197	80

Table 33: Reported ER_{50} values based on shoot fresh/dry weight and visual injury

Species	Shoot fresh weight	Shoot dry weight	Visual injury at harvest
	ER_{50}	ER_{50}	ER_{50}
Ryegrass	>300	>300	>300
Oats	>300	>300	>300
Onion	>300	>300	>300
Oilseed rape	>300	>300	>300
Soybean	44.91	25.64	35.12
Sugar beet	>300	>300	>300
Carrot	134.64	112.91	218.72
Lettuce	147.42	145.49	159.74
Tomato	185.49	133.76	133.51
Cucumber	>300	>300	>300

Onion – Exponential

Soybean – Exponential (fresh weight) Rodbard (dry weight) Rodbard (visual injury at harvest)

CONCLUSION

All species displayed visual injury except oats and oilseed rape.

Based on shoot fresh weight reduction onion, with an ER_{25} value of 136.61 g clopyralid a.e./ha and an ER_{50} value of >300 g clopyralid a.e./ha (the highest rate tested), was the most sensitive monocotyledon species to pre-emergence application of GF-1966.

Based on shoot fresh weight reduction soybean, with an ER_{25} value of 18.66 g clopyralid a.e./ha and an ER_{50} value of 44.91 g clopyralid a.e./ha, was the most sensitive dicotyledon species to pre-emergence application of GF-1966.

Based on shoot fresh weight the NOER value for all monocotyledon species was 300 g clopyralid a.e./ha (the highest rate tested).

Based on shoot fresh weight soybean, with a NOER value of 9.38 g clopyralid a.e./ha was the most sensitive dicotyledon species to pre-emergence application of GF-1966.

Based on shoot dry weight the ER_{25} and ER_{50} values for all monocotyledon species were >300 g clopyralid a.e./ha (the highest rate tested).

Based on shoot dry weight soybean, with an ER_{25} value of 10.15 g clopyralid a.e./ha and an ER_{50} value of 25.64 g clopyralid a.e./ha, was the most sensitive dicotyledon species to pre-emergence application of GF-1966.

Based on shoot dry weight the NOER value for all dicotyledon species was 300 g clopyralid a.e./ha (the highest rate tested).

Based on shoot dry weight soybean, with a NOER value of 9.38 g clopyralid a.e/ha was the most sensitive dicotyledon species to pre-emergence application od GF-1966.

Based on visual injury at harvest the ER_{50} value for all monocotyledon species was >300 g clopyralid a.e./ha (the highest rate tested).

Based on visual injury at harvest soybean, with an ER_{50} value of 35.12 g clopyralid a.e./ha, was the most sensitive dicotyledon species to pre-emergence application of GF-1966.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Soybean	*Glycine max*	GF-1966	21 days	Shoot dry weight ER_{50}	25.64	g clopyralid a.e./ha
Soybean	*Glycine max*	GF-1966	21 days	Visual injury ER_{50}	35.12	g clopyralid a.e./ha

A 2.6.2.2 GF-1966: Vegetative Vigour Test Terrestrial Non Target Plants

Comments of zRMS:	The study was performed according to OECD TG 227 and principles of GLP. The validity criteria are met. Few deviations to the guideline were noted: 1. The Study Plan states that the synthetic sandy loam mix should have a pH value of 7 to 8. The pH value of the synthetic sandy loam mix used for all species was 6.9. 2. The Study Plan states that relative humidity in the glasshouse should be 70 % (+/-25 %). Throughout the field phase of this study, the minimum relative humidity fell below 45 % (70 % • 25 %).

	According to the author of the study: "*Relative humidity is a measure of the moisture level in the air in the glasshouse. Moisture is dependent on the number of plants in the glasshouse and the amount of watering they receive which is a situation that can vary over a period of time. As the plants are watered by putting water into saucers, the relative humidity can also be affected by when they are watered and can potentially decrease before being re-watered. Consequently, on some occasions, relative humidity can be slightly below or slightly above 70 % (+A 25 %). However, for this study this was not to the detriment of the plants. This deviation should not impact on the validity of the study. This is evidenced by the quality of plants at harvest*." 4. According to the author of the study: "*The Study Director was requested by the sponsor …. to calculate ER50 values based on visual injury assessments at harvest. These values generated. This was not included in the study plan*." These deviations had no impact on the validity of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/2
Report	Davies, C; 2019; GF-1966 Vegetative Vigour Test Terrestrial Non Target Plants (based on OECD Guideline 227)-2019; Stockbridge Technology Centre Ltd, Cawood, Selby, North Yorkshire, YO8 3TZ, UK,; Lab Study No. STC/19/E1261; DAS Study No. 190287 ; 10 October 2019; Unpublished
Guideline(s):	OECD TGD 227
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable.
Duplication (if vertebrate study)	Not applicable

Test Item(s)

Test item (Common name):	GF-1966 Clopyralid
Purity:	Clopyralid 720 g a.e./kg
Description (physical state):	Soluble granule (SG)
Lot/batch no.:	GF-1966 - D062EAKA04 (TSN309356)

Test System

Monocotyledonous species:	*Lolium perenne* (ryegrass), *Avena sativa* (oats) and *Allium cepa* (onion)
Dicotyledonous species:	*Brassica napus* (oilseed rape), *Glycine max* (soybean), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato) and *Cucumis sativus* (cucumber)

Study type:	Greenhouse study assessing Vegetative Vigour
Parameters measured:	Number of dead plants at 7, 14 and 21 days after treatment application Shoot fresh weight at 21 days after treatment application Shoot dry weight at 21 days after treatment application Phytotoxicity rating system, if used: at 7, 14 and 21 days (harvest) after treatment application. Visual injury scale 0% = no visual injury, 1-39% = slight visual injury, 40-69% = moderate visual injury, 70-99% = severe visual injury, 100% = all plants dead
Growth conditions:	Temperature (range):17.0°C to 30.3°C Photoperiod: natural day-length plus supplementary lighting with 5000 lux to extend to 16 hours Light intensity (range): 402-1789 µmol/m^2/second Relative humidity: 18% to 89% Water regime and schedules: Following treatment pots placed in plastic saucers and water applied to saucers. Plants inspected daily and watered according to crop requirements. Final watering applied one day before harvest Water source/type: mains water Pest control method /fertilisation, if used: NPK base fertiliser applied to soil mix if required. Liquid feeding applied if required.
Growth medium:	Soil type: sandy loam Details of nutrient medium, if used:

	<u>All crops:</u> Sand 75.52%, silt 15.55% and clay 8.93% Organic matter 1.3% pH:6.9
Test concentrations:	<u>For ryegrass, oats, onion, oilseed rape, sugar beet and cucumber:</u> 18.75, 37.5, 75, 150, and 300 g clopyralid a.e./ha <u>For soybean, carrot, lettuce and tomato:</u> 4.69, 9.38, 18.75, 37.5, 75, 150 and 300 g clopyralid a.e./ha
Analytical verification:	<u>For all crops</u>: Highest treatment H 300 g clopyralid a.e./ha Clopyralid recovery 100% of nominal
Test material application:	Method: Application using a gas pressurised Oxford Precision Sprayer with a 2m boom fitted with 4 fan tip 110^{o} standard nozzles (ISO size: F110-01) mounted on a battery powered track sprayer
Seeds:	Source: commercial seed lots Method of seeding: by hand Prior seed treatment/sterilisation: none Number of plants per replicate pot: 1 for soybean, sugar beet, tomato and cucumber, 3 for oilseed rape, carrot and lettuce, and 5 for ryegrass, oats and onion Growth stage at application: 2 to 4 true leaves
Number of control replicates:	4 (ryegrass, oats and onion), 7 (oilseed rape, carrot and lettuce) and 20 (soybean, sugar beet, tomato and cucumber)
Number of test concentration replicates:	4 (ryegrass, oats and onion), 7 (oilseed rape, carrot and lettuce) and 20 (soybean, sugar beet, tomato and cucumber)

Methodology

Seeds were sown on a range of dates to produce plants at the required growth stage at treatment application. The test item was added to water to give the highest treatment rate and then diluted in sequence to produce the lower rates for the application to 10 species. All treatment applications were made using a track sprayer calibrated to deliver 200 L water/ha (± 10%) starting with the water only control. After treatment application the pots were removed to a glasshouse and laid out in randomized blocks. All pots were placed in saucers with water applied directly into the saucers to avoid leaching. Plants were assessed for visual injury and plant death. Shoot fresh and dry weights were recorded at harvest (21 days after treatment application).

$ER_{25,}$ ER_{50} and NOER values for each species were calculated using shoot fresh and dry weight expressed as the percentage of the untreated control and capped at 100% using JMP v.8 statistical package.

ER_{50} values based on visual injury assessments at harvest were calculated using JMP v.8 statistical package.

RESULTS AND DISCUSSION

Plant quality was excellent prior to treatment application. Plants of each species were vigorous with good foliage colour. Results are summarised in the following tables.

Table 34: Observations of % survival, % visual injury and shoot fresh and dry weight (g): Monocotyledonous species

	Lolium perenne (Ryegrass)				*Avena sativa* (Oats)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	5.22	1.04	100	0	17.22	3.42
18.75	100	0	4.67	0.95	100	0	17.26	3.48
37.5	100	0	4.56	1.06	100	0	17.20	3.41
75	100	0	4.31	0.87	100	0	16.84	3.39
150	100	0	4.32	0.90	100	0	18.83	3.63
300	100	0	5.65	1.15	100	0	20.45	3.82

	Allium cepa (Onion)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	30.36	3.30
18.75	100	0	29.59	3.56
37.5	100	0	28.78	3.64
75	100	0	27.54	3.11
150	100	0	33.74	3.84
300	100	0	34.07	3.92

Table 35: Observations of % survival, % visual injury and shoot fresh and dry weight (g): Dicotyledonous species

	Brassica napus (Oilseed rape)				*Glycine max* (Soybean)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	44.86	7.66	100	0	6.13	1.87
4.69	-	-	-	-	100	15.00	6.38	1.90
9.38	-	-	-	-	100	28.25	5.26	1.46
18.75	100	0	44.05	7.44	100	38.25	5.05	1.30
37.5	100	0	43.23	7.37	100	55.0	3.56	0.79
75	100	0	44.26	7.81	90.0	74.75	2.42	0.47
150	100	0	39.02	6.65	45.0	90.25	1.22	0.26
300	100	0	43.02	7.27	10.0	98.00	0.39	0.06

	Beta vulgaris (Sugar beet)				*Daucus carota* (Carrot)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	20.53	3.10	100	0	13.75	2.64
4.69	-	-	-	-	100	3.29	13.62	2.54
9.38	-	-	-	-	100	8.29	14.07	2.66
18.75	100	0	20.56	3.01	100	10.71	14.86	2.69
37.5	100	0	21.51	3.22	100	15.71	12.04	2.20
75	100	0	20.75	3.04	100	32.86	9.33	1.80
150	100	0	19.02	2.77	100	65.71	8.22	1.61
300	100	3.20	19.32	2.85	100	72.14	5.36	1.20

	Lactuca sativa (Lettuce)				*Lycopersicon esculentum* (Tomato)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	46.96	7.22	100	0	33.56	6.29
4.69	100	0	41.27	6.97	100	13.50	31.52	5.19
9.38	100	4.29	37.79	5.92	100	28.25	34.72	5.51
18.75	100	11.43	30.62	5.27	100	47.25	25.67	3.88
37.5	42.9	69.29	13.78	2.34	100	68.50	11.27	1.53
75	38.1	75.71	15.83	2.53	100	75.25	10.31	1.31
150	0	100	0	0	70.00	90.00	3.37	0.41
300	0	100	0	0	5.00	99.50	0.10	0.01

	Cucumis sativus (Cucumber)			
Treatment	Survival	Visual injury	Shoot fresh weight	Shoot dry weight
Control	100	0	48.76	8.86
18.75	100	2.05	48.84	8.37
37.5	100	5.45	49.59	8.41
75	100	8.35	52.94	8.83
150	100	15.25	51.79	7.79
300	100	20.50	50.82	7.19

Table 36: Reported ER_{50} values for shoot fresh weight, shoot dry weight and visual injury as g clopyralid a.e./ha

Species	Shoot fresh weight	Shoot dry weight	Visual injury
	ER_{50}	ER_{50}	ER_{50}
Ryegrass	>300	>300	>300
Oats	>300	>300	>300
Onion	>300	>300	>300
Oilseed rape	>300	>300	>300
Soybean	55.96	32.69	28.78
Sugar beet	>300	>300	>300
Carrot	192.80	264.28	110.67
Lettuce	26.44	32.27	31.28
Tomato	35.40	24.18	21.74
Cucumber	>300	>300	>300

Shoot fresh weight: lettuce – log-linear, soybean- – Jonckheere-Terpstra Step-Down Test Shoot dry weight: tomato-rodbard, soybean-– Jonckheere-Terpstra Step-Down Test
Visual injury: tomato-Rodbard

CONCLUSION

All species except ryegrass, oats, onion and oilseed rape displayed visual injury.
Based on shoot fresh weight reduction

All monocotyledon species had ER_{25} and ER_{50} values of >300 g clopyralid a.e./ha (the highest rate tested).

The most sensitive dicotyledon species to post-emergence application of GF-1966 was lettuce with an ER_{25} value of 8.92 g clopyralid a.e./ha and an ER_{50} value of 26.44 g clopyralid a.e./ha.

All monocotyledon species had a NOER value of 300 g clopyralid a.e./ha (the highest rate tested).

The most sensitive dicotyledon species to post-emergence application of GF-1966 was soybean with a NOER value of 4.69 g clopyralid a.e./ha (the highest rate tested).

Based on shoot dry weight reduction

All monocotyledon species had ER_{25} and ER_{50} values of >300 g clopyralid a.e./ha the highest rate tested).

The most sensitive dicotyledon species to post-emergence application of GF-1966 was tomato with an ER_{25} value of 10.75 g clopyralid a.e./ha and an ER_{50} value of 24.18 g clopyralid a.e./ha.

All monocotyledon species had a NOER value of 300 g clopyralid a.e./ha (the highest rate tested).

The most sensitive dicotyledon species to post-emergence application of GF-1966 was soybean with a NOER value of 4.69 g clopyralid a.e./ha (the highest rate tested).

Based on visual injury assessments at harvest

All monocotyledon species had ER_{50} values of >300 g clopyralid a.e./ha (the highest rate tested).

The most sensitive dicotyledon species to post-emergence application of GF-1966 was tomato with an ER_{50} value of 21.74 g clopyralid a.e./ha.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Lettuce	*Lactuca sativa*	GF-1966	21 days	shoot fresh weight ER_{50}	26.44	g clopyralid a.e./ha
Tomato	*Lycopersicon esculentum*	GF-1966	21 days	shoot dry weight ER_{50}	24.18	g clopyralid a.e./ha
Tomato	*Lycopersicon esculentum*	GF-1966	21 days	Visual injury ER_{50}	21.74	g clopyralid a.e./ha

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

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

No studies, other than those already evaluated during the EU Review of clopyralid, have been presented in support of this submission.

A 2.8 KCP 10.8 Monitoring data

Monitoring studies are not available for clopyralid and are not considered necessary in light of the acceptable risk concluded for all non-target organisms from uses of EF-1136