FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: Diflufenikan 500 SC

Product name(s): -

Chemical active substance:

diflufenican, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Pestila Sp. z o.o. / ProAgri International Sp. z o.o.

Submission date: January 2023, September 2023

MS Finalisation date: September 2023, January 2024

Version history

When	What
09.2023	Change in GAP table
09.2023	Initial assessment by zRMS
01.2024	The final Registration Report

Table of Contents

9 Ecotoxicology (KCP 10) ... 6

9.1 Critical GAP and overall conclusions ... 7
9.1.1 Overall conclusions ... 9
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) ... 9
9.1.1.2 Effects on aquatic organisms (KCP 10.2) ... 10
9.1.1.3 Effects on bees (KCP 10.3.1) ... 10
9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2) ... 11
9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5) ... 11
9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6) ... 12
9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7) ... 12
9.1.2 Grouping of intended uses for risk assessment ... 12
9.1.3 Consideration of metabolites ... 12
9.2 Effects on birds (KCP 10.1.1) ... 13
9.2.1 Toxicity data ... 13
9.2.1.1 Justification for new endpoints ... 13
9.2.2 Risk assessment for spray applications ... 14
9.2.2.1 First-tier assessment (screening/generic focal species) ... 14
9.2.2.2 Higher-tier risk assessment ... 14
9.2.2.3 Drinking water exposure ... 14
9.2.2.4 Effects of secondary poisoning ... 15
9.2.2.5 Biomagnification in terrestrial food chains ... 16
9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed ... 16
9.2.4 Overall conclusions ... 16
9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2) ... 17
9.3.1 Toxicity data ... 17
9.3.1.1 Justification for new endpoints ... 17
9.3.2 Risk assessment for spray applications ... 17
9.3.2.1 First-tier assessment (screening/generic focal species) ... 18
9.3.2.2 Higher-tier risk assessment ... 18
9.3.2.3 Drinking water exposure ... 18
9.3.2.4 Effects of secondary poisoning ... 19
9.3.2.5 Biomagnification in terrestrial food chains ... 20
9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed ... 20
9.3.4 Overall conclusions ... 20
9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3) ... 20
9.5 Effects on aquatic organisms (KCP 10.2) ... 21
9.5.1 Toxicity data ... 21
9.5.1.1 Justification for new endpoints ... 24
9.5.2 Risk assessment ... 24
9.5.3 Overall conclusions ... 31
9.6 Effects on bees (KCP 10.3.1) ... 32
9.6.1 Toxicity data ... 32

9.6.1.1 Justification for new endpoints ... 33
9.6.2 Risk assessment ... 33
9.6.2.1 Hazard quotients for bees ... 33
9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies) ... 35
9.6.3 Effects on bumble bees ... 35
9.6.4 Effects on solitary bees ... 35
9.6.5 Overall conclusions ... 35
9.7 Effects on arthropods other than bees (KCP 10.3.2) ... 35
9.7.1 Toxicity data ... 35
9.7.1.1 Justification for new endpoints ... 36
9.7.2 Risk assessment ... 37
9.7.2.1 Risk assessment for in-field exposure ... 37
9.7.2.2 Risk assessment for off-field exposure ... 37
9.7.2.3 Additional higher-tier risk assessment ... 38
9.7.2.4 Risk mitigation measures ... 38
9.7.3 Overall conclusions ... 38
9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4) ... 38
9.8.1 Toxicity data ... 38
9.8.1.1 Justification for new endpoints ... 39
9.8.2 Risk assessment ... 39
9.8.2.1 First-tier risk assessment ... 39
9.8.2.2 Higher-tier risk assessment ... 40
9.8.3 Overall conclusions ... 40
9.9 Effects on soil microbial activity (KCP 10.5) ... 41
9.9.1 Toxicity data ... 41
9.9.1.1 Justification for new endpoints ... 42
9.9.2 Risk assessment ... 42
9.9.3 Overall conclusions ... 43
9.10 Effects on non-target terrestrial plants (KCP 10.6) ... 43
9.10.1 Toxicity data ... 43
9.10.1.1 Justification for new endpoints ... 45
9.10.2 Risk assessment ... 45
9.10.2.1 Tier-1 risk assessment (based screening data) ... 45
9.10.2.2 Tier-2 risk assessment (based on dose-response data) ... 45
9.10.2.3 Higher-tier risk assessment ... 47
9.10.2.4 Risk mitigation measures ... 47
9.10.3 Overall conclusions ... 47
9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7) ... 48
9.12 Monitoring data (KCP 10.8) ... 48
9.13 Classification and Labelling ... 48

Appendix 1 Lists of data considered in support of the evaluation ... 50

Appendix 2 Detailed evaluation of the new studies ... 55

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates ... 55
A 2.1.1 KCP 10.1.1 Effects on birds ... 55
A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds ... 55

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) ... 55
A 2.2 KCP 10.2 Effects on aquatic organisms ... 55
A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes ... 55
A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms ... 68
A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms ... 68
A 2.3 KCP 10.3 Effects on arthropods ... 86
A 2.3.1 KCP 10.3.1 Effects on bees ... 86
A 2.3.2 KCP 10.3.2 Effects on non-target arthropods ... 107
A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna ... 118
A 2.4.1 KCP 10.4.1 Earthworms ... 118
A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms) ... 120
A 2.5 KCP 10.5 Effects on soil nitrogen transformation ... 121
A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants ... 123
A 2.6.1 KCP 10.6.1 Summary of screening data ... 124
A 2.6.2 KCP 10.6.2 Testing on non-target plants ... 124
A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants ... 132
A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna) ... 133
A 2.8 KCP 10.8 Monitoring data ... 133

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-pods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Poland	Winter wheat, Winter triticale Winter rye	F	weeds (for details please refer to dRR Section B0 and B3)	broadcast spraying	BBCH 10-29 Autumn application post emer-gence	a) 1 b) 1	NR	0.2 – 0.3 L/ha a) 0.3 L/ha b) 0.3 L/ha	100-150 g a) 150 g b) 150 g	100-400 L/ha	NR	NR	A	A	R	A	A	A	R
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

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

9.1.1 Overall conclusions

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

Birds

Effects on birds for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. However further data on Diflufenikan 500 SC is not relevant as data for each active substance on toxicity to birds are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for Diflufenikan 500 SC with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of Diflufenikan 500 SC to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substances, as well as SV_{90} and SV_m.

Drinking water exposure (leaf scenario) has not been estimated since Diflufenikan 500 SC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures. Drinking water exposure (puddle scenario) has not been performed since the ratio of effective application rate to relevant endpoint does not exceed 50 (Koc < 500 L/kg).

Exposure for earthworm-eating birds and fish-eating birds via secondary poisoning has also been estimated since log P_{ow} of diflufenican is above the trigger value of 3.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to birds from the proposed use of Diflufenikan 500 SC. No risk management measures are required.

Terrestrial vertebrates (other than birds)

Effects on mammals for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. However further data on Diflufenikan 500 SC is not relevant as data for each active substance on toxicity to mammals are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for Diflufenikan 500 SC with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of Diflufenikan 500 SC to terrestrial vertebrates other than birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with diflufenican, as well as SV_{90} and SV_m.

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

Exposure for earthworm-eating mammals and fish-eating mammals via secondary poisoning has also been estimated since log P_{ow} of diflufenican is above the trigger value of 3.

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

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Effects on aquatic organisms for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. The studies on effects of Diflufenikan 500 SC on algae, Daphnia and aquatic plants were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC.

Risk assessments for Diflufenikan 500 SC with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290.

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance/reference formulation, metabolites and formulation Diflufenikan 500 SC. PECsw Step 3/RAC values for active substance were less than 1 for few scenarios including scenarios relevant for Poland so further evaluation with Step 4 PECsw was performed.

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that Diflufenikan 500 SC used according to proposed GAP does not pose unacceptable risk to aquatic organisms provided 5m buffer zone is applied.

Classification of Diflufenikan 500 SC was done on the basis of formulation Diflufenikan 500 SC studies' results as well as active substance and co-formulants properties. The proposed classification of the product Diflufenikan 500 SC is:

Aquatic Acute 1, H400
Aquatic Chronic 1, H410

9.1.1.3 For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that Diflufenikan 500 SC used at according to proposed GAP does not pose unacceptable risk to aquatic organisms provided that 5m buffer zone is applied. -31

zRMS comments:

The risk assessment for aquatic organisms is agreed by the zRMS.
The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).
For fish, aquatic invertebrates and algae acceptable acute and chronic risk for a.s.- diflufenican and its metabolites could be concluded already for Step 4 PEC_{sw} values.
The initial risk assessment was based on the worst case PEC values and the results of laboratory toxicity testing. The PEC_{sw} Step 1-2 and Step 3 and 4 (for a.s.) were used.

Refinement risk assessment for algae was corrected by RMS.
Justification: RAC is Regulatory Acceptable Concentration. For diflufenican it is 0.00042 mg diflufenikan/mL set by the toxicity endpoint of Algae (*Scenedesmus subspicatus*) reproduction EC_{50}=0.0042 mg diflufenikan/mL, Reference to EFSA Scientific Report (2007) 122,1-84, Conclusion on the peer review of Diflufenican.

Algae: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data with mitigation of spray drift and run-off for the use of Diflufenikan 500 SC in winter cereals

Scenario	No-spray buffer (m)	-	-	5	-	10	-	20
	Vegetated filter strip (m)	-	5	5 VFS	10	10 VFS	20	20 VFS
	Nozzle reduction (%)	PEC_{sw} (µg/L)						
D1/ditch	None	0.9800	0.3381	0.3381	0.3381	0.3381	0.3381	0.3381
D1/stream	None	0.8417	0.3076	0.3076	0.2122	0.2122	0.2122	0.2122
D2/ditch	None	1.064	0.6678	0.6678	0.6678	0.6678	0.6678	0.6678
D2/stream	None	0.8931	0.4213	0.4213	0.4213	0.4213	0.4213	0.4213
D3/ditch	None	0.9482	0.2571	0.2571	0.1363	0.1363	0.07082	0.07082
D4/pond	None	0.03818	0.03672	0.03672	0.03409	0.03409	0.03186	0.03186
D4/stream	None	0.8223	0.3004	0.3004	0.1593	0.1593	0.1307	0.1307
D5/pond	None	0.03305	0.02862	0.0286	0.02064	0.02064	0.01385	0.01385
D5/stream	None	0.8872	0.3241	0.3241	0.1719	0.1719	0.08930	0.08930
D6/ditch	None	0.9589	0.4453	0.4453	0.4453	0.4453	0.4453	0.4453
R1/pond	None	0.08077	0.07881	0.02838	0.07531	0.02040	0.07234	0.01362
R1/stream	None	0.6251	0.4292	0.2284	0.4292	0.1211	0.4292	0.06293
R3/stream	None	0.8678	0.5038	0.3171	0.5038	0.1887	0.5038	0.1246
R4/stream	None	0.6290	0.6166	0.2298	0.6166	0.1219	0.6166	0.06331
RAC (µg/L)=~~0.75~~ RAC (µg/L) = 0.42		PEC/RAC ratio						
D1/ditch	None	**2,333333**	0,805	0,805	0,805	0,805	0,805	0,805
D1/stream	None	**2,004048**	0,732381	0,732381	0,505238	0,505238	0,505238	0,505238
D2/ditch	None	**2,533333**	**1,59**	**1,59**	**1,59**	**1,59**	**1,59**	**1,59**
D2/stream	None	**2,126429**	**1,003095**	**1,003095**	**1,003095**	**1,003095**	**1,003095**	**1,003095**
D3/ditch	None	**2,257619**	0,612143	0,612143	0,324524	0,324524	0,168619	0,168619
D4/pond	None	0,090905	0,087429	0,087429	0,081167	0,081167	0,075857	0,075857
D4/stream	None	**1,957857**	0,715238	0,715238	0,379286	0,379286	0,31119	0,31119
D5/pond	None	0,07869	0,068143	0,068095	0,049143	0,049143	0,032976	0,032976
D5/stream	None	**2,112381**	0,771667	0,771667	0,409286	0,409286	0,212619	0,212619
D6/ditch	None	**2,283095**	**1,060238**	**1,060238**	**1,060238**	**1,060238**	**1,060238**	**1,060238**
R1/pond	None	0,19231	0,187643	0,067571	0,17931	0,048571	0,172238	0,032429
R1/stream	None	**1,488333**	**1,021905**	0,54381	**1,021905**	0,288333	**1,021905**	0,149833
R3/stream	None	**2,06619**	**1,199524**	0,755	**1,199524**	0,449286	**1,199524**	0,296667
R4/stream	None	**1,497619**	**1,468095**	0,547143	**1,468095**	0,290238	**1,468095**	0,150738

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

The aquatic risk assessment was based on exposure to diflufenican alone. The tests with the formulation Diflufenikan 500 SC suggest lower toxicity to aquatic organisms compared to technical diflufenican. Therefore it was agreed that the risk from the formulation would be covered by the risk assessment for active substance.
The Diflufenikan 500 SC applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures. **zRMS is of the opinion, that relevant mitigation measures will be proposed at the Member State level.**

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that Diflufenikan 500 SC used at according to proposed GAP does not pose unacceptable risk to aquatic organisms provided that 5m vegetative buffer zone is applied.

9.1.1.4 Effects on bees (KCP 10.3.1)

Effects on bees for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. The studies on effects of Diflufenikan 500 SC on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC.

Risk assessments for Diflufenikan 500 SC with the proposed use pattern was carried out according to the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the latest Draft EFSA Guidance for risk assessment for bees EFSA Journal 2013; 11(7):3295.

The risk of Diflufenikan 500 SC to honeybees was assessed from Hazard Quotients (HQ) and Exposure Toxicity Ratio (ETR) between toxicity endpoints, estimated from acute oral and contact studies with active ingredient and formulated product as well as the maximum single application rate of 0.3 L/ha (150 g as/ha).

All the Hazard Quotients and Exposure Toxicity Ratios were considerably less than the respective triggers, indicating that Diflufenikan 500 SC does not pose an unacceptable risk to bees. No risk management measures are required.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

Effects on non-target arthropods for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. However, the provision of further data on the formulation is not considered essential, because it is possible to extrapolate from data for the reference formulation. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

Risk assessments for Diflufenikan 500 SC with the proposed use pattern was carried out according to the guidance for risk assessment for arthropods “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and in consideration of the recommendations of the guidance document ESCORT 2.

The risk of Diflufenikan 500 SC to non-target arthropods was assessed from in-field and off-field HQ between toxicity endpoints, estimated from laboratory studies with the reference formulation that is similar to Diflufenican 500 SC as well as the maximum single application rate. The in-field and off-field PER values were considerably less than the maximum tested rate with effects below 50%, indicating that the product Diflufenican 500 SC poses a low risk to non-target arthropods. It can be concluded that Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk management measures are required.

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

Effects on earthworms and other soil micro-organisms for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. The studies on effects of Diflufenikan 500 SC on earthworms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC.

Risk assessments for Diflufenikan 500 SC with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).

Earthworms and collembola *Folsomia candida*
The risk of Diflufenikan 500 SC to earthworms and other soil macro-organisms i.e. *Hypoaspis aculeifer* and *Folsomia candida* was assessed from toxicity exposure ratios (TERs) between the selected toxicity endpoint for the active ingredient, metabolites and the formulated product Diflufenikan 500 SC as well as the maximum soil PECs.

The acute and chronic TER values were greater than the trigger of 10 and 5 respectively indicating application of Diflufenikan 500 SC does not pose unacceptable risk to earthworms and other soil macro-organisms. No risk management measures are required.

Micro-organisms

The risk of Diflufenikan 500 SC to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and the formulated product Diflufenikan 500 SC with predicted application concentrations (PECs) or application rate for active substance, metabolites and the formulation.

According to the performed risk assessment it was assessed that the application of Diflufenikan 500 does not pose unacceptable risk to soil micro-organisms. No risk management measures are required.

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

Effects on non-target terrestrial plants for Diflufenikan 500 SC were not evaluated as part of the EU review of diflufenican. The studies on seedling emergence and vegetative vigour for Diflufenikan 500 SC were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC.

The risk of Diflufenikan 500 SC to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation Diflufenikan 500 SC and off-field predicted environmental rate.

According to the performed risk assessment it was assessed that the application of Diflufenikan 500 SC does not pose unacceptable risk to non-target plants. No risk management measures are required.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

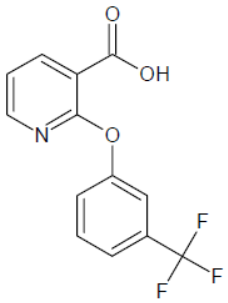
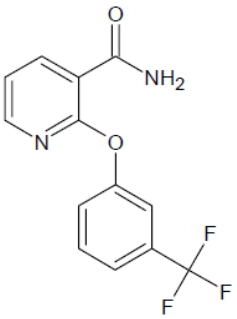
Table 9.1-2: Critical use pattern of Diflufenikan 500 SC grouped according to criterion

Grouping according to criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	winter cereals	application rate	NR

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of Diflufenikan 500 SC is indicated in the table.

Table 9.1-3 Metabolites of diflufenican

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments relevant for PEC calculation	Exposure assessment required due to
AE B107137	283		soil: 16.8% water/sediment: 35.7%	PECs: yes PECsw: yes PECsed: no
AE 0542291	282		soil: 26.3% water/sediment: 0.01%	PECs: yes PECsw: yes PECsed: no

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. However, the provision of further data on the Diflufenikan 500 SC is not considered essential, because it is possible to extrapolate data from the active substances. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Diflufenican	Acute	**LD_{50} > 2150 mg as/kg bw per day**	EFSA Scientific Report (2007) 122, 1-84
Bobwhite quail	Diflufenican	Long-term	**NOEL=91.84 mg as/kg bw per day**	

9.2.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

zRMS comments: Avian toxicity data presented in Table 9.2-1 are in general in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 122, 1-84 for diflufenican.
It is noted that the acute toxicity study for **Diflufenican 500 SC** for birds is not provided. However, the vertebrate toxicity testing must be performed only when crucial for the evaluation.
Therefore, the provision of further data on the formulation **Diflufenikan 500 SC** is not considered essential, because risk to mammals may be sufficiently assessed using the EU agreed endpoints and new studies should not be conducted in regards of animal welfare.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of Diflufenikan 500 SC in cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)				
Active substance/product	diflufenican				
Application rate (g/ha)	1 × 150				
Acute toxicity (mg/kg bw)	2150				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small omnivorous bird	158.8	1	23.82	90.3
Reprod. toxicity (mg/kg bw/d)	91.84				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small omnivorous bird	64.8	1 × 0.53	5.15	17.8

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

zRMS comments:
The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438.
The presented above birds risk assessment is accepted by the zRMS. All TER values exceed the relevant triggers indicating Diflufenikan 500 SC that does not pose an unacceptable risk to birds following applications according to

recommended use pattern. On the basis of performed calculations, acceptable acute and long-term risk to birds may be concluded from proposed uses of Diflufenikan 500 SC.

9.2.2.2 Higher-tier risk assessment

Not relevant.

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 Diflufenikan 500 SC 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 above 3000, diflufenican belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	150		
Acute toxicity (mg/kg bw)	=	2150	quotient =	0.07
Reprod. toxicity (mg/kg bw/d)	=	91.84	quotient =	1.63

zRMS comments:
Screening evaluation of the risk resulting from exposure to diflufenican via drinking water is agreed by the zRMS. It is not necessary to conduct a drinking water risk assessment for birds.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of diflufenican is above the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.

Table 9.2-3: Assessment of the risk for earthworm-eating birds due to exposure to diflufenican bioaccumulation in earthworms (secondary poisoning) (winter cereals)

Parameter	diflufenikan	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	~~0.1977~~ **0.5980**	~~21 TWA PECs~~ Average plateau concentration PECs (dRR Part B8)*
log P_{ow} / P_{ow}	4.2 / 15848.9	EFSA Scientific Report (2007) 122, 1-84
Koc	3090.6	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	3.09	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~0.61~~ 1.85	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~0.64~~ 1.94	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	91.84	-
TER_{lt}	~~143.5~~ 47.34	-

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

Risk assessment for fish-eating birds via secondary poisoning

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

Table 9.2-4: Assessment of the risk for fish-eating birds due to exposure to diflufenican via bioaccumulation in fish (secondary poisoning) (winter cereals)

Parameter	diflufenikan	comments
PEC_{sw} (twa = 21 d) (mg/L)	~~0.009654~~ 0.004192	~~Step 1 21d TWA PECsw (dRR Part B8)~~ Step 2
BCF_{fish}	1596	1596
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	~~15.41~~ 6.69	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~2.45~~ 1.06	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	91.84	-
TER_{lt}	~~37.49~~ 86.64	-

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

Not relevant. First-tier risk assessment confirmed that Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable acute and long term/reproductive risk to birds. No risk mitigations are required.

zRMS comment:

The acute and chronic risks of **Diflufenikan 500 SC** to birds were assessed based on the toxicity exposure ratios (TER) between toxicity endpoints, estimated from study with active ingredient, and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that **Diflufenican 500 SC** does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low and acceptable.

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

Effects on mammals of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. However, the provision of further data on the Diflufenikan 500 SC is not considered essential, because it is possible to extrapolate data from the active substances. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Diflufenican	Acute	LD_{50}>5000 mg as/kg bw	EFSA Scientific Report

Species	Substance	Exposure System	Results	Reference
Rat	Diflufenican	Long-term	**NOEL=35.5 mg as/kg bw/d**	(2007) 122, 1-84

9.3.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

zRMS comments:
Avian toxicity data presented in Table 9.3-1 are in general in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 122, 1-84 for diflufenican. Accepted.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use **Active substance/product** **Application rate (g/ha)**	winter cereals (Use-No. 1) diflufenican 1 × 150				
Acute toxicity (mg/kg bw) **TER criterion**	>5000 10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small herbivorous mammal	118.4	1	17.8	280.9
Reprod. toxicity (mg/kg bw/d) **TER criterion**	35.5 5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small herbivorous mammal	48.3	1 × 0.53	3.8	9.3

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

zRMS comments:
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).

The presented above mammals risk assessment is agreed by the zRMS. All TER_a values exceed the relevant triggers indicating that **Diflufenican 500 SC** does not pose an unacceptable acute risk to mammals following applications according to recommended use pattern.

9.3.2.2 Higher-tier risk assessment

Not relevant.

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 above 3000, diflufenican belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	150		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	35.5	quotient =	4.23

zRMS comments:
Screening evaluation of the risk resulting from exposure to diflufenican via drinking water is agreed by the zRMS. It is not necessary to conduct a drinking water risk assessment for mammals.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of diflufenican is above the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

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

Table 9.3-3: Assessment of the risk for earthworm-eating mammals due to exposure to diflufenican via bioaccumulation in earthworms (secondary poisoning) (winter cereals)

Parameter	diflufenican	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	~~0.1977~~ **0.5980**	~~21 TWA PECs~~ Average plateau concentration PECs (dRR Part B8)*
log P_{ow} / P_{ow}	4.2 / 15848.9	EFSA Scientific Report (2007) 122, 1-84

Parameter	diflufenican	comments
Koc	3090.6	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	3.09	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~0.61~~ 1.85	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~0.78~~ 2.37	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	35.5	-
TER_{lt}	~~45.51~~ 14.98	-

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

Risk assessment for fish-eating mammals via secondary poisoning

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

Table 9.3-4: Assessment of the risk for fish-eating mammals due to exposure to diflufenican via bioaccumulation in fish (secondary poisoning) (winter cereals)

Parameter	diflufenican	comments
PEC_{sw} (twa = 21 d) (mg/L)	~~0.009654~~ 0.004192	~~Step 1 21d TWA PECsw (dRR Part B8)~~ Step 2
BCF_{fish}	1596	EFSA Scientific Report (2007) 122, 1-84
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	~~15.41~~ 6.69	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~2.19~~ 0.95	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	35.5	-
TER_{lt}	~~16.22~~ 37.37	-

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

Not relevant. First-tier risk assessment confirmed that Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable acute and long term/reproductive risk to mammals. No risk mitigations are required.

zRMS comment:

The acute and chronic risks of **Diflufenikan 500 SC** to mammals were assessed based on toxicity exposure ratios (TER) between toxicity endpoints, estimated from study with active ingredient, and maximum and the refined residues occurring on food items. No additional assessment for formulation was required.

All TER values exceed the relevant triggers indicating that **Diflufenikan 500 SC** does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk.
The risk to earthworm- and fish-eating animals from secondary poisoning is low and acceptable.

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

Not relevant.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with diflufenican, its metabolites and representative formulation. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on effects of Diflufenikan 500 SC on *Daphnia*, algae and aquatic plants were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Diflufenican				
C. carpio	diflufenican	96 h	**LC_{50} > 0.0985 mg/L***	EFSA Scientific Report (2007) 122, 1-84
Oncorhynchus mykiss	diflufenican	35 d	**NOEC = 0.015 mg/L**	
Oncorhynchus mykiss	FOE 5043 and diflufenican WG 60 (39.6% flufenacet, 18.8% diflufenican)	96 h	LC_{50} = 12.3 mg/L	
Daphnia magna	diflufenican	48 h	**EC_{50} > 0.24 mg/L***	
Daphnia magna	diflufenican	21 d	**NOEC = 0.052 mg/L**	
Daphnia magna	FOE 5043 and diflufenican WG 60 (39.6% flufenacet, 18.8% diflufenican)	48 h	EC_{50} > 100 mg/L	
Chironomus riparius spiked water	diflufenican	28 d	NOEC = 0.10 mg/L	
Chironomus riparius spiked sediment	diflufenican	28 d	**NOEC = 2.0 mg/kg sediment**	
(*Scenedesmus subspicatus*) Without sediment	diflufenican	72 h	**E_bC_{50} = 0.00025 mg/L** E_rC_{50} = 0.00045 mg/L NOEC = 0.0001 mg/L	
(*Scenedesmus subspicatus*) With sediment	diflufenican	72 h	E_bC_{50} = 0.0024 mg/L E_rC_{50} = 0.0047 mg/L NOEC = 0.00076 mg/L	
(*Scenedesmus subspicatus*) Without sediment	diflufenican	72 h	E_bC_{50} = 0.00046 mg/L E_rC_{50} = 0.00122 mg/L **Maximum concn. from which recovery possible 0.0042 mg/L** NOEC = 0.00015 mg/L	
(*Selenastrum capricornutum*)	FOE 5043 and diflufenican SC 600 (401.5g flufenacet/L, 217.0g diflufenican/L) i.e. 'Herold SC'	72 h	E_bC_{50} = 0.0024 mg/L E_rC_{50} = 0.0063 mg/L	

Species	Substance	Exposure System	Results	Reference
Lemna gibba	diflufenican	14 d	E_bC_{50} = 0.056 mg/L **EC_{50} = 0.039 mg/L** frond density	
Lemna gibba G3	FOE 5043 and diflufenican SC 600 (401.5g flufenacet/L, 217.0g diflufenican/L) i.e. 'Herold SC'	7 d	E_bC_{50} = 0.258 mg/L dry weight EC_{50} = 0.307 mg/L frond counts	
Metabolites				
Oncorhynchus mykiss	AE B107137	96 h	**LC_{50} > 17.3 mg/L***	EFSA Scientific Report (2007) 122, 1-84
Daphnia magna	AE B107137	48 h	**EC_{50} > 20.4 mg/L***	
(*Scenedesmus subspicatus*) Without sediment	AE B107137	72 h	**E_bC_{50} >20.4 mg/L*** E_rC_{50} >20.4 mg/L*	
Chironomus riparius	AE C522392	72 h	NOEC = 1.0 mg/kg sediment	
Daphnia magna	AE 0542291	48 h	**EC_{50} > 10 mg/L**	
(*Scenedesmus subspicatus*) Without sediment	AE 0542291	72 h	**E_bC_{50} = 36 mg/L** E_rC_{50} = 66 mg/L	
(*Pseudokirchneriella subcapitata*)	AE 592370	72 h	E_bC_{50} = 39 mg/L E_rC_{50} = 58 mg/L	
(*Pseudokirchneriella subcapitata)*	AE C522392	72 h	E_bC_{50} = 3.4 mg/L E_rC_{50} = 16 mg/L	
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
*above the visual limit of solubility

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Diflufenikan 500 SC

Species	Substance	Exposure System	Results	Reference
Daphnia magna	Diflufenikan 500 SC	48 h, ss	EC_{50} > 100 mg/L nom (**41.88 mg as/Lnom**)*	KCP 10.2.1.3/01 / Czarnecka M / 2022 / W-07-22
Raphidocelis subcapitata	Diflufenikan 500 SC	72 h	E_rC_{50}=0.589 µg/L nom (**0.247 µg as/L nom**)*	KCP 10.2.1.3/01 / Czarnecka M/ 2022/

Species	Substance	Exposure System	Results	Reference
			E_yC_{50}=0.138 µg/L nom (0.058 µg as/L nom)*	W-08-22
Lemna gibba	Diflufenikan 500 SC	7 d, ss	Frond number E_rC_{50}=0.5645 mg/L nom (**0.1783 mg as/L mm**) E_yC_{50}=0.0524 µg/L nom (0.0208 mg as/L mm) Dry weight E_rC_{50}>5 mg/L nom (1.5636 mg as/L mm) E_yC_{50}=0.6194 µg/L nom (0.1928 mg as/L mm)	KCP 10.2.1.4/01 / Czarnecka M/ 2022/ W-09-22
Myriophyllum spicatum	Diflufenikan 500 SC	14d, s	Shoot length: E_rC_{50} > 200 mg/kg nom (83.75 mg as/kg nom) E_yC_{50}=91.16 mg/kg nom (38.17 mg as/kg nom) Fresh weight: E_rC_{50} =403.15 mg/kg nom (168.82 mg as/kg nom) E_yC_{50}=24.45 mg/kg nom (10.24 mg as/kg nom) Dry weight: E_rC_{50} >200 mg/kg nom (83.75 mg as/kg nom) E_yC_{50}>200 mg/kg nom (83.75 mg as/kg nom)	KCP 10.2.1.4/02 / Czarnecka M/ 2022/ W-06-22
Higher-tier studies (micro- or mesocosm studies)				
-				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
*value calculated with density of Diflufenikan 500 SC is 1.194 g/ml and nominal content of diflufenican 500 g/L

9.5.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Diflufenikan 500 SC. Details of studies and results are included in Table 9.5-2. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. Risk assessment was performed with active substance endpoints and formulation endpoints.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for diflufenican for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

		Active substance						**Plant protection product**				**Active substance**
		Fish acute	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Acuatic plants**	**Inverteb. acute**	**Algae**	**Acuatic plants**		**Sediment dwelling organism**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***		*Chironomus riparius*
Endpoint (µg/L)		LC_{50} >98.5	NOEC 15	EC_{50} >240	NOEC 52	E_bC_{50} 0.25	EC_{50} 39	EC_{50} >41880	E_bC_{50} 247	EC_{50} 178.3		NOEC 2000
AF		100	10	100	10	10	10	100	10	10		10
RAC (µg/L)		>0.985	1.5	>2.4	5.2	0.025	3.9	>418.8	24.7	17.83		200
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**										**PEC $_{gl-max}$ (µg/kg)**	
Step 1												
-	11.144	**11.31**	**7.43**	**4.64**	**2.14**	**445.76**	**2.86**	0.03	0.45	0.63	308.869	**1.54**
Step 2												
N-Europe	5.148	**5.23**	**3.43**	**2.15**	0.99	**205.92**	**1.32**	0.01	0.21	0.29	155.266	0.78
S-Europe	4.192	**4.26**	**2.79**	**1.75**	0.81	**167.68**	**1.07**	0.01	0.17	0.24	125.853	0.63
Step 3												
D1/ditch	0.9800	0.99	0.65	0.41	0.19	**39.20**	0.25	0.00	0.04	0.05	6.804	0.03
D1/stream	0.8417	0.85	0.56	0.35	0.16	**33.67**	0.22	0.00	0.03	0.05	3.317	0.02
D2/ditch	1.064	**1.08**	0.71	0.44	0.20	**42.56**	0.27	0.00	0.04	0.06	5.327	0.03

		Active substance						Plant protection product				Active sub-stance
		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Acuatic plants	Inverteb. acute	Algae	Acuatic plants		Sediment dwelling organism
D2/stream	0.8931	0.91	0.60	0.37	0.17	**35.72**	0.23	0.00	0.04	0.05	3.087	0.02
D3/ditch	0.9482	0.96	0.63	0.40	0.18	**37.93**	0.24	0.00	0.04	0.05	0.5024	0.00
D4/pond	0.03818	0.04	0.03	0.02	0.01	**1.53**	0.01	0.00	0.00	0.00	0.4584	0.00
D4/stream	0.8223	0.83	0.55	0.34	0.16	**32.89**	0.21	0.00	0.03	0.05	0.1745	0.00
D5/pond	0.03305	0.03	0.02	0.01	0.01	**1.32**	0.01	0.00	0.00	0.00	0.2877	0.00
D5/stream	0.8872	0.90	0.59	0.37	0.17	**35.49**	0.23	0.00	0.04	0.05	0.2449	0.00
D6/ditch	0.9589	0.97	0.64	0.40	0.18	**38.36**	0.25	0.00	0.04	0.05	2.412	0.01
R1/pond	0.08077	0.08	0.05	0.03	0.02	**3.23**	0.02	0.00	0.00	0.00	1.030	0.01
R1/stream	0.6251	0.63	0.42	0.26	0.12	**25.00**	0.16	0.00	0.03	0.04	0.9975	0.00
R3/stream	0.8678	0.88	0.58	0.36	0.17	**34.71**	0.22	0.00	0.04	0.05	44.32	0.22
R4/stream	0.6290	0.64	0.42	0.26	0.12	**25.16**	0.16	0.00	0.03	0.04	0.7582	0.00

Risk assessment refinement for fish

The PEC/RAC for fish D2 ditch scenario can be refined by application buffer zones. Details in table below.

Table 9.5-4: Fish: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data with mitigation of spray drift and run-off for the use of Diflufenikan 500 SC in winter cereals

	No-spray buffer (m)	**-**	**-**	**5**	**-**	**10**	**-**	**20**
	Vegetated filter strip (m)	**-**	**5**	**5 VFS**	**10**	**10 VFS**	**20**	**20 VFS**
	Nozzle reduction (%)							
Scenario		**PEC_{sw} (µg/L)**						
D2/ditch	None	1.064	0.6678	0.6678	0.6678	0.6678	0.6678	0.6678
RAC (µg/L)=0.985		**PEC/RAC ratio**						
D2/ditch	None	**1.08**	0.68	0.68	0.68	0.68	0.68	0.68

The acute exposure for fish D2 ditch scenario is acceptable provided the 5m buffer zone is applied. No risk management is required for other scenarios.

Risk assessment refinement for algae

The higher tier risk refinement was done with mesocosm endpoint of NOEC = 0.75 µg/L and AF=1. Summary and details of the mesocosm study are in Appendix 2. The mesocosm study was evaluated as acceptable for algae risk refinement. In the mesocosm study the product was applied to directly to the water body of the system in spring as the most powerful to detect possible effects. Moreover, the classes on the taxon level used for determination of endpoints are based on the most sensitive taxon found in the community.

Table 9.5-5: Algae: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data with mitigation of spray drift and run-off for the use of Diflufenikan 500 SC in winter cereals (mesocosms endpoint)

Scenario	**No-spray buffer (m)**	**-**	**-**	**5**	**-**	**10**	**-**	**20**
	Vegetated filter strip (m)	**-**	**5**	**5 VFS**	**10**	**10 VFS**	**20**	**20 VFS**
	Nozzle reduction (%)	**PEC_{sw} (µg/L)**						
D1/ditch	None	0.9800	0.3381	0.3381	0.3381	0.3381	0.3381	0.3381
D1/stream	None	0.8417	0.3076	0.3076	0.2122	0.2122	0.2122	0.2122
D2/ditch	None	1.064	0.6678	0.6678	0.6678	0.6678	0.6678	0.6678
D2/stream	None	0.8931	0.4213	0.4213	0.4213	0.4213	0.4213	0.4213
D3/ditch	None	0.9482	0.2571	0.2571	0.1363	0.1363	0.07082	0.07082
D4/pond	None	0.03818	0.03672	0.03672	0.03409	0.03409	0.03186	0.03186
D4/stream	None	0.8223	0.3004	0.3004	0.1593	0.1593	0.1307	0.1307
D5/pond	None	0.03305	0.02862	0.0286	0.02064	0.02064	0.01385	0.01385
D5/stream	None	0.8872	0.3241	0.3241	0.1719	0.1719	0.08930	0.08930
D6/ditch	None	0.9589	0.4453	0.4453	0.4453	0.4453	0.4453	0.4453
R1/pond	None	0.08077	0.07881	0.02838	0.07531	0.02040	0.07234	0.01362
R1/stream	None	0.6251	0.4292	0.2284	0.4292	0.1211	0.4292	0.06293
R3/stream	None	0.8678	0.5038	0.3171	0.5038	0.1887	0.5038	0.1246

R4/stream	None	0.6290	0.6166	0.2298	0.6166	0.1219	0.6166	0.06331
RAC (µg/L)=~~0.75~~		**PEC/RAC ratio**						
D1/ditch	None	~~**1.31**~~	~~0.45~~	~~0.45~~	~~0.45~~	~~0.45~~	~~0.45~~	~~0.45~~
D1/stream	None	~~**1.12**~~	~~0.41~~	~~0.41~~	~~0.28~~	~~0.28~~	~~0.28~~	~~0.28~~
D2/ditch	None	~~**1.42**~~	~~0.89~~	~~0.89~~	~~0.89~~	~~0.89~~	~~0.89~~	~~0.89~~
D2/stream	None	~~**1.19**~~	~~0.56~~	~~0.56~~	~~0.56~~	~~0.56~~	~~0.56~~	~~0.56~~
D3/ditch	None	~~**1.26**~~	~~0.34~~	~~0.34~~	~~0.18~~	~~0.18~~	~~0.09~~	~~0.09~~
D4/pond	None	~~0.05~~	~~0.05~~	~~0.05~~	~~0.05~~	~~0.05~~	~~0.04~~	~~0.04~~
D4/stream	None	~~**1.10**~~	~~0.40~~	~~0.40~~	~~0.21~~	~~0.21~~	~~0.17~~	~~0.17~~
D5/pond	None	~~0.04~~	~~0.04~~	~~0.04~~	~~0.03~~	~~0.03~~	~~0.02~~	~~0.02~~
D5/stream	None	~~**1.18**~~	~~0.43~~	~~0.43~~	~~0.23~~	~~0.23~~	~~0.12~~	~~0.12~~
D6/ditch	None	~~**1.28**~~	~~0.59~~	~~0.59~~	~~0.59~~	~~0.59~~	~~0.59~~	~~0.59~~
R1/pond	None	~~0.11~~	~~0.11~~	~~0.04~~	~~0.10~~	~~0.03~~	~~0.10~~	~~0.02~~
R1/stream	None	~~0.83~~	~~0.57~~	~~0.30~~	~~0.57~~	~~0.16~~	~~0.57~~	~~0.08~~
R3/stream	None	~~**1.16**~~	~~0.67~~	~~0.42~~	~~0.67~~	~~0.25~~	~~0.67~~	~~0.17~~
R4/stream	None	~~0.84~~	~~0.82~~	~~0.31~~	~~0.82~~	~~0.16~~	~~0.82~~	~~0.08~~

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

In accordance with higher tier risk assessment with mesocosm studies it can be concluded that the risk for algae is acceptable provided:
- 5m buffer strip is applied for scenarios: D1 ditch, D1 stream, D2 ditch, D2 stream, D3 ditch, D4 stream, D5 stream, D6 ditch and R3 stream.
No risk mitigations measures are required for scenarios: D4 pond, D5 pond, R1 pond, R1 stream and R4 stream.

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE B107137 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Acuatic plants**
Test species		***Onchorhynchus mykiss***	-	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***
Endpoint		EC_{50}	NOEC	EC_{50}	NOEC	E_bC_{50}	EC_{50}
(µg/L)		>17 300	-	>20 400	-	>20 400	-
AF		100	10	100	10	10	10
RAC (µg/L)		>173	-	>204	-	>2040	-
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
-	18.9335	0.11	-	0.09	-	0.01	0.11

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 AE 0542291 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**
Test species		***Brachydanio rerio***	-	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		EC_{50}	NOEC	EC_{50}	NOEC	E_bC_{50}
(µg/L)		-	-	> 10 000	-	36 000
AF		100	10	100	10	10
RAC (µg/L)		-	-	>100	-	3600
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
-	8.0238	-	-	0.08	-	0.00

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

9.5.3 Overall conclusions

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity end-points from studies for active substance/reference formulation, metabolites and formulation Diflufenikan 500 SC. On the basis of PECsw Step 4/RAC values it was concluded that the application of Diflufenikan 500 SC does not pose unacceptable risk for aquatic organisms provided that appropriate risk mitigations are applied.

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that Diflufenikan 500 SC used at according to proposed GAP does not pose unacceptable risk to aquatic organisms provided that 5m buffer zone is applied. -31

zRMS comments:

The risk assessment for aquatic organisms is agreed by the zRMS.
The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).
For fish, aquatic invertebrates and algae acceptable acute and chronic risk for a.s.- diflufenican and its metabolites could be concluded already for Step 4 PEC_{sw} values.
The initial risk assessment was based on the worst case PEC values and the results of laboratory toxicity testing. The PEC_{sw} Step 1-2 and Step 3 and 4 (for a.s.) were used.

Refinement risk assessment for algae was corrected by RMS.
Justification: RAC is Regulatory Acceptable Concentration. For diflufenican it is 0.00042 mg diflufenikan/mL set by the toxicity endpoint of Algae (*Scenedesmus subspicatus*) reproduction EC_{50}=0.0042 mg diflufenikan/mL, Reference to EFSA Scientific Report (2007) 122,1-84, Conclusion on the peer review of Diflufenican.

Algae: PEC calculation and acceptability of risk (PEC/RAC < 1) for diflufenican based on FOCUS Step 4 calculations and toxicity data with mitigation of spray drift and run-off for the use of Diflufenikan 500 SC in winter cereals

Scenario	No-spray buffer (m)	-	-	5	-	10	-	20
	Vegetated filter strip (m)	-	5	5 VFS	10	10 VFS	20	20 VFS
	Nozzle reduction (%)	PEC_{sw} (µg/L)						
D1/ditch	None	0.9800	0.3381	0.3381	0.3381	0.3381	0.3381	0.3381
D1/stream	None	0.8417	0.3076	0.3076	0.2122	0.2122	0.2122	0.2122
D2/ditch	None	1.064	0.6678	0.6678	0.6678	0.6678	0.6678	0.6678
D2/stream	None	0.8931	0.4213	0.4213	0.4213	0.4213	0.4213	0.4213
D3/ditch	None	0.9482	0.2571	0.2571	0.1363	0.1363	0.07082	0.07082
D4/pond	None	0.03818	0.03672	0.03672	0.03409	0.03409	0.03186	0.03186
D4/stream	None	0.8223	0.3004	0.3004	0.1593	0.1593	0.1307	0.1307
D5/pond	None	0.03305	0.02862	0.0286	0.02064	0.02064	0.01385	0.01385
D5/stream	None	0.8872	0.3241	0.3241	0.1719	0.1719	0.08930	0.08930
D6/ditch	None	0.9589	0.4453	0.4453	0.4453	0.4453	0.4453	0.4453
R1/pond	None	0.08077	0.07881	0.02838	0.07531	0.02040	0.07234	0.01362
R1/stream	None	0.6251	0.4292	0.2284	0.4292	0.1211	0.4292	0.06293
R3/stream	None	0.8678	0.5038	0.3171	0.5038	0.1887	0.5038	0.1246
R4/stream	None	0.6290	0.6166	0.2298	0.6166	0.1219	0.6166	0.06331
RAC (µg/L)=~~0.75~~ RAC (µg/L) = 0.42		PEC/RAC ratio						
D1/ditch	None	**2,333333**	0,805	0,805	0,805	0,805	0,805	0,805
D1/stream	None	**2,004048**	0,732381	0,732381	0,505238	0,505238	0,505238	0,505238
D2/ditch	None	**2,533333**	**1,59**	**1,59**	**1,59**	**1,59**	**1,59**	**1,59**
D2/stream	None	**2,126429**	**1,003095**	**1,003095**	**1,003095**	**1,003095**	**1,003095**	**1,003095**
D3/ditch	None	**2,257619**	0,612143	0,612143	0,324524	0,324524	0,168619	0,168619
D4/pond	None	0,090905	0,087429	0,087429	0,081167	0,081167	0,075857	0,075857
D4/stream	None	**1,957857**	0,715238	0,715238	0,379286	0,379286	0,31119	0,31119
D5/pond	None	0,07869	0,068143	0,068095	0,049143	0,049143	0,032976	0,032976
D5/stream	None	**2,112381**	0,771667	0,771667	0,409286	0,409286	0,212619	0,212619
D6/ditch	None	**2,283095**	**1,060238**	**1,060238**	**1,060238**	**1,060238**	**1,060238**	**1,060238**
R1/pond	None	0,19231	0,187643	0,067571	0,17931	0,048571	0,172238	0,032429
R1/stream	None	**1,488333**	**1,021905**	0,54381	**1,021905**	0,288333	**1,021905**	0,149833
R3/stream	None	**2,06619**	**1,199524**	0,755	**1,199524**	0,449286	**1,199524**	0,296667
R4/stream	None	**1,497619**	**1,468095**	0,547143	**1,468095**	0,290238	**1,468095**	0,150738

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

The aquatic risk assessment was based on exposure to diflufenican alone. The tests with the formulation Diflufenikan 500 SC suggest lower toxicity to aquatic organisms compared to technical diflufenican. Therefore it was agreed that the risk from the formulation would be covered by the risk assessment for active substance.
The Diflufenikan 500 SC applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures. **zRMS is of the opinion, that relevant mitigation measures will be proposed at the Member State level.**

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that Diflufenikan 500 SC used at according to proposed GAP does not pose unacceptable risk to aquatic organisms provided that 5m vegetative buffer zone is applied.

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

Effects on bees of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on effects of Diflufenikan 500 SC on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	diflufenican	oral, acute	LD_{50} >112.3 µg a.s./bee	EFSA Scientific Report (2007) 122, 1-84
Apis mellifera	diflufenican	contact, acute	LD_{50} >100 µg a.s./bee	
Apis mellifera	Diflufenikan 500 SC	48 h, acute oral	**LD_{50} > 200 µg/bee**	KCP 10.3.1.1.1/01 Kulec-Płoszczyca E/ 2022 / B-99-22
Bombus spp.	Diflufenikan 500 SC	48 h, acute oral	**LD_{50} > 200 µg/bumblebee** (>83.9 µg as/bumblebee)	KCP 10.3.1.1.1/02 Kulec-Płoszczyca E/ 2022 / B-100-22
Apis mellifera	Diflufenikan 500 SC	48 h, acute contact	**LD_{50} > 200 µg/bee**	KCP 10.3.1.1.2/01 Kulec-Płoszczyca E/ 2022 / B-101-22
Bombus spp.	Diflufenikan 500 SC	48 h, acute contact	**LD_{50} > 100 µg/bumblebee** (>41.9 µg as/bumblebee)	KCP 10.3.1.1.2/02 Kulec-Płoszczyca E/ 2022 / B-102-22
Apis mellifera	Diflufenikan 500 SC	Chronic oral	LDD_{50} = 1203.11 µg/bee/day (502.08 µg a.i./bee/day) NOEDD = 226.86 µg/bee/day (96.29 µg a.i./bee/day)	KCP 10.3.1.2/01 Mautino G/ 2023 / 1003.H.SAG22
Apis mellifera	Diflufenikan 500 SC	Larva, repeated exposure	LD_{50} = 1430 µg/larva (598.90 µg a.i./larva) NOED < 57.31 µg/ larva (20.48 µg a.i./bee/day)	KCP 10.3.1.4/01 Mautino G/ 2023 / 1004.H.SAG22
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Diflufenikan 500 SC. Details of studies and results are included in Table 9.6-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.6.2 Risk assessment

9.6.2.1 Hazard quotients for bees

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) and "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).

Risk assessment acc. to SANCO/10329/2002 rev.2 (final), October 17, 2002

Table 9.6-2: First-tier assessment of the risk for bees due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use **Product** **Application rate (g/ha)**	winter cereals (Use-No. 1) Diflufenikan 500 SC 1 × 358.2*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Acute oral toxicity	>200	358.2	1.79
Acute contact toxicity	>200		1.79

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*density of Diflufenikan 500 SC is 1.194 g/ml

Risk assessment acc. to EFSA Journal 2013;11(7):3295

Table 9.6-3: Screening step assessment of the risk for bees due to the use of Diflufenikan 500 SC in winter cereals downward spray (BBCH 00-14)

Intended use **Product** **Application rate (g/ha)**	winter cereals (Use-No. 1) Diflufenikan 500 SC 1 × 358.2*				
Test design	**LD_{50} (µg/bee) LDD_{50} (µg/bee/day) NOED (µg/larva)**	**Single application rate (g/ha)**	**SV**	**HQ/ ETR**	**Trigger**
Acute oral toxicity	>200	358.2	7.6	< 0.01	0.2
Acute contact toxicity	>200	358.2	-	< 1.80	42
Chronic oral toxicity	1203.11	358.2	7.6	0.002	0.03
Larva toxicity	< 57.31	358.2	4.4	0.03	0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.
*density of Diflufenikan 500 SC is 1.194 g/ml

Table 9.6-4: Screening step assessment of the risk for bumblebees due to the use of Diflufenikan 500 SC in winter cereals downward spray (BBCH 00-14)

Intended use **Product** **Application rate (g/ha)**	winter cereals (Use-No. 1) Diflufenikan 500 SC 1 × 358.2*				
Test design	**LD_{50} (µg/bee) LDD_{50} (µg/bee/day) NOED (µg/larva)**	**Single application rate (g/ha)**	**SV**	**HQ/ ETR**	**Trigger**

Acute oral toxicity	>200	358.2	11.2	< 0.02	0.036
Acute contact toxicity	>100	358.2	-	< 3.58	7

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.
*density of Diflufenikan 500 SC is 1.194 g/ml

The screening step risk assessment above has indicated no unacceptable acute risk for honeybees and bumblebees.

zRMS comments:
The risk assessment for adult bees based on the laboratory tests with diflufenican and the formulation Diflufenican 500 SC are considered acceptable.
All hazard quotients are clearly below the trigger of 50, indicating that the intended use poses a low risk to bees in the field.
The chronic toxicity test for adult bees and the chronic test for larvae have been provided for authorisation of plant protection product Diflufenican 500 SC. The studies have been accepted by zRMS.
The risk assessment for bees based on GD for bees, 2013 (however is still not implemented at EU level) is accepted by RMS. The screening step risk assessment above has indicated no unacceptable acute risk for honeybees and bumblebees.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

The acute risk of Diflufenikan 500 SC to honeybees was assessed from HQ/ETR between toxicity endpoints, estimated from acute oral and contact studies with formulated product Diflufenikan 500 SC as well as the maximum single application rate. The HQ values were considerably less than the trigger values that means product Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable acute oral and contact risk to honeybees. No risk mitigations are required.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

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

documents.

Effects on non-target arthropods of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on effects of Diflufenikan 500 SC on arthropods were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Laboratory tests with standard sensitive species				
Typhlodromus pyri (adults)	709 g diflufenican/kg WG (187.5 g as/ha)	Laboratory, glass, 48 hours	187.5 g as/ha Mortality: 7.7% Fecundity: 23% reduction	EFSA Scientific Report (2007) 122, 1-84
Aphidius rhopalosiphi (protonymphs)	709 g diflufenican/kg WG (187.5 g as/ha)	Laboratory, glass, 48 hours	187.5 g as/ha Mortality: 2.8% Fecundity: 39.8% reduction	
Further laboratory and extended laboratory studies				
Aphidius rhopalosiphi (adults)	700 g diflufenican/L	Extended laboratory plants, 48 hours	187.5 g as/ha Mortality: 0% Parasitisation: 14.3% increase	EFSA Scientific Report (2007) 122, 1-84
Aleochara Bilineata (adults)	247 g diflufenican/L	Extended laboratory, sand, 8 weeks	247 g as/ha Mortality: 0% Parasitisation: 106%	
Poecilus cupreus	250 g diflufenican/L	Extended laboratory, sand, 15 days	250 g as/ha Mortality: 0% Feeding:0%	
Aphidius rhopalosiphi	Diflufenikan 500 SC	Laboratory, glass, 48 hours	Mortality: 48h LR_{50} > 3000 ml/ha (>1500 g as/ha) Reproduction: 12d ER_{50} > 3000 ml/ha (>1500 g as/ha)	KCP 10.3.2.1/01 Mautino G/ 2023 /1016.H.SAG22
Typhlodromus pyri	Diflufenikan 500 SC	Laboratory, glass, 48 hours	Mortality: 7d LR_{50} > 3000 ml/ha (>1500 g as/ha) Fecundity: 14d ER_{50} > 3000 ml/ha (>1500 g as/ha)	KCP 10.3.2.1/02 Mautino G/ 2023 / 1017.H.SAG22
Field or semi-field tests				
Not relevant.				

9.7.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)		
Active substance	diflufenican		
Application rate (g/ha)	1 × 150		
MAF	1		
Test species Tier I	**LR_{50} (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1500	150	0.1 / Yes
Aphidius rhopalosiphi	1500		0.1 / Yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient;

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)				
Active substance/product	Diflufenican 500 SC				
Application rate (g/ha)	1 × 150				
MAF	1				
VDF	5[1] 10				
Test species Tier I	**LR_{50} (g/ha)**	**Drift rate**	**CF**	**corr. $PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1500	2.77%	10[2]	8.31 4.155	0.006 / Yes 0.00277/Yes
Aphidius rhopalosiphi	1500				0.006 / Yes 0.0027/Yes

MAF: Multiple application factor; VDF: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient

[1] value in accordance with Working Document on Risk Assessment of Plant Protection Products in the Central Zone (Version 1.0, May 2021)

[2] value for Tier I in accordance with EFSA Supporting publication 2019:EN-1673

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk of Diflufenikan 500 SC to non-target arthropods was assessed from in-field and off-field HQ between toxicity endpoints, estimated from laboratory studies with Diflufenican 500 SC as well as the maximum single application rate. The in-field and off-field HQ values were considerably less than the trigger value indicating that the product Diflufenican 500 SC poses a low risk to non-target arthropods. It can be concluded that Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk mitigations are required.

zRMS comments:
The risk assessment to non-target arthropods based on the laboratory tests with diflufenican and the formulation Diflufenican 500 SC are considered acceptable.
The HQ values were considerably less than 2. It can be concluded that used at max. application rate of 0.3 L formulation/ha (150 g diflufenikan/ha) to protect maize according to proposed GAP, does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk mitigations are required.

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

9.8.1 Toxicity data

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on effects of Diflufenikan 500 SC on earthworms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Earthworms				

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~diflufenican~~	~~14 d, acute 10% peat content~~	~~LC_{50} >500 mg a.s./kg dw[1]~~	~~EFSA Scientific Report (2007) 122, 1-84~~
Eisenia fetida	diflufenican	56 d, chronic 10% peat content	NOEC = 500 mg/kg dw[1]	
~~*Eisenia fetida*~~	~~AE B107137~~	~~14 d, acute 10% peat content~~	~~LC_{50} >500 mg a.s./kg dw[1]~~	
~~*Eisenia fetida*~~	~~AE 0542291~~	~~14 d, acute 10% peat content~~	~~LC_{50} >500 mg a.s./kg dw[1]~~	
Eisenia andrei	Diflufenikan 500 SC	8 week, chronic 10% peat content	Reproduction/Survival NOEC ≥ 500 mg/kg dw [1] (≥ 209.65 mg as/kg dw) [1]	KCP 10.4.1.1/01 / Pieczka P / 2022 / G-89-21
Other soil macro-organisms				
Hypoaspis aculeifer	Preparation (Herold SC 600)	14 d, chronic	NOEC = 5.4 mg diflufenican/kg soil[1]	EFSA Scientific Report (2007) 122, 1-84
Folsomia candida	Preparation (Diflufenican SC500)	28 d, chronic 5% peat content	NOEC = 438 mg diflufenican/kg soil[1]	
Field studies				
Not relevant.				
Litter bag test				
Not relevant.				

[1]endpoints corrected to allow for logPow of > 2

9.8.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Diflufenikan 500 SC. Details of studies and results are included in Table 9.8-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is relevant only for active substance.

The acute risk assessment for earthworms and other soil microorganisms has been performed with active substance and metabolites endpoints only. The acute toxicity study for earthworms is not required any-

more while in case of other soil macro-organisms it was assumed that risk is covered by the data for non-target arthropods and product is not applied on bare soil so requirement for other soils macro-organisms can be waived.

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 Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)		
Acute effects on earthworms			
~~**Product/active substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~diflufenican~~	~~>500~~	~~0.5980[1]~~	~~>836~~
~~AE B107137~~	~~>500~~	~~0.0240[1]~~	~~>20833~~
~~AE 0542291~~	~~>500~~	~~0.0380[1]~~	~~>13158~~
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
diflufenican	500	0.5980[1]	836.1
diflufenican as Diflufenikan 500 SC	≥ 209.65	0.5980[1]	≥ 350.6
Chronic effects on other soil macro- organisms i.e. *Hypoaspis aculeifer* and *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
diflufenican	5.4	0.5980[1]	9.0
diflufenican	438	0.5980[1]	732.4

[1] PECs, average plateau concentration as a worst case (dRR Part B8)

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk of Diflufenikan 500 SC to soil macro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substances, metabolites and Diflufenikan 500 SC with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

zRMS comment:

The long-term risks of Diflufenikan 500 SC to soil meso- and macro-organisms were assessed based on toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).

Conclusion:

Risk assessment for earthworms

Risk for earthworms is low. No additional calculations for earthworms are needed.

Risk assessment for macroorganisms other than earthworms

As stated in Commission Regulation EU No 284/2013 of 1 March 2013, "For plant protection products applied as a foliar spray, data on the relevant two non-target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on *Folsomia candida* and *Hypoaspis aculeifer* shall be required."

The formulated product Diflufenikan 500 SC is applied as a foliar spray treatment. As demonstrated above, acceptable risks are expected towards the earthworms and a low in-field and off-field risk is demonstrated for non-target arthropods - such as - *Typhlodromus pyri, Aphidius rhopalosiphi* (standard laboratory studies) in cereals (0.3 L formulation/ha, equivalent to 150 g diflufenican/ha). On the other hand, all the long-term TER values are much higher than the trigger value of 5, indicating that Diflufenican 500 SC poses low acute risk also for earthworms. Therefore, the risk assessment for macroorganisms other than earthworms is not required. In addition, the risk assessment for *Folsomia candida* and *Hypoaspis aculeifer* based on the data from EFSA Scientific Report (2007) 122, 1-84 (however for similar formulation) was performed by Applicant.

All the long-term TER values are much higher than the trigger value of 5, indicating that Diflufenican 500 SC poses low ~~acute~~ chronic risk to earthworms and macroorganisms other than earthworms (*Folsomia candida, Hypoaspis aculeifer*)) when applied according to the proposed use rates (cereals).

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

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

Effects on soil microorganisms of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on effects of Diflufenikan 500 SC on microorganisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	diflufenican	28 d	no effects > 25% at: 187.5 & 937.5 g a.s. /ha	EFSA Scientific Report (2007) 122, 1-84 & DAR
N-mineralisation	AE B107137	28 d	no effects > 25% at: 0.36 mg/ kg dw	
N-mineralisation	AE 0542291	28 d	no effects > 25% at: 0.36 mg/ kg dw	
N-mineralisation	Diflufenikan 500 SC	28 d	no effects > 25% at: 2.4 mg/ kg dw (1 mg as/kg dw) 12 mg/ kg dw (5 mg as/kg dw)	KCP 10.5/01 / Pieczka P / 2022 / G-90-21
~~C-mineralisation~~	~~diflufenican~~	~~28 d~~	~~no effects > 25% at:~~ ~~187.5 & 937.5 g a.s. /ha~~	~~EFSA Scientific Report (2007) 122, 1-84 & DAR~~
~~C-mineralisation~~	~~AE B107137~~	~~28 d~~	~~no effects > 25% at:~~ ~~0.36 mg/ kg dw~~	
~~C-mineralisation~~	~~AE 0542291~~	~~28 d~~	~~no effects > 25% at:~~ ~~0.36 mg/ kg dw~~	

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Diflufenikan 500 SC. Details of studies and results are included in Table 9.9-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
diflufenican as Diflufenikan 500 SC	1 & 5 (at 28 d)	0.5980[1]	yes

AE B107137	0.36 (at 28 d)	0.0240[1]	yes
AE 0542291	0.36 (at 28 d)	0.0380[1]	yes
Product/active substance	**Max. rate with effects ≤ 25 % (g/ha)**	**Application rate (g/ha)**	**Risk acceptable?**
diflufenican	187.5 & 937.5	150	yes

[1] PECs, average plateau concentration as a worst case (dRR Part B8)

9.9.3 Overall conclusions

The risk of Diflufenikan 500 SC to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substances, metabolites and Diflufenikan 500 SC with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of Diflufenikan 500 SC at maximum rate of 0.3 L/ha (150 g as/ha) does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

zRMS comments:

The diflufenican had no significant effect on soil micro-organisms at 1.25 mg a.s./kg dry soil. This is about 2 times higher than the maximum $PEC_{soil;accu.}$ Of 0.5980 mg a.s./kg dry soil following the worst-case application to cereals. According to the performed risk assessment it was concluded that the application of Diflufenikan 500 SC at maximum rate of 0.3 L/ha (150 g as/ha) does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required. This supports the conclusion that under field conditions, use of Diflufenican 500 SC at the proposed rates poses no unacceptable risk to non-target soil microorganisms.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of Diflufenikan 500 SC were not evaluated as part of the EU assessment of diflufenican. The studies on seedling emergence and vegetative vigour for Diflufenikan 500 SC were submitted in this dossier and deemed acceptable for evaluation and authorisation of Diflufenikan 500 SC. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance Exposure System	Results	Reference
Cucumis sativa	AE F088657 00	*Raphanus sativus*	EFSA Scientific Re-

Species	Substance Exposure System	Results	Reference
Brassica napus *Raphanus sativus* *Glycine max* *Beta vulgaris* *Helianthus annuus* *Lycopersicon esculentum* *Avena sativa* *Allium cepa* *Lolium perenne*	SC42 21 d pre-emergence	ER_{50}=415.9 g a.s. /ha *Cucumis sativa, Brassica napus, Glycine max, Beta vulgaris, Helianthus annuus, Lycopersicon esculentum, Avena sativa, Allium cepa, Lolium perenne* ER_{50}>428 g a.s. /ha	port (2007) 122, 1-84
	AE F088657 00 SC42 21 d post-emergence	*Beta vulgaris* ER_{50}=174.8 g a.s. /ha *Lycopersicon esculentum* ER_{50}=290.8 g a.s. /ha *Cucumis sativa, Brassica napus, Raphanus sativus, Glycine max, Helianthus annuus, Avena sativa, Allium cepa, Lolium perenne* ER_{50}>428 g a.s. /ha	
Sunflower *Helianthus annuus*	Diflufenikan 500 SC 21 d Seedling emer-gence	ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage > 300 ml/ha	KCP 10.6.2/01 / Pieczka P/ 2022 / G-92-21
Pea *Pisum sativum*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage > 300 ml/ha	
Cabbage *Brassica oleracea var. capitata*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight = 88.5 ml/ha ER_{50} plant damage = 66 ml/ha	
Carrot *Daucus carota*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage > 300 ml/ha	
Onion *Allium cepa*		ER_{50} plant number 20.5 ml/ha ER_{50} shoot length 25.4 ml/ha ER_{50} dry weight = 18.6 ml/ha **ER_{50} plant damage = 17.5 ml/ha**	
Perennial ryegrass *Lolium perenne*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length =179.3 ml/ha ER_{50} dry weight = 53.9 ml/ha ER_{50} plant damage = 129.8 ml/ha	
Sunflower *Helianthus annuus*	Diflufenikan 500 SC 21 d Vegetative vigour	ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage =207.5 ml/ha	KCP 10.6.2/02 / Piec-zka P/ 2022 / G-91-21
Pea *Pisum sativum*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage > 300 ml/ha	
Cabbage *Brassica oleracea var. capitata*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight = 120 ml/ha	

Species	Substance Exposure System	Results	Reference
		ER_{50} plant damage > 300 ml/ha	
Carrot *Daucus carota*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight = 120 ml/ha ER_{50} plant damage > 300 ml/ha	
Onion *Allium cepa*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight = 148.4 ml/ha **ER_{50} plant damage = 83.5 ml/ha**	
Perennial ryegrass *Lolium perenne*		ER_{50} plant number > 300 ml/ha ER_{50} shoot length > 300 ml/ha ER_{50} dry weight > 300 ml/ha ER_{50} plant damage > 300 ml/ha	
Field studies			
Not relevant.			-

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Diflufenikan 500 SC. Details of studies and results are included in Table 9.10-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)			
Product	Diflufenikan 500 SC			
Application rate (ml/ha)	1 × 300			
MAF	1			
Test species	**ER_{50} (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER criterion: TER ≥ 5**

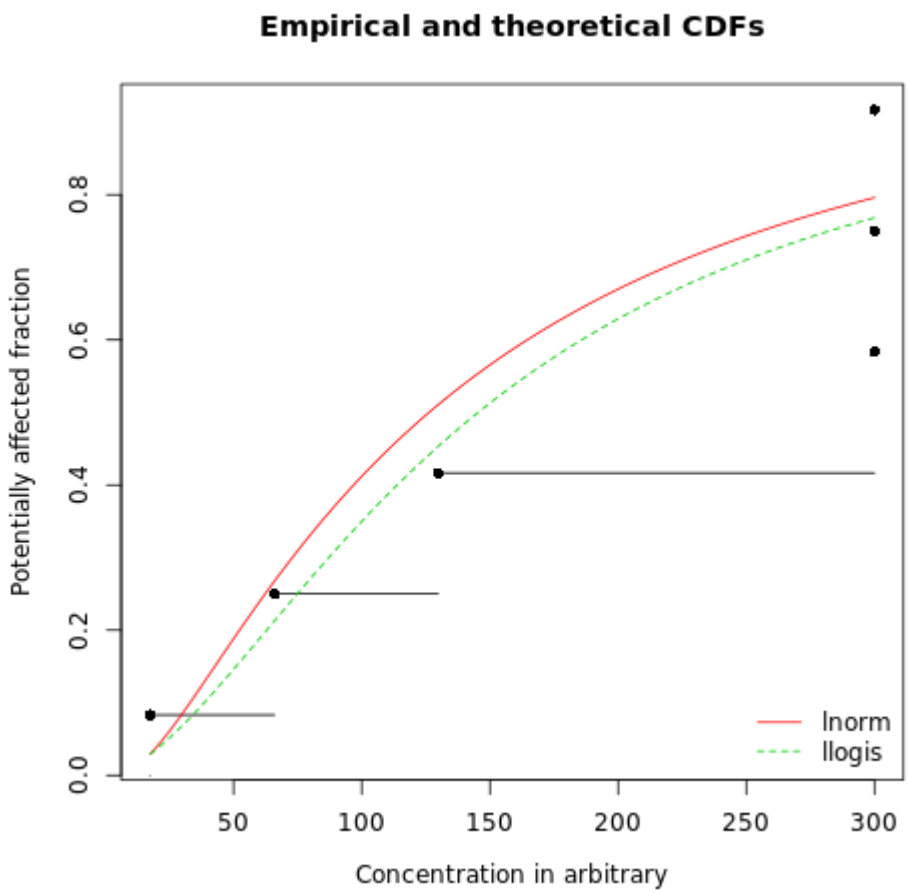
Seedling emergence (Tier I)				
Allium cepa	17.5	2.77	8.31	**2.1**
Vegetative vigour (Tier I)				
Allium cepa	83.5	2.77	8.31	10

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

Since the risk assessment with the worst endpoint of seedling emergence study with Diflufenican 500 SC failed, the refined risk assessment was performed using the HC5 value. The Species Sensitivity Distribution has been calculated using the Mosaic tool.

Table 9.10-3: Species and endpoints used foe HC5 calculation for seedling emergence study with Diflufenican 500 SC

Species	**E_rC_{50} (ml/ha)**	
Helianthus annuus	>300	Median HC_5 = 23 ml/ha n = 6
Pisum sativum	>300	
Brassica oleracea var. capitata	66	
Daucus carota	>300	
Allium cepa	17.5	
Lolium perenne	129.8	



Log normal distribution (log-likelihood = -37.8)
meanlog: 4.8 [4 ; 5.7]
sdlog: 1 [0.4 ; 1.5]

Log logistic distribution (log-likelihood = -37.9)
shape: 1.5e+02 [62 ; 3.4e+02]
scale: 1.7 [1 ; 4.8]

Table 9.10-4: HC5 with specified confidence interval

HC	Log-normal	Log-logistic
HC5 (ml/ha)	**23 [7.3 ; 96]**	25 [6 ; 1e+02]
HC10 (ml/ha)	33 [12 ; 1.2e+02]	39 [12 ; 1.3e+02]
HC20 (ml/ha)	52 [21 ; 1.5e+02]	63 [23 ; 1.7e+02]
HC50 (ml/ha)	1.3e+02 [53 ; 2.9e+02]	1.5e+02 [62 ; 3.4e+02]

Table 9.10-5: Assessment of the risk for non-target plants due to the use of Diflufenikan 500 SC in winter cereals (worst case scenario, max. application rate 0.3 L/ha)

Intended use	winter cereals (Use-No. 1)			
Product	Diflufenikan 500 SC			
Application rate (ml/ha)	1 × 300			
MAF	1			
Test species	**HC5 (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER criterion: TER ≥ 1**
Seedling emergence (Tier I)				
Allium cepa	23	2.77	8.31	2.7

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not relevant.

9.10.3 Overall conclusions

The risk of Diflufenikan 500 SC to non-target plants was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation Diflufenikan 500 SC with application rate of Diflufenikan 500 SC. According to the performed risk assessment it was assessed that the application of Diflufenikan 500 SC used in accordance with GAP, does not pose unacceptable risk to non-target plants. No risk mitigations are required.

zRMS comments:

The refinement risk assessment using the HC_5 value was not accepted by zRMS. Sufficient representative toxicity data according to SANCO/10329/2002 rev 2 final must be available, the minimum requirement is n ≥ 6 for NTTP. We have only 6 points including one point that is an extreme outlier from the data set, however it is the lowest endpoint based on phytotoxicity effect for *Allium cepa* (17.5 mL/ha).

Diflufenican 500 SC is a herbicide due to in our opinion n should be at least 10 for SSD. For 3 species we have the same toxicity endpoints such as >300 mL/ha (the same sensitivity). It means that we have

only 4 points to draw the dose-effect curve based on practical side of calculations. There was not enough detailed information regarding the statistical parameters of the determined SSD curve. On the other hand *for Lolium perenne* the lowest toxicity endpoint should be based on dry weight not phytotoxicity effect. In this case, the second SSD should be performed based on dry weight parameter. **The refinement risk assessment for SSD should be considered by MSs level.**

The risk assessment calculations for non-target plants was performed by zRMS based on deterministic approach:

Risk assessment for non-target terrestrial plants due to the use of Diflufenican 500 SC in sugar beet considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use **Active substance/product** **Application rate** **MAF**		winter cereals (Use-No. 1) Diflufenikan 500 SC 1 × 300 1
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL product/ha)**
1	2.77	8.31
5	0.57	1.71
Toxicity value ER_{50} =17.5 mL formulation/ha		**TER** **criterion: TER ≥ 5**
1		**2.12**
5		10.23

Based on the predicted rates of **Diflufenikan 500 SC** in off-field areas, the TER values describing the risk for non-target plants following exposure to Diflufenikan 500 SC according to the GAP of the formulation Diflufenikan 500 SC achieve the acceptability criteria TER ≥ 5, with applying:
- 5 m without use of drift reducing nozzles

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

Not available.

9.12 Monitoring data (KCP 10.8)

Not available.

9.13 Classification and Labelling

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

data is proposed for the formulation:

Table 9.13-1: Justified proposals for classification and labelling for Diflufenikan 500 SC according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS09
Signal word:	Warning
Hazard statement(s):	Very toxic to aquatic life. [H400] Very toxic to aquatic life with long lasting effects. [H410]
Precautionary statement(s):	Collect spillage [P391]
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads). [SP 1] To protect aquatic organisms, respect an 5m unsprayed buffer zone of to surface water bodies. [SPe 3]

Table 9.13-2: Summary of evaluation of the ecotoxicological studies for Diflufenikan 500 SC

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	E_yC_{50}=0.0524 µg/L	Y	Aquatic Acute 1, H400	KCP 10.2.1.4/01 / Czarnecka M/ 2022/ W-09-22
Chronic toxicity to aquatic organisms	no data for formulation, classification based on composition	Y	Aquatic Chronic 1, H410	Please refer to dRR Part C

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1.3/01	Czarnecka M	2022	Diflufenikan 500 SC *Daphnia magna*, Acute Immobilisation Test Study Code: W-07-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.2.1.3/01	Czarnecka M	2022	Diflufenikan 500 SC *Raphidocelis subcapitata SAG 61.81 (*formerly *Pseudokirchneriella subcapitata),* Growth inhibition test Study Code: W-08-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.2.1.4/01	Czarnecka M	2022	Diflufenikan 500 SC *Lemna gibba* CPCC 310 Growth inhibition test Company Report No: W-09-22 Source: Institute of Industrial Organic Chemistry Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.2.1.3/02	Czarnecka M	2022	Diflufenikan 500 SC Water-sediment *Myriophyllum spicatum* toxicity test Study Code: W-06-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP	N	Pestila* ProAgri**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.3.1.1.1/01	Kulec-Płoszczyca E	2022	Diflufenikan 500 SC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test Study Code: B-99-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.1.1.1/02	Kulec-Płoszczyca E	2022	Diflufenikan 500 SC Bumblebees (Bombus spp.), Acute Oral Toxicity Test Study Code: B-100-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.1.1.2/02	Kulec-Płoszczyca E	2022	Diflufenikan 500 SC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test Study Code: B-101-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.1.1.2/02	Kulec-Płoszczyca E	2022	Diflufenikan 500 SC Bumblebees (Bombus spp.), Acute Contact Toxicity Test Study Code: B-102-22 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.1.2/01	Mautino G.	2023	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Chronic Oral Toxicity Test Study Code: 1003.H.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.1.4/01	Mautino G.	2023	Effects of DIFLUFENIKAN 500 SC (diflufenican 500 g/L) on Honeybees (*Apis mellifera* L.) in the	N	Pestila*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			laboratory – Larval Toxicity Test Following Repeated Exposure Study Code: 1004.H.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished		ProAgri**
KCP 10.3.2.1/01	Mautino G.	2023	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on Typhlodromus pyri in the laboratory – Standard laboratory test Study Code: 1017.1H.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila* ProAgri**
KCP 10.3.2.1/02	Mautino G.	2023	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on *Aphidius rhopalosiphi* in the laboratory– Standard laboratory test Study Code: 1016.1H.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila* ProAgri**
KCP 10.4.1.1/01	Pieczka P	2022	Diflufenikan 500 SC Earthworm reproduction test Study Code: G-89-21 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.5/01	Pieczka P	2022	Diflufenikan 500 SC Soil Microorganisms: Nitrogen Transformation Test Study Code: G-90-21 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**
KCP 10.6.2/01	Pieczka P	2022	Diflufenikan 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study Code: G-92-21	N	Pestila* ProAgri**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished		
KCP 10.6.2/02	Pieczka P	2022	Diflufenikan 500 SC Terrestial Plant Test: Vegetative Vigour Test Study Code: G-91-21 Source: Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland GLP Unpublished	N	Pestila* ProAgri**

*Pestila Spółka z ograniczoną odpowiedzialnością (short name: Pestila Sp. z o.o.)
**ProAgri Spółka z ograniczoną odpowiedzialnością or ProAgri International Spółka z ograniczoną odpowiedzialnością (short name: ProAgri Sp. z o.o. or ProAgri International Sp. z o.o.)

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

Not relevant. No studies submitted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 KCP 10.2.1.1 Acute toxicity to fish

Not relevant. No studies submitted.

A 2.2.1.2 KCP 10.2.1.2 Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study is considered valid. All validity criteria were met: - the percentage of immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%), - the dissolved oxygen concentrations in the test vessels were within the range of 8.3 – 9.0 mg/L (criterion: not less than 3 mg/L). - the dissolved oxygen concentrations in the test vessels were within the range of 8.3 – 9.0 mg/L (criterion: not less than 3 mg/L). **Deviation in the study:** In the experimental part of study, no deviations occurred from the OECD Guideline for the Testing of Chemicals No. 202 (2004): 'Daphnia sp., Acute Immobilisation Test', EU Method C.2. 'Daphnia sp., Acute Immobilisation Test', other references given in section 8 and the SOP's listed in section 9 of the report and the study plan. One deviation from the study plan concerning the name of one of the Sponsor occurred. In the study plan the name of one of the Sponsor was given without quotation mark (Pestila Sp. z o. o.). In fact, the correct name of the Spon-

	sor is „Pestila" Sp. z o. o. The deviation did not have any impact on the results generated during the study. **Agreed toxicity endpoints**: 48-h EC_{50} = 100 mg formulation/L (48-h EC_{50} = 41.88 mg diflufenikan/L)

Reference:	KCP 10.2.1.2/01
Report	Diflufenikan 500 SC *Daphnia magna*, Acute Immobilisation Test, Czarnecka M.; 2022; Study code: W-07-22
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	The concentration of diflufenican was chemically analyzed using the high performance liquid chromatography (HPLC) with Diode Array Detection.

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

3. Test organism

Species:	*Daphnia magna* Straus
Source:	neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Age:	no older than 24 h, not the first brood progeny
Feeding:	during the test daphnia were not fed
Test units:	glass beakers with a capacity of 150 mL (one parent per vessel)

4. Environmental conditions:

Medium:	Elendt M7
Dissolved oxygen concentration in the control:	8.5 – 9.0 mg/L
pH:	7.26 – 7.97
Medium temperature:	20.5 – 21.8°C
Lighting:	daily cycle 16 h light : 8 h dark, fluorescent light source

STUDY DESIGN AND METHOD

The aim of the study was to demonstrate that the test item concentration causing 50% immobilisation of Daphnia magna, i.e. the EC50 value after 48 h of exposure . The acute *Daphnia magna* immobilisation test for Diflufenican 500 SC was conducted according to OECD Guideline 202. Immobilisation of *Daphnia magna* exposed to the test item, Diflufenican 500 SC was investigated during a 48-hour semi-static test. The definitive test was performed with a single test item concentration of 100 mg/L as a limit test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control per replicate. Four replicates were used for the test item concentration and the control, each with five *Daphnia magna*. The *Daphnia magna* were observed for immobilisation after 24 and 48 h of exposure and any abnormal behaviour or appearance. The Daphnia magna were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel. No immobilisation of Daphnia magna was observed during the period of exposure, neither in the control, nor in the test item concentration of 100 mg/L. Good condition of daphnia culture was confirmed by the study with the reference substance, potassium dichromate.

The concentration of diflufenican was chemically determined using the high performance liquid chromatography (HPLC) with Diode Array Detection. In fresh samples at exposure initiation and at the renewal, the determined concentrations of diflufenican were in the range of 101.5 – 105.3% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. In spent samples at renewal and at exposure termination, the determined concentrations of diflufenican were in the range of 99.5–101.0% of the nominal concentration. Therefore, the concentrations of diflufenican were stable under test conditions. The endpoint value was determined based on the nominal test item concentration.

Test design:	definitive test: control and tested concentration prepared in 4 replicates each, with 5 daphnia introduced into each replicate
Type of the exposure:	semi-static
Exposure time:	2 days (48 hours) with renewal after 24 h
Tested concentrations, definitive test:	control (0 mg/l), 100.0 mg/l (limit test)
Dates:	start of the study 09.02.2022 start of the experimental part: 16.03.2022 end of the experimental part: 18.03.2022 end of the study: 09.05.2022
Statistic:	ToxRat Professional Version 3.3.0 commercial software
Validity criteria:	- the percentage of immobilisation of Daphnia magna in the control was 0% (criterion: not more than 10%) - the dissolved oxygen concentrations in the test vessels were within the range of 8.3 – 9.0 mg/L (criterion: not less than 3 mg/L)

RESULTS

The effect of the test item on immobilisation of *Daphnia magna* was assessed. The test item concentration used in the definitive test was determined on the basis of the preliminary test results. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.
No immobilisation of *Daphnia magna* was observed during the period of exposure, neither in the control, nor in the test item concentration of 100 mg/L. The immobilization of *Daphnia magna* after 24 h and 48 h of exposure is given in table below. Particles of the test item in the form of sediment were observed at the bottom of test vessels for the test item concentration of 100 mg/L after 24 and 48 hours of exposure.

In fresh samples at exposure initiation and at the renewal, the determined concentrations of diflufenican were in the range of 101.5 – 105.3% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. In spent samples at renewal and at exposure termination, the determined concentrations of diflufenican were in the range of 99.5–101.0% of the nominal concentration. Therefore, the concentrations of diflufenican were stable under test conditions.

The endpoint values were determined based on the nominal test item concentration. Since the immobilisation of *Daphnia magna* was 0%, no statistical analysis was needed.

Table KCP 10.2.1.2-2: Immobilization of daphnia after 24 h and 48 h– definitive test

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

CONCLUSION

The EC_{50}/48 h is higher than 100 mg/L. The endpoint value based on nominal test item concentration.

A 2.2.1.3 KCP 10.2.1.3 Effects on aquatic algae

Comments of zRMS:	The study is considered valid. All validity criteria were met: - the biomass in the control increased by a factor of 136.2 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.7% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 18.6% (criterion: it must not exceed 35%). In the experimental part of the study, no deviations occurred from the OECD Test Guidelines No. 201 (2006): 'Freshwater alga and cyanobacteria, Growth inhibition test', EU Method C.3. 'Freshwater algae and cyanobacteria, growth inhibition test', the references given in section 8, SOP's listed in section 9 of the report, and the study plan. **Agreed toxicity endpoints:**

Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [µg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 5.0	0.833 (0.684 – 1.017)	0.589 (0.480 – 0.724)
E_rC_{20}	1.054 (0.557 – 1.835)	0.230 (0.160 – 0.301)	0.226 (0.153 – 0.294)
E_rC_{10}	0.153 (0.030 – 0.331)	0.117 (0.071 – 0.167)	0.137 (0.080 – 0.193)
LOEC	0.128	>5.0	≤0.051
NOEC	0.051	≥5.0	<0.051

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]

Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [µg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	>5.0 (2.78 – >5.0)	0.184 (0.162 – 0.209)	0.138 (0.126 – 0.151)
E_yC_{20}	0.188 (0.043 – 0.387)	0.051 (0.040 – 0.063)	0.055 (0.046 – 0.063)
E_yC_{10}	0.031 (0.002 – 0.098)	0.026 (0.019 – 0.034)	0.034 (0.027 – 0.041)
LOEC	0.128	≤0.051	≤0.051
NOEC	0.051	<0.051	<0.051

(–) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]

E_rC_{50}=0.589 µg/L nom
(0.247 µg as/L nom)
E_yC_{50}=0.138 µg/L nom
(0.058 µg as/L nom)

Reference:	KCP 10.2.1.3/01
Report	Diflufenikan 50 SC *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudo-kirchneriella subcapitata*), Growth inhibition test; Czarnecka M.; 2022; Study Code: W-08-22
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024

Chemical analysis/stability:	The concentrations of the active substance in the test item were chemically determined using the validated high performance liquid chromatographic method with MS/MS detection. Samples of all test item concentrations and the control collected at exposure initiation and samples of all test item concentrations and the control collected at exposure termination were chemically determined.
2. Vehicle and/or positive control:	vehicle control: AAP medium, positive control: 3,5-dichlorophenol
3. Test organism	
Species:	*Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*)
Source:	cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology
Age:	three days prior to the start of the test
Test units:	flask of a volume 250 mL
4. Environmental conditions:	
Medium:	AAP medium
Medium temperature:	22.6 – 23.3 ºC
pH:	7.37 – 8.06
Lighting:	mean light intensity: 6493 – 6970 lux; constant illumination and shaking

STUDY DESIGN AND METHOD

The aim of the study was to determine the test item concentrations causing 50% inhibition of growth rate and yield of the algae, *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) (ErC50 and EyC50 after 72 hours of exposure, respectively). The definitive test was performed using the test item concentrations and the control. The cell density in the three-day-old algal pre-culture was determined by counting the number of cells in the Bürker chamber under a microscope. It was 3.431 x 106 cells/mL. The pre-culture was used to inoculate each test concentration and the control in order to obtain the initial algae cells density of 1 x 104 cells per mL. Each test item concentration was split up into three replicates, whereas the control was split up into six replicates. From all test item concentrations and the control, samples in a volume of 300 mL were collected and transferred for chemical determinations at exposure initiation. In the definitive test, the absorbance for each replicate of each test item concentration and the control was measured after 24, 48 and 72 h of exposure. In the definitive test, microscopic observations of algae cell morphology were performed at exposure termination. The endpoint values were determined on the basis of the nominal test item concentrations.

Test design:	tested concentrations in three replicates, control in six replicates, flasks arranged randomly
Type of the exposure:	static
Exposure time:	72 hours
Inoculum:	1 x 10^4 cells/mL

Tested concentrations, definitive test:	5, 2, 0.8, 0.32, 0.128 and 0.051 mg/L
Dates:	start of the study 14.03.2022 start of the experimental part: 18.07.2022 end of the experimental part: 21.07.2022 end of the study: 08.08.2022
Statistic:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, Step-down Jonckheere-Terpstra Test Procedure.

CONCLUSION

The effect of the test item on the green algae growth was assessed. The range of the test item concentrations used in the definitive test was determined on the basis of the preliminary test results. The growth inhibition was estimated on the basis of the density of the algae cells determined in the definitive test.

Table KCP 10.2.1.3-1: Freshwater alga growth inhibition test – final results

Parameter	Growth rate	Yield
EC_{50} – 72 h [mg/L]	0.589	0.138
LOEC – 72 h [mg/L]	≤0.051	≤0.051
NOEC – 72 h [mg/L]	< 0.051	< 0.051

A 2.2.1.4 KCP 10.2.1.4 Effects on aquatic macrophytes

Comments of zRMS:	The study is considered valid. All validity criteria were met: In the definitive test, the following validity criteria specified in the OECD Guideline No.221/ EU method C.26. were met: - the doubling time of frond number in the control was 1.7 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 17.1), - the average specific growth rate in the control between day 0 and day 7 was 0.405 d^{-1} (minimum requirement: higher than 0.275 d^{-1}). **Agreed toxicity endpoints:**

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:

The E_rC_{50}/7 d value is 0.5645 mg/L (95% confidence interval 0.3667 – 0.9213).
The E_rC_{20}/7 d value is 0.0275 mg/L (95% confidence interval 0.0115 – 0.0503).
The E_rC_{10}/7 d value is 0.0057 mg/L (95% confidence interval 0.0016 – 0.0132).
For growth rate, the NOEC/7 d value is 0.015 mg/L, whereas the LOEC/7 d value is 0.048 mg/L.

The E_yC_{50}/7 d value is 0.0524 mg/L (95% confidence interval 0.0343 – 0.0785).
The E_yC_{20}/7 d value is 0.007 mg/L (95% confidence interval 0.0029 – 0.0123).
The E_yC_{10}/7 d value is below 0.0047 mg/L.
For yield, the NOEC/7 d value is lower than 0.0047 mg/L, whereas LOEC/7 d value is lower or equal to 0.0047 mg/L.

Endpoints based on the dry weight:

The E_rC_{50}/7 d value is higher than 5.0 mg/L.
The E_rC_{20}/7 d value is 0.7340 mg/L (95% confidence interval 0.4588 – 1.1333).
The E_rC_{10}/7 d value is 0.0826 mg/L (95% confidence interval 0.0314 – 0.1553).
For growth rate, the NOEC/7 d value is 0.015 mg/L, whereas the LOEC/7 d value is 0.048 mg/L.

The E_yC_{50}/7 d value is 0.6194 mg/L (95% confidence interval 0.3947 – 1.0382).
The E_yC_{20}/7 d value is 0.0355 mg/L (95% confidence interval 0.0143 – 0.0657).
The E_yC_{10}/7 d value is 0.0080 mg/L (95% confidence interval 0.0021 – 0.0187).
For yield, the NOEC/7 d value is 0.015 mg/L, whereas LOEC/7 d value is 0.048 mg/L.

The endpoint values based on the geometric means of determined concentrations of diflufenican:

Endpoints based on the frond number:

The E_rC_{50}/7 d value is 0.1783 mg/L (95% confidence interval 0.1183 – 0.2862).
The E_rC_{20}/7 d value is 0.0111 mg/L (95% confidence interval 0.0048 – 0.0198).
The E_rC_{10}/7 d value is 0.0026 mg/L (95% confidence interval 0.0007 – 0.0058).
For growth rate, the NOEC/7 d value is 0.0063 mg/L, whereas the LOEC/7 d value is 0.021 mg/L.

The E_yC_{50}/7 d value is 0.0208 mg/L (95% confidence interval 0.0141 – 0.0299).
The E_yC_{20}/7 d value is 0.0033 mg/L (95% confidence interval 0.0015 – 0.0056).
The E_yC_{10}/7 d value is below 0.0021 mg/L.
For yield, the NOEC/7 d value is lower than 0.0021 mg/L, whereas LOEC/7 d value is lower or equal to 0.0021 mg/L.

Endpoints based on the dry weight:

The E_rC_{50}/7 d value is higher than 1.5636 mg/L.
The E_rC_{20}/7 d value is 0.2304 mg/L (95% confidence interval 0.1465 – 0.3518).
The E_rC_{10}/7 d value is 0.0294 mg/L (95% confidence interval 0.0117 – 0.0536).
For growth rate, the NOEC/7 d value is 0.0063 mg/L, whereas the LOEC/7 d value is 0.021 mg/L.

The E_yC_{50}/7 d value is 0.1928 mg/L (95% confidence interval 0.1273 – 0.312).
The E_yC_{20}/7 d value is 0.0142 mg/L (95% confidence interval 0.0061 – 0.025).
The E_yC_{10}/7 d value is 0.0036 mg/L (95% confidence interval 0.001 – 0.008).
For yield, the NOEC/7 d value is 0.0063 mg/L, whereas the LOEC/7 d value is 0.021 mg/L.

Frond number

E_rC_{50}=0.5645 mg formulation/L nom
(**0.1783 mg diflufenikan/L mm**)
E_yC_{50}=0.0524 µg formulation/L nom
(0.0208 mg diflufenikan/L mm)

Dry weight

E_rC_{50}>5 mg formulation/L nom
(1.5636 mg diflufenikan/L mm)
E_yC_{50}=0.6194 µg formulation/L nom
(0.1928 mg diflufenikan/L mm)

Reference:	KCP 10.2.1.4/01
Report	Diflufenikan 500 SC *Lemna gibba*, Growth Inhibition Test; Czarnecka M.; 2022; Study Code: W-09-22
Guideline(s):	Yes, OECD 221
Deviations:	Deviations concerning determination the ErC50 value after 7 days of exposure based on dry weight. Despite the fact that the range of concentrations was selected on the basis of the preliminary test, the above mentioned value could not be determined. The deviation did not have any impact on the results generated during the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	The concentrations of diflufenican were chemically determined. The concentrations of diflufenican were chemically analyzed using a high performance liquid chromatography (HPLC) with Diode Array Detection (DAD).
2. Vehicle and/or positive control:	vehicle: 20X AAP medium, positive control: 3,5-dichlorophenol

3. Test organism

Species:	duckweed (*Lemna gibba*) specification CPCC 310
Source:	from the Institute of Industrial Organic Chemistry, Branch Pszczyna obtained from the University of Waterloo, Canadian Phycological Culture Centre, Ontario, Canada
Test units:	150 ml glass crystallizers

4. Environmental conditions:

Medium:	20X AAP medium
Medium temperature:	22.9 – 23.5ºC
pH:	7.52 – 9.02
Lighting:	7003 – 7505 lux; constant illumination

STUDY DESIGN AND METHOD

Lemna gibba growth inhibition test was performed according to OECD Guideline No 221. The aim of the study was to determine the influence of test item Diflufenikan 500 SC on growth of gibbous duckweed *Lemna gibba*. The growth of *Lemna gibba* exposed to the test item, Diflufenikan 500 SC, was investigated in a 7 day semi-static test with daily renewals. The test was performed in glass crystallizers containing 150 mL of either the test item concentration or the control. The initial frond number in each test item concentration and the control was nine. The following test item concentrations were used: 5.0, 1.56, 0.49, 0.15, 0.048, 0.015, and 0.0047 mg/L plus the control. The total number of fronds in each test vessel was counted twice during exposure (day 2 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time. The concentrations of diflufenican were chemically determined. The concentrations of diflufenican were chemically analyzed using a high performance liquid chromatography (HPLC) with Diode Array Detection (DAD). The endpoint values were determined based on the nominal test item concentrations, nominal concentrations of diflufenican, and geometric means of determined concentrations of diflufenican.

Test design:	tested concentrations in three replicates, control in six replicates, 9 fronds on every replicate
Type of the exposure:	semi-static
Exposure time:	7 days
Tested concentrations, definitive test:	5.0, 1.56, 0.49, 0.15, 0.048, 0.015 and 0.0047 mg/l (2.0965, 0.6541, 0.2055, 0.0629, 0.0201, 0.0063, 0.0020 mg diflufenican/L)
Dates:	start of the study 01.03.2022 start of the experimental part: 23.03.2022 end of the experimental part: 01.04.2022 end of the study: 19.07.2022
Statistic:	Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals), Dunnett's Multiple t-test Procedure, ToxRat Professional commercial software Version 3.3.0

CONCLUSION

In the growth inhibition test on *Lemna gibba*, the endpoint values were determined on the basis of the nominal concentrations of the test item. They are given below.

Table KCP 10.2.1.4-1: *Lemna gibba* growth inhibition test-final results

Rated value	ErC_{50} [mg/L]	LOEC [mg/l]	NOEC [mg/l]
Growth rate – frond number	0.5645	0.048	0.015
Growth rate – dry weight	not detected (>5)	0.048	0.015
Rated value	EyC_{50} [mg/L]	LOEC [mg/l]	NOEC [mg/l]
Yield – frond number	0.0524	≤0.0047	≤0.0047

Yield – dry weight	0.6194	0.048	0.015

Comments of zRMS:	The study is considered valid. All validity criteria were met: - the mean total shoot length in the control in comparison with the mean total shoot length at exposure initiation increased 3.4-fold. The criterion of at least doubling the total shoot length was met; - the mean fresh weight in the control in comparison with the mean fresh weight for representative group at exposure initiation increased 4.0-fold. The criterion of at least doubling the fresh weight was met; - the plants in the control were without visual symptoms of chlorosis and during the exposure phase no contamination with algae, fungi or bacteria on the plants, on the sediment surface or in the test medium was observed; - the mean coefficient of variation for yield based on fresh weight in replicates of the control in a period from exposure initiation to termination was 20.8%; did not exceed 35%. **Deviations of the study:** The deviation concerned determination of exact the EC values. The range of concentrations was selected on the basis of the preliminary exposure test results. However, due to flat concentration-response obtained in the definitive test exact the EC values could not be determined. The EC_{50} values were estimated on the basis of percentages of growth rate and yield inhibitions and considered as higher than 200 mg/kg (the highest test item concentration used for exposure). In the definitive test, all validity criteria were met, the deviations did not have any impact on the results generated during the study. **Agreed toxicity endpoints:** The endpoint values determined on the basis of the nominal test item concentrations are given below: Endpoint values calculated on the basis of the total shoot length: The E_rC_{50}/14 d value is higher than 200 mg/kg. The NOEC/14 d value for growth rate is 0.6 mg/kg. The LOEC/14 d value for growth rate is 1.9 mg/kg. The E_yC_{50}/14 d value is 91.16 mg/kg (95% confidence limit: 19.23->200). The NOEC/14 d value for yield is 0.19 mg/kg. The LOEC/14 d value for yield is 0.6 mg/kg. Endpoint values calculated on the basis of the fresh weight: The E_rC_{50}/14 d value is 403.15 mg/kg (95% confidence limit: 81.66 – >200). The NOEC/14 d value for growth rate is 0.6 mg/kg. The LOEC/14 d value for growth rate is 1.9 mg/kg. The E_yC_{50}/14 d value is 24.45 mg/kg (95% confident limit: 7.87 – 163.23), The NOEC/14 d value for yield is 0.19 mg/kg. The LOEC/14 d value for yield is 0.6 mg/kg. Endpoint values calculated on the basis of the dry weight: The E_rC_{50}/14 d and E_yC_{50}/14 d values are higher than 200 mg/kg. The NOEC/14 d value for growth rate and yield is 0.19 mg/kg. The LOEC/14 d value for growth rate and yield is 0.6 mg/kg.

Reference:	KCP 10.2.1.4/02
Report	Diflufenikan 500 SC Water-sediment *Myriophyllum spicatum* toxicity test; Czarnecka M.; 2022; Study Code: W-06-22
Guideline(s):	Yes, OECD 239
Deviations:	The deviation concerned determination of exact the EC values. The range of concentrations was selected on the basis of the preliminary exposure test results. However, due to flat concentration-response obtained in the definitive test exact the EC values could not be determined. The EC50 values were estimated on the basis of percentages of growth rate and yield inhibitions and considered as higher than 200 mg/kg (the highest test item concentration used for exposure). In the definitive test, all validity criteria were met, the deviations did not have any impact on the results generated during the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	In order to determine stability of diflufenican in aqueous phase and sediment, the stability test was performed. The analysis were performed after preparation of concentrations and 7 days after test initiation. Based on stability test results, the definitive test was planned to be performed in test spiking of sediment design.

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

3. Test organism

Species:	*Myriophyllum spicatum*
Source:	standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology
Test units:	glass beakers of 11 cm diameter and 24 cm height, medium volume 2L

4. Environmental conditions:

Medium:	aerated test medium Smart and Barko and a conditioned sediment 309-350 µS/cm conductivity

pH:	pH in control: 7.6 – 8.69
Dissolved oxygen:	87.5 – 98.2%
Lighting:	10.81 – 11.36 klux in a daily cycle of 16 h day and 8 h night
Temperature:	19-20.7°C

STUDY DESIGN AND METHOD

The growth of watermilfoil Myriophyllum spicatum exposed to the test item, Diflufenikan 500 SC for 14 days was studied in a water-sediment system, in static test design, in conditions required for the vegetative growth. The toxicity test consisted of a rooting phase (7 days) and an exposure phase (14 days). The plants (representative group) were exposed in a set of nominal test item concentrations and control. Exposure via sediment was used since diflufenican was not stable in water. Three plants rooted and transplanted into a pot with spiked sediment were placed in a beaker and overlaid with test medium. The test item was applied into sediment of water-sediment system. The impact of the test item on the plants growth was assessed based on total shoot length, fresh weight and dry weight of plants. In the tested range of the test item concentrations the inhibition of growth rate for total shoot length ranged from 5.1 to 33.6%, for fresh weight ranged from 3.5 to 40.9%, for dry weight ranged from 11.4 to 29.4% in comparison with plants in the control. The inhibition of yield for total shoot length ranged from 15.7 to 48.7%, for fresh weight ranged from 5.5 to 57.3%, for dry weight ranged from 18.8 to 48.4% in comparison with plants in the control.

Test design:	all test item concentrations were performed in four replicates (12 plants per replicate) and control in six (18 plants per replicate)
Test type:	sediment spiked
Type of the exposure:	static
Exposure time:	7 days rooting phase, 14 days exposure phase
Tested concentrations, definitive test:	200, 62.5, 19.5, 6.1, 1.9, 0.6 and 0.19 mg/L
Dates:	start of the study 27.07.2022 start of the experimental part: 09.08.2022 end of the experimental part: 03.09.2022 end of the study: 24.10.2022
Statistic:	Probit analysis using linear max. likelihood regression and analyses by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Step-down Jonckheere-Terpstra Test Procedure

CONCLUSION

The impact of the test item on the plants growth was assessed based on total shoot length (i.e. sum of each side shoot length and main shoot length), fresh weight and dry weight of plants. In the tested range of the test item concentrations the inhibition of growth rate for total shoot length ranged from 5.1 to 33.6%, for fresh weight ranged from 3.5 to 40.9%, for dry weight ranged from 11.4 to 29.4% in comparison with

plants in the control. The inhibition of yield for total shoot length ranged from 15.7 to 48.7%, for fresh weight ranged from 5.5 to 57.3%, for dry weight ranged from 18.8 to 48.4% in comparison with plants in the control. At exposure termination in the control the plants were healthy, with green leaves and stems, without discolorations with very good developed roots, anchored in sediment. In the test item concentrations of 0.19, 0.6 and 1.9 mg/kg, no visible changes were observed in comparison with plants in the control. In the test item concentration of 6.1 mg/kg, few short roots were observed. In the test item concentrations of 19.5, 62.5 and 200 mg/kg distorted and discoloration of apices, and few short roots were observed.

Table KCP 10.2.1.3-2: *Myriophyllum spicatum* toxicity test -final results

Rated value	ErC_{50} [mg/kg]	NOEC [mg/kg]	LOEC [mg/kg]	EyC_{50} [mg/kg]	NOEC [mg/kg]	LOEC [mg/kg]
Fresh weight	403.15	0.6	1.9	24.45	0.19	0.6
Dry weight	200	0.19	0.6	-	-	-
Total shoots length yield after 14 days	200	0.6	1.9	91.16	0.19	0.6

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

Not relevant. No studies submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

For the higher tier risk assessment form aquatic organisms, mesocosm study with formulation similar to Diflufenikan 500 SC was used. Tested formulation Diflufenican 500 SC is similar to Diflufenikan 500 SC and have the same formulation type hence the mesocosm study can be used to support the risk assessment of Diflufenikan 500 SC. The study was used for re-registration of Legato 500 SC and is no longer protected. Summary of the study is included below and was copied form the Registration Report of Legato 500 SC.

Comments of zRMS:	This study has not been assessment by RMS.

Reference:	KCP 10.2.3/01
Report	Outdoor aquatic mecosocm study with Diflufenican 500 SC (500 g a.s./L); Hommen, U., 2010; Study Code: MAK-005/4-52
Guideline(s):	CLASSIC workshop (Giddings et al. 2002)
GLP	Yes
Dates of work:	27.05.2009 - 21.01.2010
Test substance:	Diflufenican 500 SC, Batch no: D-178, Diflufenican 500 g/L
Test facility:	Fraunhofer Institute, Molecular Biology and Applied Ecology, Schmallenberg, Germany

Executive Summary

A study sponsored by Agan Chemicals Manufacturers Ltd., Ashdod 77102, Israel, was performed at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in cooperation with the Institut

fur Gewasserschutz MESOCOSM GmbH (MESOCOSM GmbH) to evaluate effects of Diflufenican 500 SC (500 g a.s./L) on algae, macrophytes, zooplankton and benthic macroinvertebrates in outdoor mesocosms.
The test item was applied to the outdoor mesocosm community on May 27, 2009. This was done in accordance with the recommendations of the CLASSIC workshop (Giddings et al. 2002), where application to a developing system in spring is regarded as most powerful to detect possible effects.
The test item was applied to a series of stainless steel enclosures in a large lined basin located at the MESOCOSM GmbH facility in Homberg/Ohm, Germany. The basin was filled and allowed to equilibrate for > 6 months before application. Fifteen enclosures, each containing approximately 2000< L of water with a sediment layer, were used to assess the impact on the indigenous species assemblages of macrophytes, phytoplankton, periphyton, zooplankton and macrozoobenthos. The enclosures were set on April 21, 2009.
The nominal initial water concentrations of Diflufenican were 0.25, 0.75, 1.5, 3.0, and 9.0 µg a.s./L with two replicate enclosures per concentration. Five untreated enclosures were used as controls. Using the toxicological approach in order to allow extrapolation to the field situation with different entry paths possible, the test item was applied directly into the water column on May 27, 2009. The in-life phase was terminated 12 weeks after the application.
Water samples from the treated enclosures were analysed for Diflufenican (LOQ: 0.1 µg/L) and its main metabolite 2-(3-Trifluoro-methyl-phenoxy)-nicotinic acid (TFMP-Na(acid), LOQ: 0.01 µg/L) by GC/NCI-MS/MS. Sediment samples were only taken from the enclosures of the three highest treatment levels and analysed for Diflufenican and TFMP-Na(acid).
All enclosures were monitored for water parameters (temperature, oxygen concentration, pH, conductivity, nutrients) macrophytes, phytoplankton, periphyton, zooplankton, and macrozoobenthos at weekly or bi-weekly intervals.
All water parameters were close to the range of oligotrophic and mesotrophic natural pond water during the entire study. No biologically significant differences were observed at the two measured water depths.

Diflufenican measurements in the application solutions were 87 to 96 % (mean 92 %) of the nominal concentrations, and mesocosm water sampled 2 - 3 hours after the application were 71 to 106 % (mean 92 %) of the nominal concentrations.
Diflufenican concentrations in the water phase decreased comparably in all enclosures with small differences between replicates. At the higher treatment levels (1.5 µg a.s./L and higher), where 7 or 8 values above the LOQ were available for fitting, the DT50 was approximately 3 weeks.
The concentrations of the metabolite TFMP in the water increased over the first 8 weeks after application and were relatively stable in the last 4 weeks of the study. The maximum measured concentration (at nominal 9 µg a.s./L) was 2.4 µg/L.
Diflufenican was found in the sediment with up to 12 µg a.s./kg at the highest treatment level. Despite some variability (likely caused by inhomogeneous distribution in the sediment), the concentrations were relatively stable until the end of the study. TFMP was always found in concentrations below 1 µg/kg, with a trend of increasing concentrations until the end of the study.

Observed effects including time of recovery were classified according to the scheme suggested in the EU Guidance Document on Aquatic Ecotoxicology (SANCO 2002) and the refinement suggested by de Jong et al. (2008) in order to facilitate the estimation of the study specific NOEC (No Observed Effect Concentration) and NOEAEC (No Observed Ecologically Adverse Effect Concentration). The effect summary table (Table 10.2-17) lists the effect classes for the different type of endpoints and the five concentrations tested. Note that the classes on the taxon level are based on the most sensitive taxon found in the community.
The analysis of the phytoplankton community and population level based on cell counts as well as the analysis of the pigments by delayed fluorescence spectroscopy revealed direct effects of the test treatment on two taxa, *Cryptomonas* spec. and *Erkenia* spec. with NOECs of 3 and 0.75 µg a.s./L, respectively. Both species showed fast recovery (within 8 weeks after application). Thus, the effects were considered as class 3 effects. For several taxa temporary higher abundances at 9 µg a.s./L were found. For Bacillariophyceae and Xantophyceae higher abundances compared to control were also found at the end of the

study. Despite that these potential indirect effects on these taxa were not found over several consecutive weeks they were considered as class 5 because they were found more than 8 weeks after application.
On the community level, slight temporary effects were found at 1.5 µg a.s./L (considered as class 2 effects) while effects at 3 and 9 µg a.s./L were more pronounced but with recovery (class 3). Pigment analysis revealed to be less sensitive than cell counts with no consistent NOECs found.

For the periphyton, no effects were considered up to 0.75 µg a.s./L (class 1) At 1.5 µg a.s./L slight temporary indirect effects on green algae could not be excluded (class 2) while temporary direct effects on blue-green algae at 9 µg a.s./L were possible (class 3). Due to significantly higher abundances of chromophytes at 3 and 9 µg a.s./L at the end of the study, class 5B is considered for this periphyton group.
The submerged macrophytes showed no effects of the test item up to the highest treatment level (class 1). For *Lemna* sp., an indirect effect over the last six weeks of the study cannot be excluded for 3 and 9 µg a.s./L, which was considered as a class 5B effect despite that a clear trend to full recovery was given. Total photosynthesis as indicated by oxygen concentration, pH values and conductivity of the enclosure water was not affected by the test item. Diurnal measurements of the oxygen concentration showed normal patterns and the oxygen level did not fall below 7 mg/L, which is not considered to be critical for e.g. fish living in small lentic waters.
The zooplankton was strongly dominated by rotifers, followed by copepods. No direct effects, indicated by a treatment related decrease of abundance directly after application, were observed except for one taxon, Cylopidae, where significantly lower abundances compared to controls were found (NOEC of 3 µg a.s./L) five and six weeks after application For a few other taxa significantly higher abundances than in the control were found on single dates, e.g. for *Hexarthra* with a NOEC of 0.25 µg a.s./L on two single dates. Only for *Daphnia,* a significant positive (in the sense of increase in abundance) effect was found (NOEC 0.75 µg a.s./L) consistently over a few samplings. On the zooplankton community level effects were not significant up to 1.5 µg a.s./L. At 3 µg a.s./L slight effects were found, which were only significant for the evenness on one day. More frequently, deviations from control were found at 9 µg a.s./L.
No zooplankton taxon or community metric showed long-term effects up to the highest treatment level. No indications of direct or indirect adverse effects of the test item were found on either the community or on the population level of the macrozoobenthos.
As the analysis of the application solutions and the water samples taken a few hours after application confirmed the intended exposure, the effects can be related to the nominal (initial) concentrations. A mean DT50 of 22 days was found in the 3 highest treatment levels.

0.75 µg a.s./L is considered as the general NOEC for the study because at this concentration no indication of direct effects on any taxon were found. Also the community structure of phytoplankton, zooplankton and macrozoobenthos was not affected. Only for the rotifer *Hexartha* temporary higher abundances were found. However, this increase in abundance was statistically significant only at two single and not consecutive sampling dates and not correlated to the test concentration. In addition, it did not significantly affect the community structure. Thus, it was considered as a class 2 effect and not relevant for the determination of the study specific NOEC.

1.5 µg a.s./L is considered to be the study specific NOEAEC because no long-term effects were found at this concentration while at the next higher concentration significant differences to controls at the end of the study could not be excluded.

The **remaining uncertainty can be regarded as small** because the study has been conducted under outdoor conditions in a well situated large mesocosm pond system with a large number of species. The test item has been applied directly into the water column in order to account for different entry paths of the test item into a water body.

Table KCP 10.2.3-1: Summary of effect classes observed for several endpoints in the outdoor mesocosm study with Diflufenican 500 SC (based on the most sensitive taxon per group)*

	Nominal initial concentration [µg a.s./L]				
	0.25	0.75	1.5	3	9
Phytoplankton					
Community structure	1	1	2	3A	3A
Abundance of taxa	1	1	3A	3A	5B+
Pigments	1	1	1	2+	2+
Periphyton					
Pigment analysis	1	1	2+	5B+	5B+
Macrophytes					
Lemna	1	1	1	5B	5B
Rooted macrophytes	1	1	1	1	1
Photosynthetic activity					
O2, pH, Conductivity	1	1	1	1	1
Zooplankton					
Community structure	1	1	1	1	2
Abundance of taxa	1	2+	3A+	3A+	3A+
Macrozoobenthos					
Community structure	1	1	1	1	1
Abundance of taxa	1	1	1	1	1
Study		**NOEC**	**NOEAEC**		

*Effect classes according to the Guidance Document on Aquatic Ecotoxicology (SANCO 2002) and de Jong et al. (2008): 1 = effect could not be demonstrated, 2 = slight and or temporary effect without statistical significance over at least two consecutive samplings, 3A = pronounced short-term effect with recovery within 8 weeks after application or total period of effects < 8 weeks, 5B = pronounced effects without full recovery within the study, A += increase of abundance.

I. Materials and Methods

A. Materials

1. Test Material: Diflufenican 500 SC

Description: Suspension Concentrate

Lot/batch #: D-178

Concentration/Purity: Diflufenican 500 g/L (509.3 g/L analyzed)

Stability of test compound: Expiry date: December 2010

2. Test site, duration

Location: Institut fur Gewasserschutz MESOCOSM GmbH Homberg/Ohm, Germany

Soil type/Substrate: The pond sediment was collected from the lake at a depth of approximately 0.2 m to 0.7 m below the water surface.

Test date/duration: May 27, 2009
The in-life phase was terminated 12 weeks after the application.

General climatic conditions: The measured climatic conditions at the outdoor facility site in Homberg / Ohm correspond to Middle and Northern European conditions.

3. Application

Mode of application: Test item was applied to a series of stainless steel enclosures in a large lined basin

Dosage: 0.25, 0.75, 1.5, 3.0, and 9.0 µg a.s./L (nominal)
Application scheme: Test item was applied directly into the water column
Condition of application: --
Climatological conditions: Generally no wind, No precipitation, Sunshine with some clouds, Temperature ranging from 17°C to 21°C.

4. Test design

Type & size: 15 stainless steel enclosures (approximately 2000 L) with a sediment layer

Test system: Stainless-steel enclosures (mesocosms) each with a diameter of approximately 143 cm (surface approximately 1.60 m2) and a depth of approximately 150 cm. The nominal depth of the water body was 120 cm ± 10 %, resulting in a total volume of approximately 2000 L. The stainless steel enclosures were pressed into the sediment of an artificial pond (diameter of approximately 7.68 m, water volume approx. 57000 L) Within each enclosure, a sediment layer of about 10-15 cm height rested on a clay layer of about 5-10 cm. The water depth was 1.20 m within a range of ± 10 %.

Pre-treatment: --
Post-treatment: --
Untreated control: Five untreated enclosures were used as controls.
Replications: 2
Statistics: The potential of recovery was assessed based on the results of the Williams-tests (NOEC calculation) and on the interpretation of population dynamics in diagrams showing means per treatment level over the course of the study. Recovery was assumed if an endpoint (population abundance or a community-related measure) after a direct effect showed a clear increase and reached the level of the controls again.
Dose-respone: --

5. Biological systems

Test organisms: Phytoplankton, Periphyton, Marcrophytes, Zooplankton and Macrozoobenthos
Community: --

6. Sampling

General features: At least two water samples (20 – 25 mL, unsieved) were taken from all enclosures after application. The sampling was a depth-integrated sampling (a minimum water column of 80 cm, inner diameter of approximately 40 mm). For each sampling time and enclosure, one water sample was analysed; another sample of the same size was stored at 4°C as reserve-sample. Sediment samples were taken from the enclosures with the three highest test item concentrations (two replicates) and from one untreated enclosure. At least three sample cores per enclosure were separated into two horizons (approximately 0 to 5 cm and the lower part) and bulked to provide a combined sample for each horizon. Physical water parameters were measured weekly at two levels beneath the water surface (30 cm and 80 cm); chemical parameters were measured biweekly. The following water parameters were measured: Temperature, pH, conductivity, content of oxygen, ammonium, nitrate, phosphate, and water hardness.

Actual concentration:	The analysis of Diflufenican and its main metabolite TFMP-Na(acid) in water and sediment was performed by GC/NCI-MS/MS after applying adapted sample preparation techniques. The LOQs were 0.1 µg Diflufenican/L and 0.01 µg TFMP-Na(acid) /L for the matrix water; and 1 µg Diflufenican/kg dry matter and 0.1 µg TFMPNa(acid) /kg dry matter in the matrix sediment.
Biological sampling:	phytoplankton, periphyton, macrophytes, zooplankton, and macrozoobenthos

II. Results and Discussion

A. Chemical analysis

The initial exposure to the test item was verified in two ways.
The individual application solutions for each enclosure were measured for their concentration of a.s. before application. These concentrations deviated by less than 20 % of the nominal concentrations (87 to 96 % with a mean of 92 %).

The measurements of integrated water samples taken 2 – 3 hours after application yielded concentrations of 71 to 106 % of the nominal a.s. concentrations with a mean of 92 %. One reason for the variability might be that the test item was not yet homogenously distributed in the water column. Control samples revealed concentrations below the LOQ of 0.1 µg/L.

In conclusion, the initial exposure to the test item met at least the nominal loading of the enclosures. Thus, effects in the following sections are related to the nominal a.s. concentrations Diflufenican concentrations in the water phase decreased comparably in all enclosures. The dissipation half-life time (DT503) in the water phase ranged between 14 and 24 d. However, at nominal concentrations of 1.5 µg a.s./L and higher, where 7 or 8 values were above the LOQ, DT50 was always above 20 d with a mean of 22 d.

The concentrations of the metabolite TFMP-NA (acid) in the water increased over the first 8 weeks after application and were relatively stable in the last 4 weeks of the study. The maximum measured concentration (at nominal 9 µg a.s../L) was 2.4 µg TFMP-NA(acid)/L.

Diflufenican and TFMP-NA (acid) were not found in the control samples (< LOQ of 0.1 µg/L).

Sediment samples were only taken and analysed from enclosures of the three highest treatment levels (nominal 1.5, 3 and 9 µg a.s./L).

Except for two potential outliers (3 µg a.s./L B on day 26 and 1.5 µg a.s./L B on day 54) the measured Diflufenican concentrations were related to the treatment level. The absolute maximum concentration found was around 12 µg/kg. Despite some variability which is likely caused by inhomogeneous distribution in the sediment, the concentrations were more or less stable with means of 3.6, 5.5 and 8.4 µg/kg at nominal 1.5, 3 and 9 µg a.s./L, respectively.

TFMP-NA (acid) was always found in concentration below 1 µg/kg with a trend of increasing concentrations until the end of the study.

B. Physical and chemical analysis

All physical and chemical parameters were within the range of oligotrophic and mesotrophic natural pond water (Schwoerbel, 1999) at the start of and during the study.

The physical parameters showed no biologically significant differences at the two measured water depths. Water temperatures ranged from around 16°C (June) to around 22°C (July) over the study. There was no significant difference between the levels at 30 and 80 cm below surface.

There were no apparent treatment related effects in the measured water hardness (usually 4 – 6 ° dH with a trend to decrease to values around 3 ° dH at the end of the study), nitrate (always < 0.4 mg/L), and ammonium (always < 0.1 mg/L).

The total phosphate concentration of the water was usually around or below 0.2 mg/L with a general trend to increase at the end of the study. The absolute maximum concentration found was 0.7 mg/L.

The water level during the study remained within 1.2 m ± 10 %. No adjustment of the water level was made during the study.

C. Phytoplankton

Presence and dominance of taxa
In total, 117 phytoplankton taxa were differentiated in the 195 samples. The dominating classes were Chrysophyceae, Cryptophyceae, Bacillariophyceae, Cyanophyceae, and Chlorophyceae. Less than 0.1 % of the cells found belonged to other classes (Dinophyceae, Xanthophyceae, Euglenophyceae, Zygnematophyceae).
Eight taxa were found with more than 1 % of the total counts over all samples and built together approximately 94 % of total counts. The clearly dominating species was the Chrysophyte *Erkenia subaequiciliata*. More than 60 % of all cells counted belonged to this species. The next most abundant taxon, *Chroomonas acuta* / *Rhodomonas* sp., was far less frequently found (< 10 % of all cells). For further details please refer to the original study report.

Dynamics of the total phytoplankton
The highest phytoplankton densities in all enclosures were observed around the day of application with up to around 60 000 cells / mL. Later the abundance varied within the range of 1000 to 10 000 cells/mL. At days 12 and 19 significantly higher abundance was found with NOECs of 1.5 and 3 µg a.s./L, respectively, indicating a potential short-term indirect effect on total phytoplankton. However, the difference from the range of the controls was only slight and only for 9 µg a.s./L consistent over two consecutive samplings. Apart from a single NOEC of 0.75 µg a.s./L on day 33 for a decrease, no significant differences to controls were found until the end of the study.

Number of species and diversity
On average around 20 phytoplankton taxa were found per sample (range 8 - 33). The number of taxa showed no clear treatment related effect. Only at 9 µg a.s./L, significant differences to controls were found on two single dates (day 2 and 47). Shannon index and evenness increased generally within the first weeks after the application while staying relatively stable over the last 10 weeks of the study.

On the first three samplings (day -13 to day 2) the diversity in the highest treatment level was significantly higher in the enclosure treated on day 0 with 9 µg a.s./L. Considering the lower total abundance at this time at 9 µg a.s./L, it is likely the one or more dominant taxa were less abundant. On day 12 and 19 the opposite pattern was found – increased abundance and reduced diversity, likely caused by a stronger bloom of dominant taxa at 9 µg a.s./L.

Thus, effect class 1 was assumed for phytoplankton diversity up to 3 µg a.s./L with slight short-term effects at 9 µg/L (class 2).

Similarity
The similarity analysis confirmed that the deviation of the phytoplankton community structure at the highest treatment level from the control at the start of the study especially if the absolute cell number are

considered (Steinhaus' index). Based on relative abundances the Stander's index revealed less difference before application but clearly reduced similarity within the first week after application at 3 and 9 µg a.s./L. By day 21, Stander's index indicated recovery – similarity between the 3 and 9 µg a.s./L enclosures and the controls were close to the within similarity of the control again. Both indices showed not clear treatment related effects over the last weeks of the study.

Principal Response Curves

The PRCs for the phytoplankton community did not display a significant part of the total variance of the data set (p=0.202) but showed decreased abundances at 3 and 9 µg a.s./L directly after application. However, as mentioned before, the enclosures treated with 9 µg a.s./L showed significant deviations from controls also before the test item application. In addition, the lower treatment levels showed deviations from the controls, e.g. around day 40, but these were considered as no treatment related due to the missing dose-response relation.

The dominating *Erkenia subaequiciliata* showed the highest correspondence to the PRCs, while other Chrysophyceae had negative species weights in the PRC analysis, indicating an opposition pattern of dynamics compared to *Erkenia*.

Redundancy analysis per sampling date revealed significant treatment effects from day -13 to day 33. The Williams test was applied to the sample scores of the first axis of Principal Component Analyses (PCA) per sampling date in order to calculate community NOECs. A NOEC of 0.75 µg a.s./L was calculated for day 19 while 1.5 µg a.s./L was the lowest consistent NOEC (significant effects found at 3 µg a.s./L on days 12 and 19). No significant effects were found over the last 6 weeks of the study.

Summary of effects on the phytoplankton community level

The NOECs found for endpoints related to the phytoplankton community. The lowest consistent NOEC is 1.5 µg a.s./L (based on PCA).

The effects on the total algae density were classified as a type 1 (no effects) up to 3 µg a.s./L with class 2 considered for 9 µg a.s./L based on the slight and short term increase on days 12 and 19.

Considering diversity, similarity and ordination analysis together slight effects of the treatment on phytoplankton community are considered at the 1.5 µg a.s./L level (class 2) while effects are considered to be more pronounced but temporary at 3 and 9 µg a.s./L (class 3A).

Table KCP 10.2.3-2: NOECs [µg a.s./L] for the phytoplankton community

Day	-13	-2	2	6	12	19	26	33	40	47	54	71	83	Cons.
Total phytoplankton	9	9	9	9	1.5	3+	9	0.75	9	9	9	9	9	3+
N (species)	9	9	9	9	9	9	9	9	9	3	9	9	9	
Shannon	3+	3+	3+	9	3	3	9	9	9	9	9	9	9	3
Evenness	3+	3+	3+	9	3	1.5	9	9	9	9	9	9	9	3
Similarity (estimated)	3	3	3	1.5	3	3	9	9	9	9	9	9	9	3
Ordination (PCA)	1.5	3	3	3	1.5	0.75	9	3	9	9	9	9	9	1.5

* no statistical tests, NOECs estimated based on the diagrams (Study report, Figure 12)
NOECs calculated by the Williams-test (p<0.05, one-sided), values with a + in brackets indicate an increase; bold numbers indicate a significant effect at least at the highest treatment level (9 µg a.s./L), grey shading indicates consistent effects over at least two samplings. cons. = consistent NOEC, significant effect at the next higher treatment level over at least two consecutive samplings

Abundance of phytoplankton classes

The dominant algae classes were Chrysophyceae, Cryptophyceae, Bacillariophyceae, Cyanophyceae, and Chlorophyceae. Other classes were found only sporadically with relatively low abundances and are not considered further here except where they showed statistically significant differences to controls.

The dynamics of the dominating Chrysophyceae are similar to those of the total phytoplankton. Significant differences to controls were found on three separate sampling dates with NOECs of 0.75 µg a.s./L. No clear dose-response relation was observed and thus, the differences are not considered to be treatment related.

For Cryptophyceae significantly higher abundances were found at 9 µg a.s./L on day 12 and 19. Despite that the abundance in one of the two replicates of 9 µg a.s./L started to increase just before application, all four replicates of 3 and 9 µg a.s./L are (slightly) above the range of the controls on day 12 and 19.

Also for Bacillariophyceae significantly higher abundances at 9 µg a.s./L were found within the first three weeks after application. Abundance at 3 µg a.s./L was only slightly above the range of controls on day 12. Also at the end of the study (day 71 and 83) abundance at 9 µg a.s./L was higher than in the other enclosures, but because this was caused by only one of the two replicates, this was statistically not significant.

No treatment related effects were found for Cyanophyceae and Chlorophyceae.

For Xantophyceae, NOECs of 3 µg a.s./L were revealed for day 47 to 83 but this was based on a few cells found in one replicate of 9 µg a.s./L while in the other replicates, as well as in 10 other enclosures including the controls, no cells were found.

<u>Abundance of phytoplankton species</u>
NOEC calculations were conducted for every species (respectively the lowest determined taxonomic level) and each sampling date. Due to the large number of taxa, the focus here will be on species with consistent NOECs (over at least two samplings after application) of 3 µg a.s./L or lower.

Only for two taxa (*Cryptomoas erosa + ovata*, Crypotophyceae and *Erkenia subaequiciliata*, Chrysophyceae) a consistent decrease of abundance was found.

For *Cryptomonas* a clear pronounced decrease of abundance over the first four weeks after application at 9 µg a.s./L while the population grew in controls and all other treatment levels. Within four weeks (until day 54) the populations at 9 µg a.s./L could recover back to the range of controls.

All enclosures showed highest abundances of *Erkenia* in the weeks before the application. While in the controls abundance decreased until day 12 and remained around 100 cells/mL until day 40, the decrease was earlier and more pronounced at 3 and 9 µg a.s./L. Later (day 19 – 40) also the lower treatment levels showed abundances below the range of the controls. However, this was not clearly related to the treatment level (slight effects at 0.75 µg a.s./L while larger deviations at 0.25 µg a.s./L) and thus the lowest consistent NOEC was 0.75 µg a.s./L (days 19 and 26). The higher treatment levels (3 and 9 µg a.s./L) started to recover within the third week after application and reached control abundances again around day 40.

For several taxa, significantly higher abundances were found at 9 µg a.s./L over at least two samplings: larger Centrales, *Chroomonas acuta / Rhodomonas* sp., *Gomphonema* sp., *Nitzschia acicularis, N. palea, Oszillatoria* sp., *Peronielle* sp. *Stichococcus bacillaris, Stylochrysalis* sp. and *Tetrachlorella ornate*. Often these differences were only found over two samplings for single taxa and some of the taxa were only found in very low abundances but all together, on each sampling date at least for one taxon significantly higher abundances at 9 µg a.s./L were observed.

<u>Phytoplankton pigment analysis</u>

Phytoplankton samples were additionally analysed by Delayed Fluorescence Excitation Spectroscopy, a technique to quantitatively estimate phytoplankton composition by determining photosynthetic activity in living cells.

No consistent statistically significant effects could be found. In the first 3 weeks after application, significantly higher pigment activity was found on single dates for blue-greens, greens and chromophytes resulting in NOECs of 3 µg a.s./L for green and blue-green algae and of 1.5 µg a.s./L for chromophytes. On day 54 significantly lower chlorophyll a concentrations and chromophyte and cryptophyte activity was found at 9 µg a.s./L.

The diagrams indicate that there might have been short-term indirect effects on blue-greens and chromophytes. Pigments characterising green algae were found only in low concentrations and with high variability. Thus, interpretation is difficult, e.g. on day 40 the highest concentration was measured at 0.75 µg a.s./L.

The difference to controls found for chromo- and cryptophytes as well as chlorophyll a, on day 54 was most pronounced for cryptophytes (NOEC = 3 µg a.s./L). However, this was not found on the samplings two weeks before and after day 54 and it does also not correspond to the findings for Cryptophyte cel numbers shown before.

Summary of the phytoplankton

The analysis of the community and population level based on cell counts as well as the analysis of the pigments by delayed fluorescence spectroscopy revealed direct effects of the test treatment on two taxa, *Cryptomonas* and *Erkenia* with NOECs of 3 and 0.75 µg a.s./L. Both species showed fast recovery (within 8 weeks after application). Thus, the effects were considered at class 3 effects. For several taxa temporary higher abundances at 9 µg a.s./L were found. For Bacillariophyceae and Xantophyceae higher abundances compared to control were also found at the end of the study. Despite that these potential indirect effects on these taxa were not observed over several weeks, they were conservatively considered as class 5B because they were found more than 8 weeks after application.
On the community level, slight temporary effects were found at 1.5 µg a.s./L (considered as class 2 effects) while effects at 3 and 9 µg a.s./L were more pronounced but with recovery (class 3). Pigment analysis revealed to be less sensitive than cell counts with no consistent NOECs found.
Thus, the general NOEC for phytoplankton community is considered to be 0.75 µg a.s./L based on likely direct effects on one species (*Erkenia*) while effects restricted to not more than 8 weeks after the application were observed up to 3 µg a.s./L (NOEAEC).

D. Periphyton

Periphyton was analysed approximately every two weeks by delayed fluorescence excitation spectroscopy.

Potential indication of direct effects were found for blue-greens only (NOEC = 3 µg a.s./L on day 12, while significantly higher values were found on day 55 for chlorophyll and green algae (NOEC = 0.75 µg a.s./L) and on day 82 for chlorophyll a and chromophytes (NOEC = 3 and 1.5 µg a.s./L respectively.

The diagrams (refer to study report) confirm a potential inhibition of the growth of blue-greens directly after application at 9 µg a.s./L which was considered to be a class 3 effect because abundance was below the range of the controls until day 40.

The potential indirect effects on chlorophyll a and green algae on day 55 above 0.75 µg a.s./L seem to be very small and thus, they are considered as class 2 effects. However, on the last sampling date, chlorophyll a values at 9 µg a.s./L significantly above the mean of the controls which was considered as a class 5B effect.

At the end of the study, chromophyte abundance at 3 and 9 µg a.s./L was clearly higher than in the controls and thus, also considered as class 5B effect. No effects were found for the Cryptophytes.

Thus, the NOEC for the Periphyton is considered to be 0.75 µg a.s./L while indirect effects at the end of the study could not be excluded at concentrations above 1.5 µg a.s./L (NOEAEC).

Table KCP 10.2.3-3: NOECs [µg a.s./L] calculated for DF excitation spectroscopy of periphyton samples

Day	-1	12	26	40	55	72	82
Chlorophyll a	9	9	9	9	**0.75+**	9	3+
Blue-greens	9	**3**	9	9	9	9	9
Greens	**3+**	9	9	9	**0.75+**	9	9
Chromophytes	9	9	9	9	9	9	**1.5+**
Cryptophytes	**3**	9	9	9	9		9

NOECs calculated by the one-sided Williams-test. Bold numbers indicate a significant difference to controls at least at the highest treatment level of 9 µg a.s./L, grey fields indicate significant differences over more than one sampling date and (+) indicates significantly higher values compared to controls

E. Macrophytes

Submerged macrophytes growing in the sediment were mapped at least biweekly. The following species were found during the study: *Ceratophyllum demersum*, *Potamogeton natans* and *Zannichellia palustris*. The alga *Chara intermedia* and filamental algae were also considered within the functional group of macrophytes.

Macrophyte coverage increased within the first 8 weeks after application up to 14 – 43 %. Over the last few weeks, total coverage remained stable.

Except for *Potamogeton* on the last sampling day, no significant differences to controls in macrophyte growth were found after the application. However, *Potamogeton* covered always less than 2 % of the surface area and the differences between treatment levels at the end of the study were not clearly related to the treatment level and seem to be within the variability observed before. Also for the other species no treatment-related trends were found. *Zannichellia* was found only in one enclosure at the end of the study and therefore it is not considered further here.

Thus, there were no effects of the test item on the growth of the submerged macrophytes (class 1). The duck weed *Lemna* sp. was not included in the estimation of the coverage but individual plants were counted in a small isolated area per enclosure. The significantly higher abundances at 9 µg a.s./L on day 13 can hardly be seen in the diagram but over the last 6 weeks of the study significantly lower frond numbers were found at 3 and 9 µg a.s./L. The decrease of frond numbers in these enclosures started in the fifth week after application and was more pronounced at 3 than at 9 µg a.s./L. In the last 3 weeks of the study frond numbers increased again indicating recovery but the mean level of controls was not reached. Considering the clearly unaffected growth in the first four weeks of the study and the short generation time of *Lemna*, the reduced numbers later are unlikely attributed to a direct effect of the test item. However, the potential indirect effect on *Lemna* in the last weeks of the study was considered to be a class 5B effect. This is considered to be a conservative classification because the dynamics in the last weeks suggests that recovery would have occurred 1 or 2 weeks after day 84.

In summary, the submerged macrophytes showed no effects of the test item up to the highest treatment level (class 1). For *Lemna* sp., an indirect effect cannot be excluded for 3 and 9 µg a.s./L, which was considered as a class 5B effect despite that a clear trend to full recovery was given.

Table KCP 10.2.3-4: Effect classification for the macrophytes

	Nominal initial concentration [µg a.s./L]				
Macrophytes	**0.25**	**0.75**	**1.5**	**3A**	**9**
Submerged macrophytes	1	1	1	1	1
Lemna sp.	1	1	1	**5B**	**5B**

1 = no effect, 5B =pronounced effects without full recovery within the study.

F. Zooplankton

Presence and frequency of taxa

In total 42 zooplankton taxa respectively live stages (nauplia, copepodits and adults of copepods) could be differentiated in the 180 samples. Some taxa were combined for further analysis if it was not clear that they belonged to different species. Finally, 36 taxa were used for further analysis. Rotatoria were by far the most abundant group with about 62 % of the individuals found belonging to this class, followed by Copepoda (37 %). Phyllopoda, Insecta (*Chaoborus* sp.) Ostracoda, and Branchiura (*Argulus* sp.) were found only rarely with together less than 1 % in the total zooplankton.

Nearly 99 % of the individuals found in all the samples belonged to the six taxa with dominance (proportion over all samples) above 1 %. The most abundant taxa were the rotifer *Keratella quadrata* (52 % of all individuals, 432 Ind/L on average) and nauplia (32 %, not differentiated into cyclopoid and diaptomid nauplia), followed by *Polyarthra* (rotifer), Cyclopoidae (copepodits and adults) and the rotifers *Hexarthra* and *Synchaeta*. Daphnia was found with a mean abundance of 2.7 Ind/L and with 0.3 % of the total zooplankton.

Keratella quadrata, *Polyarthra*, nauplia, cyclopoidae, and larvae of the phantom midge *Chaoborus* were found in 95 – 100 % of the samples.

Dynamics of the total zooplankton

Total abundance varied usually within 200 and 1000 Ind/L over the study. Except for day 21 with a NOEC of < 0.25 µg a.s./L for an increase in abundance no significant differences to controls were found. Despite not being statistically significant, abundance at 9 µg a.s./L was above the range of controls over the first four weeks after application.

Number of species and diversity

Up to 17 taxa were found in the samples of the controls and between 8 and 17 taxa were found per treatment level. The mean number of taxa was more or less stable over the study and no treatment-related effects were found. Also for the Shannon index no significant effects were found. Only the evenness was significantly higher at 3 and 9 µg a.s./L in day 35 and 43.

Similarity

Similarity analysis revealed no treatment related differences in zooplankton community structure. Only on day 28 the Stander's index for similarity between controls and 9 µg a.s./L is slightly lower than between control and the other treatments.

Principal response curves
The PRCs correspond to the results of the similarity analysis indicating no treatment related effects on the zooplankton community structure. In total the PRCs do not display a significant part of the total variance of the zooplankton data set (p=0.064 in Monte-Carlo permutation tests): 32 % of the total variance is explained by time while 27 % is explained by the treatment. From this 27 %, 31 % are shown by the PRCs.

The highest species weights were calculated for the rotifer *Synchaeta*, indicating that their dynamics showed a similar pattern to the PRCs. Lower but still considerable positive weights were found for the rotifers *Polyarthra* and *Hexarthra*, *Daphnia* and Diaptomidae. In contrast to these species, the cladocerans *Chydorus* and *Simalocephalus* had negative weights, which indicate an opposite pattern to the one shown in the PRCs.

Ordination analysis per sampling date revealed significant treatment effects only on three isolated sampling dates. For two of them, a Community NOEC of 3 µg a.s./L was derived via principal component analysis.

Summary of effects on the community level

The different analyses on the zooplankton community level indicated consistent statistically significant treatment related effects only for the evenness at 9 µg a.s./L. However, these slight deviations were only given for two sampling dates. Other community level endpoints did not indicate any adverse treatment related effects. Thus, slight and temporary effects found only at 9 µg a.s./L were considered as class 2 effects.

Abundance of main taxonomic groups
Regarding the abundance of main zooplankton groups no consistent significant differences to controls (over at least two samplings) were found. NOECs of 3 µg a.s./L were found for Branchiura and Rotatoria only on single dates at 9 µg a.s./L for short-term increases of abundance. Copepod (and due to their dominance also crustaceans in total) abundance was significantly higher at all treatment levels on day 21, while no significant differences to controls were found at all other dates.

Ostracoda were too rare for a meaningful analysis.
Rotifers in total showed no treatment-related effects. Abundances were slightly above the range of controls at 9 µg a.s./L from day 8 to 28 (with significance only on day 21).

The Copepoda abundance indicates no treatment related effects, the statistical finding on days 21 is related to a short-term drop of the mean of controls while the abundance in the treated enclosures stays stable and still within the range of controls. Thus, this NOEC is not considered to indicate an (indirect) effect of the treatment.

Phyllopoda were much rarer than rotifers and copepods with mean abundances usually below 10 Ind/L. No indication of a treatment effect is given.

Branchiura (only *Argulus*) and Insecta (only *Chaoborus*) were relatively rare but no indication of effects was found. The statistical findings for Branchiura are not considered to be treatment related due to the high variability of data caused by low numbers.

Analysis of the population level
The Williams-test applied to each zooplankton taxon and sampling date revealed only a few significant differences to controls considering the high number of tests conducted. NOECs found even before the application demonstrate that differences can be found also only by chance. Therefore, NOECs were considered as reliable only if they were consistent over at least two consecutive sampling dates or if the population dynamics in the diagrams indicate a trend. Consistent NOECs were only found for two taxa,

Cyclopoidae (sum of copepodits and adults) with a NOEC of 3 µg a.s./L on day 35 and 42 and *Daphnia* with a NOEC of 0.75 µg a.s./L for higher abundances from day 21 to day 35.

Cyclopoidae show directly after application a slightly more pronounced increase of abundance at 9 µg a.s./L before the mean abundance at this treatment levels falls below the range of controls on days 35 and 43. Thus, a direct effect on the copepodit and adult cyclopoids is unlikely, but the NOEC of 3 µg a.s./L might be caused by effects on nauplia (less nauplia developing to copepodits) or other indirect effects (e.g. reduced food, increased competition).

Daphnia appeared relatively late in the study, i.e. in the controls the populations started to increase after day 42. However, in the enclosures treated with 1.5 µg a.s./L or more, *Daphnia* was found earlier in considerable numbers (counts were higher than the shown numbers of Ind./L because more than 1 L was evaluated for zooplankton determination). Although there is considerable variability between replicates, the data are considered as an indication of possible indirect effects on daphnids.

In the following, the population dynamics of the most dominant taxa are shown. For all these taxa the Williams test indicated no consistent effects over at least two samplings and also the diagrams show no indication of direct or indirect treatment effects.

For *Keratella quadrata*, the means per treatment level were usually close to the one of the controls. Deviations were based on single replicates, i.e. replicate A of the 1.5 µg a.s./L treatment level on day 8. In the second half of the study abundance decreased down to very low levels in one of the controls (D) only. There is no indication of a direct toxicant effect on Nauplia directly after application and also later in the study no treatment related differences to controls were found.

The rotifer *Polyarthra* showed a trend to higher abundances at 3 and 9 µg a.s./L over the first 7 weeks after application, with statistical significance on days 21 and 43.

The increase in abundance of *Hexarthra* at 0.75 µg a.s./L and higher after day 35 might indicate an indirect effect, but the difference to controls was only significant on days 43 and 71, variability between replicates was partly high (i.e. for the 3 µg a.s./L treatment) and it was not correlated to the test concentration. Until the end of the study mean abundances at 3 and 9 µg a.s./L decreased back to the range of the controls.

For *Synchaeta* no indication of treatment related effects were found.

<u>Summary for the zooplankton</u>

The zooplankton was strongly dominated by rotifers followed by copepods. No direct effects, indicated by a treatment related decrease of abundance directly after application, were observed. Only for one taxon, Cyclopidae, significantly lower abundances compared to controls were temporary found at 9 µg a.s./L (class 3A effect).

For a few other taxa significantly higher abundances were found on isolated, not consecutive dates, e.g. for *Hexarthra* with NOECs of 0.25 µg a.s./L These deviations to controls were considered as class 2 effects.

Only for *Daphnia*, a significant positive (increase in abundance) effect was found (NOEC 0.75 µg a.s./L) consistently over a few samplings, which is considered as a class 3A effect at 1.5 µg a.s./L and more.

On the community level effects were not significant up to 1.5 µg a.s./L. At 3 µg a.s./L a significant effect was only found for the evenness on one day and thus not further considered. At 9 µg a.s./L consistent significant effects were only given for evenness while other diversity indices, similarity and ordination

analysis revealed no effects. Thus, community level effects at 9 µg a.s./L were considered to be class 2 effects

No taxon or community metric showed long-term effects. Thus, no class 5 effects were found for the zooplankton.

Table KCP 10.2.3-5: Effect classification for the zooplankton

	Nominal initial concentration [µg a.s./L]				
Zooplankton	**0.25**	**0.75**	**1.5**	**3A**	**9**
Community structure	1	1	1	1	2
Total abundance	1	1	1	1	2+
Rotifers	1	1	1	1	2+
Copepoda	1	1	1	1	1
Phyllopoda	1	1	1	1	1
Cyclopidae	1	1	1	1	3A
Daphnia	1	1	3A+	3A+	3A+
Hexartha	1	2+	2+	2+	2+
Branchiura	1	1	1	1	1
Chaoborus	1	1	1	1	1

Only those species are listed which were more sensitive than the taxonomic group in total.
1 = no effect, 2 = slight short-term effects, 3A = short-term effects with recovery within 8 weeks after application,
(+) = increase of abundance.

G. Macroinvertebrates

Presence and dominance of taxa

In total, 26 taxa, respectively stages (larvae or pupae) were differentiated in the 1344 samples (for details see appendix J of the report). As the level of taxonomic differentiation was very variable (from species like Asellus aquaticus to orders like Tricladia) and because the number of individuals found was often very small, the community analysis was based on the 12 orders found. The dominating taxon was *Asellus aquaticus* (Isopoda) with around 50 % of all organisms found in the artificial substrate samplers. Bivalvia, Ephemeroptera and Diptera were found with approximately 10 %, followed by Gastropoda, Hirudinea and Oligochaeta and Odonata. All other taxa were found only with a few individuals. Except for the Heteroptera, all these taxa were found in at least 80 % of the samples.

Dynamics of the total macrozoobenthos

Usually approximately 100 – 300 individuals of macroinvertebrates were found per sample. No clear treatment-related effects were found. On day 56 the mean abundance at 9 µg a.s./L was slightly (26 %) but significantly larger than the mean of controls. However, it was still within the range of controls and thus it was not considered as an effect.

Number of species and diversity

Generally, differences in the number of species (respectively the taxa) and diversity indices were small between the treatment levels and were not significant. Thus, adverse effects on macrozoobenthos diversity are considered not to have occurred up to 9 µg a.s./L.

Similarity

The similarity between the macrozoobenthos communities was relatively high over the whole course of the study, especially if the analysis was based on relative abundances. No clear treatment related effects were found.

Principal Response curves

The PRCs do not show any concentration-related effects on the community structure. Due to one missing sample, permutation analysis of the significance of the PRCs was not possible. Deviations from control after application were usually not much larger than before application and largest deviations from the control were calculated for the lower treatment levels. Therefore, and based on the results of the diversity and similarity analysis, ordination analysis per sampling date and Williams tests were not conducted and the community NOEC is assumed to be 9 µg a.s./L.

The highest species weight was calculated for the Bivalvia. For all other orders the (absolute) weights were smaller than 1 indicating small correspondence with the PRCs.

Summary of effects on the macrozoobenthos community level

In total, no indication of direct or indirect effects of the test item on the macrozoobenthos community was found except for slightly increased mean total abundance at 9 µg a.s./L on a single sampling date (8 weeks after application) which was not considered to be an effect.

Table KCP 10.2.3-6: NOECs [µg a.s./L] calculated for the macrozoobenthos community endpoints

Day	-13	-2	13	21	28	42	56	72	84	Cons.
Total macrozoobenthos	9	9	9	9	9	9	3+	9	9	
N (species)	9	9	9	9	9	9	9	9	9	
Shannon	9	9	9	9	9	9	9	9	9	
Evenness	9	9	9	9	9	9	9	9	9	
Similarity*	9	9	9	9	9	9	9	9	9	
Ordination (PCA)	9	9	9	9	9	9	9	9	9	

* NOECs for Similarity only estimated based on the diagrams

NOECs calculated by the Williams-test ($p<0.05$, one-sided), Bold numbers indicate a significant effect at least at the highest treatment level (9 µg a.s./L). Shading indicates significant effects at least over two sampling dates. A + indicates a NOEC for an increase

Abundances of macrozoobenthos orders

NOECs for the abundance of the 12 orders found in the macrozoobenthos were only found for three taxa (Acari, Odonata and Gastropoda) on single sampling dates after application and (with one exception) calculated for an increase of abundance at 9 µg a.s./L. Only for Odonata larvae on day 56 a NOEC of 3 µg a.s./L was calculated for lower abundance.

According to the diagrams of the population dynamics no indication of adverse effects is given.

The NOEC calculated for water mites (Acari) is based on one single animal found in one replicate of the 9 µg a.s./L treatment while no mites were found in all other samples. Therefore, the diagram is not shown.

For Gastropoda significantly higher abundances were found at the end of the study (day 84) at 9 µg a.s./L but without any indication of treatment-related direct or indirect effects before this.

Odonata were found with up to 20 larvae per sample but the data indicate no effects: The NOEC of 3 µg a.s./L for a higher abundance seems to be caused only by a reduced variability within the controls, because mean abundance in controls and at 9 µg a.s./L did not change very much from the sampling before application to day 14. The statistical findings later fall into the time with generally lower dragonfly numbers found and thus, they are likely caused only by chance.

The diagrams for the taxa without findings of statistically significant differences to controls do not show any treatment-related trends.

Summary of the macrozoobenthos

No adverse direct or indirect effects of the test item were found either on the community or on the population level of the macrozoobenthos (class 1).

III. Conclusions

Fate and exposure

The initial exposure to the test item met the nominal loading of the enclosures, as shown by Diflufenican measurements in the application solutions (resulting in theoretically 92 % of the nominal concentrations on average) and in enclosure water sampled 2 - 3 hours after the application (resulting in 92 % recovery of the nominal concentrations on average).

Diflufenican concentrations in the water phase decreased comparably in all enclosures with small differences between replicates. At the higher treatment levels (1.5 µg a.s./L and higher), where 7 or 98 values above the LOQ were available for fitting, the DT50 was approximately 3 weeks.

The concentrations of the metabolite TFMP-Na(acid) in the water increased over the first 8 weeks after application and were relatively stable in the last 4 weeks of the study. The maximum measured concentration (at nominal 9 µg a.s./L) was 2.4 µg metabolite/L.

Diflufenican was found in the sediment with up to 12 µg a.s./kg at the highest treatment level. Despite some variability (likely caused by inhomogeneous distribution in the sediment), the concentrations were relatively stable until the end of the study. TFMP-Na(acid) was always found in concentrations below 1 µg metabolite/kg with a trend of increasing concentrations until the end of the study.

As the analysis of the application solutions and the water samples taken a few hours after application confirmed the intended exposure, the effects are related to the nominal (initial) concentrations. A mean DT50 of 22 days was found for the active substance in the three highest treatment levels.

Effects

At the different treatment levels the following effects were found (Table 10.2-17 for classification according to SANCO 2002 and de Jong et al. 2008):

0.25 µg a.s./L: No effects were found on any of the monitored population or community level endpoints (class 1)

0.75 µg a.s./L: For the rotifer Hexarthra higher abundances were found over a few sampling dates. However, the strength of increase was not related to the test concentrations, and the difference to control was statistically significant only on two single (non-consecutive) sampling dates. Thus, this was considered as a class 2 + effect. No effects were found on any other population or community. 1.5 µg a.s./L: Slight short-term effects found on the phytoplankton community structure due do clear effects on the Cryptophyte *Erkenia*. Pigment analysis revealed a short-term positive effect on periphyton while no effects were found on macrophytes. Within the zooplankton clear short-term positive effects were found for *Daphnia* which, however, had no effects on the community structure. The macrozoobenthos was not affected. Thus, class 3A effects for decrease or increase of a few taxa were given.

3.0 µg a.s./L: Clear short-term effects were found on the phytoplankton community due to effects on Cryptophyceae, Bacillariophyceae and Chrysophyceae. For the increase of periphyton (pigment analysis) no recovery within the study period was observed. Abundance of Lemna was significantly reduced and full recovery could not be demonstrated. However, a clear population growth was observed in the last weeks of the study. Rooted macrophytes were not affected. The zooplankton community was slightly affected due to (positive) short-term effects on *Daphnia* and *Hexarthra*. No effects were found for the macrozoobenthos. Thus, due to possible indirect effects on the periphyton and Lemna at the end of the study, class 5B effect are assigned.

9.0 µg a.s./L: In addition to the effects observed at 3 µg a.s./L, effects on Baciallariophyceae (i.e. *Peroniella*), and Xantophyceae at the end of the study. Within the zooplankton, the total rotifer abundance was temporarily and slightly increased and Cyclopoida might have been negatively affected but showed recovery. Thus, class 5B is also given for the highest treatment level.

Thus, the test item had direct effects on the phytoplankton and the macrophyte *Lemna* starting at 1.5 respectively 3 µg a.s./L. Positive effects indicated by increased abundances were found for some phytoplankton taxa, the periphyton, and *Daphnia* and rotifers in the zooplankton community. Cyclopodidae might have been affected at the highest treatment level.

Table KCP 10.2.3-7: Summary of effect classes observed for several endpoints in the outdoor mesocosm study with Diflufenican 500 SC (based on the most sensitive taxon per group)*

	Nominal initial concentration [µg a.s./L]				
	0.25	**0.75**	**1.5**	**3**	**9**
Phytoplankton					
Community structure	1	1	2	3A	3A
Abundance of taxa	1	1	3A	3A	5B+
Pigments	1	1	1	2+	2+
Periphyton					
Pigment analysis	1	1	2	5B+	5B+
Macrophytes					
Lemna	1	1	1	5B	5B
Rooted macrophytes	1	1	1	1	1
Photosynthetic activity					
O2, pH, Conductivity	1	1	1	1	1
Zooplankton					
Community structure	1	1	1	2	2
Abundance of taxa	1	2+	3A+	3A+	3A+
Macrozoobenthos					
Community structure	1	1	1	1	1
Abundance of taxa	1	1	1	1	1
Study		**NOEC**	**NOEAEC**		

*Effect classes according to the Guidance Document on Aquatic Ecotoxicology (SANCO 2002) and de Jong et al. (2008): 1 = effect could not be demonstrated, 2 = slight and or temporary effect without statistical significance over at least two consecutive samplings, 3A = pronounced short-term effect with recovery within 8 weeks after application or total period of effects < 8 weeks, 5B = pronounced effects without full recovery within the study, '+'= increase of abundance.

0.75 µg a.s./L is considered as the general NOEC for the study because at this concentration no indication of direct or indirect pronounced effects on any taxon was found. Also the community structure of phytoplankton, zooplankton and macrozoobenthos was not affected. Only for the rotifer *Hexartha* slight and short-term increase of abundance was found. However, this increase in abundance was statistically significant only at two single (not consecutive) sampling dates and not correlated to the test concentration. In addition, it did not significantly affect the community structure. Thus, it was considered as a class 2 effect and not relevant for the study NOEC.

1.5 µg a.s./L is considered to be the study specific NOEAEC because no long-term effects were found at this concentration while at the next higher concentration significant differences to controls at the end of the study could not be excluded for macrophytes and the periphyton.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS: The study is considered valid. All validity criteria were met:
− the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
− the LD_{50}/24 h of the reference item (dimethoate) was 0.15 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

Agreed toxicity endpoints:

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD_{50} [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	0.0	> 200.0
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	1	3.3	
100.0	30	1	3.3	
200.0	30	2	6.7	

Reference: KCP 10.3.1.1.1/01

Report: Diflufenikan 500 SC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test; Kulec-Płoszczyca E.; 2022; Study Code: B-99-22

Guideline(s): Yes, OECD 213

Deviations: Not relevant

GLP: Yes

Acceptability: Yes

Duplication: No

(if vertebrate study)

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024

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

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna
Age:	adult worker bees about 3 weeks old
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	50% sucrose solution
Test units:	cages 5cm x 7cm x 4.5cm

4. Environmental conditions:

Temperature:	25ºC
Relative humidity:	air humidity 63-64%
Photoperiod:	dark room

STUDY DESIGN AND METHOD

The acute oral toxicity study of Diflufenikan 500 SC was conducted to determine the LD_{50}. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. Quarantine of the bees was not carried out, because within a month before the beginning of the study, insects were not treated with chemicals compounds. Before the experiment. the honeycomb with the honeybees was transferred from the apiary to an experimental room. The honeybees were removed from the comb and starved for up to two hours before the initiation of the treatment. In the definitive test, five doses of the test item i.e.: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee were used (with a separation factor of 2) plus the control. In the definitive test, three doses of the reference item, dimethoate were used. Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of 50% sucrose solution, containing the test item at

the doses mentioned above, using a micropipette. During the entire experiment, the insects were caged in groups of 10. After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute oral toxicity test finished after the 48-hour observation.

Test design:	tested doses and control in 3 replicates, 10 bees per replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	12.5, 25, 50, 100 and 200 µg/bee
Dates:	start of the study 28.07.2022 start of the experimental part: 28.07.2022 end of the experimental part: 30.08.2022 end of the study: 29.09.2022
Statistic:	ToxRat Professional 3.3.0. software, Probit analysis using linear max. likelihood regression

CONCLUSION

The acute oral toxicity study of the test item, Diflufenikan 500 SC on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below. The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee.

Table KCP 10.3.1.1.1-1: *Apis mellifera* acute oral toxicity test -final results

Dose [µg/bee]	Mortality after 48 h after the beginning of the treatment (%)	LD_{50} [µg/bee]
0.0	0.0	>200
12.5	0.0	
25	0.0	
50	3.3	
100	3.3	
200	6.7	

Comments of zRMS:	The study is considered valid. All validity criteria were met: − mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%). − mortality in the toxic reference item group (dimethoate) at the end of the test was 100.0% (criterion: ≥ 50%). Deviations in the study: The test was performed according to the OECD Guideline for the Testing of Chemicals No. 247 (2017): Bumblebee, Acute Oral Toxicity Test' [1], other references given in section 9 and the SOP's listed in section 10 of the report. In the study following deviation occurred. According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration. However, in the experiment they were replaced by calibrated glass pipettes. This deviations had no impact on the quality, integrity and final results of the study.

Agreed toxicity endpoints:

Dose		Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}/48 h	
test item [µg/bumblebee]	diflufenican [µg a.i. / bumble-bee]		[no.]	[%]	[µg/ bumblebee]	diflufenican [µg a.i. / bumblebee]
Control		50	0	0.0	> 200.0	> 83.9
200.0	83.9	50	0	0.0		
Reference item: dimethoate						
Dose [µg/bumblebee]	4.0	30	30	100.0	–	

Reference: KCP 10.3.1.1.1/02

Report: Diflufenikan 500 SC Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Kulec-Płoszczyca E; 2022; Study Code: B-100-22

Guideline(s): Yes, OECD 247

Deviations: According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration. However, in the experiment they were replaced by calibrated glass pipettes.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): Diflufenikan 500 SC

Formulation: OD (diflufenican 500 g/L)

Description (physical state): white liquid

Batch no.: 1/DIF/2022

Production date: 01.2022

Expiration date: 01.2024

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

3. Test organism

Species: bumbleebee (*Bombus* spp.)

Source: commercial supplier: Koppert Polska sp. z o.o.

Age: adult worker bumblebees

Acclimation period:	acclimatized to the test conditions for about 24 hours before starting the experiment
Diet:	50% sucrose solution
Test units:	a dark climate room

4. Environmental conditions:

Temperature:	temperature 25-25.5ºC
Relative humidity:	64-66%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The study was conducted to determine the acute oral toxicity of Diflufenikan 500 SC to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD_{50} at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200 µg test item/bumblebee, plus the control and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary test results. The bumblebees were exposed to the test item distributed in a 50% aqueous sucrose solution. The insects were selected for the exposure in terms of their sizes. The treated diet was provided in calibrated pipettes. Each pipette contained 40 µL of the sucrose solution with the test item at the tested dose. The insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 4.0 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4-5, 24 and 48 hours after the test/ reference item administration. The acute oral toxicity test finished after the 48-hour observation.

Test design:	tested dose and control in 50 replicates, 1 insect per replicates; reference item in 30 replicates, 1 insect per replicates
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	200 µg/bumblebee (limit test)
Dates:	start of the study 25.02.2022 start of the experimental part: 08.02.2022 end of the experimental part: 11.03.2022 end of the study: 19.05.2022
Statistic:	not relevant, statistical analysis was not needed due to the lack of mortality

CONCLUSION

The acute oral toxicity study of the test item, Diflufenikan 500 SC on bumblebees in the laboratory test are summarized below. The median lethal doses (LD_{50}/24 h, LD_{50}/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee i.e. > 83.9 µg diflufenican/ bumblebee.

Table KCP 10.3.1.1.1-2: *Bombus* spp. acute oral toxicity test -final results

Dose test item [µg/ bumblebee]	Dose diflufenican [µg a.i. / bumblebee]	Mortality after 48 h (%)	LD_{50}/48 h [µg/bumblebee]	LD_{50}/48 h Diflufenican [µg a.i. /

				bumblebee]
Control		0.0	**> 200.0**	**> 83.9**
200.0	83.9	0.0		

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met: − the mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10.0%), − the LD_{50}/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10–0.30 µg a.i./bee). The following validity criteria were met during the test: The study was performed according to the OECD Guideline No. 214/EU Method C.17. However, According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilisation [SOP/B/48]. This method was described in the Study Plan and the SOP/B/48. The mentioned deviation had not effect on the results of the study. **Agreed toxicity endpoints:** LD_{50} > 200 µg formulation/bee

Reference:	KCP 10.3.1.1.2/01
Report	Diflufenikan 500 SC Honeybees (*Apis mellifera* L.) Acute Contact Toxicity Test; Kulec-Płoszczyca E.; 2022; Study Code: B-101-22
Guideline(s):	Yes, OECD 214
Deviations:	According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilisation.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflifenikan 500 SC
Formulation:	SC (diflufenican 40 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024

2. Vehicle and/or positive control: vehicle water + control with surfactant (1% Triton)
positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna
Age:	adult worker bees approximately 3 weeks
Acclimation period:	the quarantine was not carried out because insects were not treated with any chemical compounds within a month before the start of the study
Diet:	50% sucrose solution
Test units:	cages 5cm x 7cm x 4.5cm

4. Environmental conditions:

Temperature:	24.5-25 ºC
Relative humidity:	63-64%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

Mortality of honeybees, *Apis mellifera*, exposed to Diflufenikan 500 SC was investigated during 48-hour test. Five doses of the test item plus two controls were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. A microapplicator was used to apply the test ite. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

Test design:	tested doses and control in three replicates, 10 bees per replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	12.5, 5, 50, 100 and 200 µg/bee
Dates:	start of the study 20.07.2022 start of the experimental part: 04.08.2022 end of the experimental part: 06.08.2022 end of the study: 29.09.2022
Statistic:	ToxRat Professional 3.3.0. software, Probit analysis using linear max. likelihood regression

CONCLUSION

The acute oral toxicity study of the test item, Diflufenikan 500 SC on bees in the laboratory test are summarized below. The median lethal doses LD_{50}/24 h and LD50/48 h are higher than the highest dose used in the test i.e. 200 µg/honeybee.

Table KCP 10.3.1.1.2-1: *Apis mellifera* acute contact toxicity test - final results

Dose [µg/bee]	Mortality after 48 h after the beginning of the treatment (%)	LD_{50} [µg/bee]
0.0 water control	0.0	>200
0.0 1% Triton control	0.0	
12.5	0.0	
25	0.0	
50	0.0	
100	0.0	
200	0.0	

Comments of zRMS:	The study is considered valid. All validity criteria were met: The following validity criteria were met: − Mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%). − Mortality in the toxic reference item group (dimethoate) at the end of the test was 90.0% (criterion: ≥ 50%). Deviations of the study: The test was performed according to the OECD Guideline for Testing of Chemicals No. 246 (2017): Bumblebee, Acute Contact Toxicity Test' [1], other references given in section 9 and the SOP's listed in section 10 of the report. According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation. **Agreed toxicity endpoints:** LD_{50} > 100 µg formulation/bumblebee (>41.9 µg diflufenikan/bumblebee)

Reference:	KCP 10.3.1.1.2/02
Report	Diflufenikan 500 SC Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test; Kulec-Płosczyca E; 2022; Study Code: B-102-22
Guideline(s):	Yes, OECD 246
Deviations:	According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
2. Vehicle and/or positive control:	vehicle water + control with surfactant (1% Triton) positive control: dimethoate
3. Test organism	
Species:	bumbleebee (*Bombus* spp.)
Source:	commercial supplier: Koppert Polska sp. z o.o.
Age:	adult worker bumblebees
Acclimation period:	acclimatized to the test conditions for about 24 hours before starting the experiment
Diet:	50% sucrose solution
Test units:	a dark climate room
4. Environmental conditions:	
Temperature:	25-25.5ºC
Relative humidity:	64-66%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The study was conducted to determine the acute contact toxicity of Diflufenikan 500 SC to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD_{50} at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 100.0 µg test item/bumblebee, plus the controls and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary range - finding test results. The bumblebees were exposed to the test item diluted in distilled water with surfactant Triton X-100 and applied to the dorsal part of the thorax, using a microapplicator. The volume was 2 µL/bumblebee. The insects were selected for the exposure in terms of their sizes. After that, the insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 10.0 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4, 24 and 48 hours after the test/ reference item administration. The acute contact toxicity test finished after the 48-hour observation.

Test design:	tested dose and controls in 50 replicates, 1 insect per replicates; reference item in 30 replicates, 1 insect per replicates
Exposure time:	acute test, 48 h

Tested concentrations, definitive test:	100 µg/bumblebee (limit test)
Dates:	start of the study 16.02.2022 start of the experimental part: 01.03.2022 end of the experimental part: 03.03.2022 end of the study: 13.05.2022
Statistic:	not relevant, statistical analysis was not needed due to the lack of mortality

CONCLUSION

The acute contact toxicity study of the test item, Diflufenikan 500 SC on bumblebees in the laboratory test are summarized below. The median lethal doses (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 100.0 µg test item/bumblebee i.e. > 41.9 µg diflufenican/bumblebee.

Table KCP 10.3.1.1.2-2: Bombuss spp. acute contact toxicity test - final results

Dose test item [µg/ bumblebee]	Dose diflufenican [µg a.i. / bumblebee]	Mortality after 48 h (%)	LD_{50}/48 h [µg/bumblebee]	LD_{50}/48 h Diflufenican [µg a.i. / bumblebee]
Control		0.0	**> 100.0**	**> 41.9**
Control + 1% surfactant		0.0		
100.0	41.9	0.0		

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met: 1. Mortality in the control group Average mortality across replicates for the control (50% w/v sucrose solution only) ≤ 15% at the end of the test (actual value was 6.67%, therefore, the validity criterion was met). 2. Mortality in the reference group Mortality rate at the end of the test period of 100% (actual value was 100.00%, therefore, the validity criterion was met). **Agreed toxicity endpoints:** LDD_{50} = 1203.11 µg formulation/bee/day (502.08 µg diflufenikan/bee/day) LDD_{20} = 454.34 µg formulation/bee/day (189.71 µg diflufenikan/bee/day) LDD_{10} = 273.10 µg formulation/bee/day (114.07 µg diflufenikan/bee/day) NOEDD = 226.86 µg formulation/bee/day (96.29 µg diflufenikan/bee/day)

Reference:	KCP 10.3.1.2/01
Report	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Chronic Oral Toxicity Test; Mautino G.; 2023; Study Code: 1003.H.SAG22
Guideline(s):	Yes, OECD 245
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - average mortality across replicates for the control (50% w/v sucrose solution only) ≤ 15% at the end of the test; - mortality in the reference group ≥ 50% at the end of the test period

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	The content of diflufenican active ingredient was determined in the feeding solutions of honey bees new born workers of the biological phase of the study.

2. Vehicle and/or positive control:	vehicle: 50% sucrose solution positive control: ROGOR L 40 ST (nominally 400 g/L dimethoate)

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	Beekeeper Paolo Farinetti, Via Montà Castino 25, 12074 Cortemilia (CN), Italy. One commercial beehive, queen-right, healthy (disease free) and adequately fed, with normal population of young adult worker individuals was placed at SAGEA Centro di Saggio s.r.l. Test Facility.
Age:	max. 2-day old
Acclimation period:	The test units were placed into an incubator, and kept under darkness at the mean environmental conditions of 33±2 °C; 50-70% RH for at least 1 day, until the beginning of the test. Bees were fed ad libitum with sucrose solution only.

Diet:	Sucrose solution in water with a final concentration of 500 g/L (50% w/v) was used as food ad libitum. The syrup was administered using a 2.5 mL syringe. The syringes were inserted into the cage via an opening in the top of the test unit. Food was daily replaced by changing the feeders until the end of test. Food consumption was adjusted for the test solutions evaporation from the feeders.
Test units:	Ventilated stainless steel cages 8.5 cm x 6.5 cm x 4.5 cm (length x height x width), front side: removable glass panel, back side: perforated with 50 ventilation holes; Ø 2 mm.

4. Environmental conditions:

Temperature:	34.56 ± 0.048 °C (34.44 – 34.63 °C)
Relative humidity:	61.8 ± 0.7% (62.4 – 60.7%)
Photoperiod:	photoperiod: 0 h light: 24 h dark

STUDY DESIGN AND METHOD

The purpose of this study was to determine the chronic oral toxicity of DIFLUFENICAN 500 SC to young adult honeybees (*Apis mellifera* L.). The study was carried out in accordance with OECD Guideline No 245. One day before test start, bees were collected from brood combs without the use of smoke and without anaesthetics. By means of a proper brush, the bees were collected in plastic containers with holes for oxygenation and immediately transported to SAGEA's laboratory. Once in the laboratory, the bees were randomly allocated to the test units (cages) after a light anaesthetisation with CO2 (2 bar for about 45 seconds). Anaesthetised bees were gently transferred to the test units by means of a plastic spoon. The study consisted of 7 treatments (5 rates of the test item, 1 control group, 1 reference item) with 3 replicates, each containing 10 bees per cage. The doses of the test and reference items were dispersed in a 50% sucrose solution in water and offered ad libitum. Feeding solutions were replaced daily by changing the feeders. Mortality was recorded daily for 10 days.

Test design:	tested dose and control in three replicates, 10 bees per replicate
Exposure time:	chronic test, 10 days
Tested concentrations, definitive test:	0.8, 2, 5, 12.5 and 31.25 µL test item/bee (400, 1000, 2500, 6250 and 15625 µg a.s./bee
Dates:	start of the study 05.09.2022 start of the experimental part: 22.09.2022 end of the experimental part: 02.10.2022 end of the study: 25.05.2023

Statistic: Software used for statistical analysis was "Agricultural Research Manager 2020" (ARM)", version 2020. Mortality data were analysed by ANOVA test and subsequently, if it is significant, by S-N-K's test, α≤0.05 and the LD50 calculated. On the mortality data the standard error was calculated. The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for mortality were calculated.

RESULTS

All study validity criteria were met.

At the end of the exposure period the cumulative mortality in the control (sucrose solution in water 50% w/v only) was 6.67% and test item DIFLUFENIKAN 500 SC values ranged from 10.00% in treatment 1593.6 mg a.i./Kg feeding solution to 100.00% in treatment 62250 mg a.i./Kg feeding solution. Reference item mortality reached the 100.00%.

Table KCP 10.3.1.2-1: Average percentage of young adult bee's mortality after 10 days

Treatment number	Treatment	Application rate (a.i. nominal intake)	Concentration (mg a.i./kg feeding solution)	Concentration (µg a.i./bee/day)	Mortality (%)	Survivors correction (%)[b]
T1	Control	Sucrose solution 50% w/v	-	-	6.67	-
T2	DIFLUFENIKAN 500 SC	400 µg a.i./bee	1593.6 mg a.i./kg	40.74 µg a.i./bee/day	10.00	3.57
T3	DIFLUFENIKAN 500 SC	1000 µg a.i./bee	3984 mg a.i./kg	96.29 µg a.i./bee/day	10.00	3.57
T4	DIFLUFENIKAN 500 SC	2500 µg a.i./bee	9960 mg a.i./kg	273.94 µg a.i./bee/day	33.33	28.57
T5	DIFLUFENIKAN 500 SC	6250 µg a.i./bee	24900 mg a.i./kg	650.56 µg a.i./bee/day	46.67	42.86
T6	DIFLUFENIKAN 500 SC	15625 µg a.i./bee	62250 mg a.i./kg	973.94 µg a.i./bee/day	100	100.00
T7	ROGOR L 40 ST	1 mg dimethoate/kg feeding solution		0.19 µg dimethoate /bee/day	100	100.00

CONCLUSION

Mortality in the control units (50% w/v sucrose solution) was 6.67% at day 10.

At the end of the exposure period the cumulative mortality in the control (sucrose solution in water 50% w/v only) was 6.67% and test item DIFLUFENIKAN 500 SC values ranged from 10.00% in treatment T2 (1593.6 mg a.i./Kg feeding solution) to 100.00% in treatment T6 (62250 mg a.i./Kg feeding solution). Reference item mortality reached the 100.00%.

A dose-response effect on young adults' mortality was observed between treatment T2, T3, T4, T5 and T6

and the control.

The 10-d NOEC (mortality) value corresponded to 3984 mg a.i./Kg feeding solution (treatment T3) and 10-d LOEC (mortality) matched with the rate of 9960 mg a.i./Kg feeding solution (treatment T3).
The 10-d LC50 was 21002 mg a.i./Kg feeding solution (95% confidence intervals 18962.52 – 23428.34 mg a.i./Kg feeding solution).

The 10-d NOEDD (mortality) value corresponded to 96.29 µg a.i./bee/day (treatment T2) and 10-d LOEDD (mortality) matched with the rate of 273.94 µg a.i./bee/day (treatment T3).

The calculated 10-d LDD50 was 502.08 µg a.i./bee/day (95% confidence intervals 455.08 – 558.14).
For the mean uptake of feeding solution/bee/day at the end of the test period (expressed as mean of the mean values), ranged from 17.20 mg (treatment T6) to 34.80 mg/bee/day (treatment T4); moreover, a 28.75 mg value of mean uptake was observed for the toxic references and the control (50% w/v sucrose solution only) showed a value of 25.99 mg/bee/day.

By considering the mean uptake in µg of a.i./bee (i.e., expressed as mean of the mean values), test item DIFLUFENIKAN 500 SC mean values (expressed as mean of the mean values) ranged from 40.74 to 937.94 µg a.i./bee/day on treatments T2 (400 µg a.i./bee) and T6 (15625 µg a.i./bee), respectively. ROGOR L40 ST showed a mean value of 0.027 µg a.i./bee/day.

Table KCP 10.3.1.2-2: Mortality of young adult bees after 10 days

Endpoints	mg a.i./kg feeding solution	mg f.p./kg feeding solution
LC_{10} [95% confidence intervals]	4536.72 mg a.i./Kg feeding solution [3903.33 – 5176.34]	10836 mg f.p./Kg feeding solution [9317.40 – 12371.79]
LC_{20} [95% confidence intervals]	7677.17 mg a.i./Kg feeding solution [6833.44 – 8542.03]	18372.74 mg f.p./Kg feeding solution [16345.69 – 20450.85]
LC_{50} [95% confidence intervals]	21002 mg a.i./Kg feeding solution 18962.52 – 23428.34]	50441.16 mg f.p./Kg feeding solution [45527.91 – 56288.46]
NOEC	3984 mg a.i./Kg feeding solution	9513 mg f.p./kg feeding solution
LOEC	9960 mg a.i./Kg feeding solution	23784 mg f.p./kg feeding solution
LDD_{10}	114.07 µg a.i./bee/day [98.02 – 130.07]	273.10 µg f.p./bee/day [234.68 – 311.41]
LDD_{20}	189.71 µg a.i./bee/day [169.11 – 210.55]	454.34 µg f.p./bee/day [404.99 – 504.26]
LDD_{50}	502.08 µg a.i./bee/day [455.08 – 558.14]	1203.11 µg f.p./bee/day [1090.42 – 1337.48]
NOEDD	96.29 µg a.i./bee/day	226.86 µg f.p./bee/day
LOEDD	273.94 µg a.i./bee/day	656.70 µg f.p./bee/day

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

Not relevant. No studies submitted. The 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) is still being reviewing hence the waiving of request for chronic studies is fully justified. The chronic studies for bees and larvae will be provided when EFSA guidance is in force.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Comments of zRMS:	The study is considered valid. All validity criteria were met. Mortality in the control group: ❖ Cumulative larval mortality from D3 to D8 was 6.25%, therefore the validity criterion was met. ❖ Adult emergence at D22 was 87.50%, therefore the validity criterion was met. Mortality in the reference group: ❖ Larval mortality was 100% at D8. **Agreed toxicity endpoint:** LD_{50} = 1430 µg formulation/larva (598.90 µg diflufenikan/larva) NOED < 57.31 µg formulation/larva (20.48 µg diflufenikan/bee/day) LOED = 57.31 µg formulation/larva (20.48 µg diflufenikan/bee/day)

Reference:	KCP 10.3.1.4/01
Report	Effects of DIFLUFENIKAN 500 SC (diflufenican 500 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Larval Toxicity Test Following Repeated Exposure; Mautino G.; 2023; Study Code: 1004.H.SAG22
Guideline(s):	Yes, OECD GD 239
Deviations:	The calculated concentrations of sample 1-T2-1004.H.SAG22S analytical extracts didn't fall within the ± 20 % of the calibration range, slightly exceeding the 784 ng/mL concentration value calculated as 80 % of the last calibration point, 979.7 ng/mL.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - in the control plate(s), cumulative larval mortality from day-3 to day-8 ≤ 15% across all replicates; - in the control plate(s), the adult emergence rate on day-22 ≥ 70% across all replicates; - test item: larval mortality ≥ 50% on day-8 across all replicates.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid

Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	The content of diflufenican active ingredient was determined in the water solutions of the biological phase of the study.
2. Vehicle and/or positive control:	vehicle: 50% sucrose solution positive control: ROGOR L 40 ST (dimethoat)
3. Test organism	
Species:	honeybee *Apis mellifera*
Source:	Beekeeper Paolo Farinetti. Three commercial beehives, queenright, healthy (disease free) and adequately fed, with normal population of young adult worker individuals (approx. 2 weeks old) was placed at SAGEA Centro di Saggio s.r.l. Test Facility.
Age:	first instar larvae
Diet:	The larval food was composed of the three following diets, adapted to the needs of the larvae at different stages of development: - Diet A (16 Aug 2022) for all theses: 50% weight of fresh royal jelly (8.800 g) + 50% weight of an aqueous solution containing 2% weight of yeast extract (0.176 g), 12% weight of glucose (1.056 g), 12% weight of fructose (1.056 g) and 6.512 g of deionized water. - Diet B (18 Aug 2022) for thesis T1 (untreated): 50% weight of fresh royal jelly (1.100 g) + 50% weight of an aqueous solution containing 3% weight of yeast extract (0.033 g), 15% weight of glucose (0.165 g), 15% weight of fructose (0.165 g) and 0.737 g of deionized water. - Diet B (18 Aug 2022) for treated theses: 50% weight of fresh royal jelly (7.700 g) + 50% weight of an aqueous solution containing 3% weight of yeast extract (0.231 g), 15% weight of glucose (1.155 g), 15% weight of fructose (1.155 g) and 3.659 g of deionized water. - Diet C (19 Aug 2022) for thesis T1 (untreated): 50% weight of fresh royal jelly (3.300 g) + 50% weight of an aqueous solution containing 4% weight of yeast extract (0.132 g), 18% weight of glucose (0.594 g), 18% weight of fructose (0.594 g) and 1.980 g of deionized water. - Diet C (19 Aug 2022) for treated thesis: 50% weight of fresh royal jelly (34.100 g) + 50% weight of an aqueous solution containing 4% weight of yeast extract (1.364 g), 18% weight of glucose (6.138 g), 18% weight of fructose (6.138 g) and 13.660 g of deionized water. After preparation, both containers of diet C has been preserved in fridge well covered with parafilm at 4 °C for two days.

Test units:	Larvae were reared in crystal polystyrene grafting cells having an internal diameter of 9 mm and a depth of 8 mm. Each cell was placed into a well of a 48 multi-well plate. The top of the grafting cell was maintained at the level of the plate by placing a piece of dental roll. The plates have been sterilized before being used.
4. Environmental conditions:	
Temperature/ relative humidity:	Mean Test conditions from day-1 to day-8: Temperature: 34.70 ± 0.09 °C (34.77 – 34.54 °C) Relative humidity: 95.2 ± 1.3% RH (97.6 – 93.7%) Photoperiod: light: darkness (except during observation and food replacement) Mean Test conditions from day-8 to day-15: Temperature: 34.52 ± 0.13 °C (34.76 – 34.39 °C) Relative humidity: 81.0 ± 1.1% (83.1 – 80.0%) Photoperiod: light: darkness (except during observation and food replacement) Mean Test conditions from day-15 to day-22: Temperature: 34.37 ± 0.14 °C (34.55 – 34.19 °C) Relative humidity: 64.1 ± 4.3% (70.1 – 59.9%) Photoperiod: light: darkness (except during observation and food replacement)

STUDY DESIGN AND METHOD

The purpose of this study was to determine the chronic oral toxicity of the DIFLUFENIKAN 500 SC (diflufenican 500 g/L) on honeybee larvae (Apis mellifera L.) consequently to a repeated exposure under laboratory conditions, providing larvae with food added with the test item. Adults' emergence at day-22 was used as the toxic endpoint. A Range finding test was initially performed followed by the Definitive Test. The Definitive Test rates were established taking into consideration the Range finding test results. The Definitive test was performed using five doses of the test item DIFLUFENIKAN 500 SC (diflufenican 500 g/L) in a geometric series, with factor 2.5 and covering the range for ED50.
Larvae were collected from three different colonies, each one representing a replicate. 16 per replicate, 48 per treatment. Test item was compared with an untreated control and a toxic standard as recommended in the guideline for a ED50 approach. Reference item was ROGOR L 40 ST (dimethoate 400 g/L). From day-3 to day-6, test and reference items were dispersed in the diet, following the OECD 239 scheme, at the suitable concentrations. Larval mortality was recorded at the time of feeding from day-4 to day-8, moreover from day-8 to day-22 pupal mortality was evaluated and on day-22, the number of emerged adults was counted.

Test design:	16 larvae x 3 colonies = 48 larvae
Exposure time:	chronic test, exposition: 4 days (from D3 to D6), duration of the test: 22 days
Tested concentrations, definitive test:	0.041 µL/larva (20.48 µg a.i./larva) 0.10 µL/larva (51.20 µg a.i./larva) 0.26 µL/larva (128 µg a.i./larva) 0.64 µL/larva (320 µg a.i./larva) 1.60 µL/larva (800 µg a.i./larva)

Dates: start of the study 05.09.2022
start of the experimental part: 03.10.2022
end of the experimental part: 24.10.2022
end of the study: 08.06.2023

Statistic: Software used for statistical analysis was "Agricultural Research Manager" (ARM)", 2022.5.
Mortality data were analysed by ANOVA test, subsequently, the pair-wise S-N-K's test (α≤0.05) was used and the ED50 calculated where possible.
The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for adults' emergence rate were calculated.

RESULTS

From day-3 to day-8, larvae were exposed to the test item DIFLUFENICAN 500 SC and reference item. The diet volume and composition were adapted on a daily basis.

Table KCP 10.3.1.4-1: Number of bee's larvae alive from day-2 to day-8

Treatment no.	Treatment	Application rate (Nominal intake)	Test item concentration in the larval diet	Bee's larvae alive						
				D2	D3	D4	D5	D6	D7	D8
T1	Control	-	-	48	48	48	48	47	45	45
T2	DIFLUFENIKAN 500 SC	0.041 µL f.p.*/larva (20.48 µg a.i.**/larva)	266.24 µL f.p./Kg of diet (133.12 mg a.i./Kg of diet)	48	48	47	44	43	42	42
T3	DIFLUFENIKAN 500 SC	0.10 µL f.p./larva (51.20 µg a.i./larva)	665.60 µL f.p./Kg of diet (332.80 mg a.i./Kg of diet)	48	48	48	47	45	42	42
T4	DIFLUFENIKAN 500 SC	0.26 µL f.p./larva (128 µg a.i./larva)	1664 µL f.p./Kg of diet (832 mg a.i./Kg of diet)	48	48	44	41	37	36	36
T5	DIFLUFENIKAN 500 SC	0.64 µL f.p./larva (320 µg a.i./larva)	4160 µL f.p./Kg of diet (2080 mg a.i./Kg of diet)	48	48	45	41	38	35	33
T6	DIFLUFENIKAN 500 SC	1.60 µL f.p./larva (800 µg a.i./larva)	10400 µL f.p./Kg of diet (5200 mg a.i./Kg of diet)	48	48	46	44	38	34	32
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	48	48	38	16	0	0	0

D = day
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

Table KCP 10.3.1.4-2: Cumulative mortality of bee's larvae from day-3 to day-8

Treatment		Application rate (Nominal intake)	Test item concentration in the larval diet	Cumulative %mortality					p^a
				D4	D5	D6	D7	D8	
T1	Control	-	-	0.00	0.00	2.08	6.25	6.25	c
T2	DIFLUFENIKAN 500 SC	0.041 µL f.p.*/larva (20.48 µg a.i.**/larva)	266.24 µL f.p./Kg of diet (133.12 mg a.i./Kg of diet)	2.08	8.33	10.42	12.50	12.50	c
T3	DIFLUFENIKAN 500 SC	0.10 µL f.p./larva (51.20 µg a.i./larva)	665.60 µL f.p./Kg of diet (332.80 mg a.i./Kg of diet)	0.00	2.08	6.25	12.50	12.50	c
T4	DIFLUFENIKAN 500 SC	0.26 µL f.p./larva (128 µg a.i./larva)	1664 µL f.p./Kg of diet (832 mg a.i./Kg of diet)	8.33	14.58	22.92	25.00	25.00	b
T5	DIFLUFENIKAN 500 SC	0.64 µL f.p./larva (320 µg a.i./larva)	4160 µL f.p./Kg of diet (2080 mg a.i./Kg of diet)	6.25	14.58	20.83	27.08	31.25	b
T6	DIFLUFENIKAN 500 SC	1.60 µL f.p./larva (800 µg a.i./larva)	10400 µL f.p./Kg of diet (5200 mg a.i./Kg of diet)	4.17	8.33	20.83	29.17	33.33	b
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	20.83	66.67	100	100	100	a

D = day
[a], S-N-K test (P≤0.05) on the data at day-8
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

At day-8, the cumulative mortality for DIFLUFENIKAN 500 SC ranged from 12.50% to 33.33% on treatments T2 and T3 and T6, respectively. The highest mortality was observed on treatment T7 (reference item ROGOR L 40 ST) with a total mortality that reached 100% and the control showed a cumulative mortality of 6.25%.

Larval mortality was evaluated from day-3 to day-8 after an exposure period of 3 days (from day-3 to day-6). Pupal mortality was calculated in percentage from D8 to D22.

Table KCP 10.3.1.4-3: Percent pupal mortality at day-15 and day-22 from day-8

Treatment		Application rate (Nominal intake)	Test item concentration in the larval diet	% pupae mortality at D15	Correct-ed mor-tality at D15	% pupal mor-tality at D22	p^a	Cor-rected mor-tality at D22[b]
T1	Control	-	-	2.38	-	4.31	b	-
T2	DIFLUFENIKAN 500 SC	0.041 µL f.p.*/larva (20.48 µg a.i.**/larva)	266.24 µL f.p./Kg of diet (133.12 mg a.i./Kg of diet)	4.60	2.28	10.07	ab	6.03
T3	DIFLUFENIKAN 500 SC	0.10 µL f.p./larva (51.20 µg a.i./larva)	665.60 µL f.p./Kg of diet (332.80 mg a.i./Kg of diet)	11.95	9.81	10.26	ab	6.22
T4	DIFLUFENIKAN 500 SC	0.26 µL f.p./larva (128 µg a.i./larva)	1664 µL f.p./Kg of diet (832 mg a.i./Kg of diet)	8.33	6.10	3.03	b	-1.33
T5	DIFLUFENIKAN 500 SC	0.64 µL f.p./larva (320 µg a.i./larva)	4160 µL f.p./Kg of diet (2080 mg a.i./Kg of diet)	2.78	0.41	12.42	ab	8.48
T6	DIFLUFENIKAN 500 SC	1.60 µL f.p./larva (800 µg a.i./larva)	10400 µL f.p./Kg of diet (5200 mg a.i./Kg of diet)	18.33	16.34	19.44	a	15.82
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	-	-	-	-	-

D = day
a, S-N-K test (P≤0.05)
b, mean mortality corrected by Schneider-Orelli's formula
-, not applicable
*f.p.: formulated product **a.i.: active ingredient

At day-15, mortality for DIFLUFENIKAN 500 SC ranged from 2.78% (corrected value: 0.41%) to 18.33% (corrected value: 16.34%) on treatments T4 and T6. Mortality in the control corresponds to 2.38%.

At day-22, pupal mortality ranged from 3.03% (corrected value: -1.33%) to 19.44% corrected value (15.82%) on treatments T2 and T6, respectively. Pupal mortality in the control group corresponded to 4.31%.

Adults' emergence and percent reduction in the adults' emergence in comparison to the control were calculated at day-22.

Table KCP 10.3.1.4-4: Adults' emergence at day-22

Treatment		Application rate (Nominal intake)	Test item concentration in the larval diet	Emergence rate (%)	p^a	Er (%)[b]
T1	Control	-	-	87.50	a	-
T2	DIFLUFENIKAN 500 SC	0.041 µL f.p.*/larva (20.48 µg a.i.**/larva)	266.24 µL f.p./Kg of diet (133.12 mg a.i./Kg of diet)	75.00	b	14.29
T3	DIFLUFENIKAN 500 SC	0.10 µL f.p./larva (51.20 µg a.i./larva)	665.60 µL f.p./Kg of diet (332.80 mg a.i./Kg of diet)	68.75	b	21.43
T4	DIFLUFENIKAN 500 SC	0.26 µL f.p./larva (128 µg a.i./larva)	1664 µL f.p./Kg of diet (832 mg a.i./Kg of diet)	66.67	bc	23.81
T5	DIFLUFENIKAN 500 SC	0.64 µL f.p./larva (320 µg a.i./larva)	4160 µL f.p./Kg of diet (2080 mg a.i./Kg of diet)	58.33	c	33.33
T6	DIFLUFENIKAN 500 SC	1.60 µL f.p./larva (800 µg a.i./larva)	10400 µL f.p./Kg of diet (5200 mg a.i./Kg of diet)	43.75	d	50.00
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	0.00	e	100.00

a, S-N-K test (P≤0.05) on the data at day-8
b, Er = emergence % reduction in comparison to the control
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

Emergence rate for DIFLUFENIKAN 500 SC ranged from 43.75% when the highest dosage (5200.00 mg a.i./Kg of diet) was applied, to 75.00% with treatment T2. Control showed value of 87.50%, while reference item 0.00%.

Percent reduction in emergence (Er%) for the test item ranged from 14.29% to 50.00% for the lowest (treatment T2) and highest (treatment T6) dosages, respectively.

The NOED value for the test item DIFLUFENIKAN 500 SC was lower than the lowest dosage applied (<20.48 µg a.i./larva) and NOEC was <133.12 mg a.i./kg diet. LOED value correspond to test the item dosage of 20.48 µg a.i./larva and LOEC to 133.12 mg a.i./kg diet.

The estimated 22-d ED_{50} for DIFLUFENIKAN 500 SC was 598.90 µg a.i./larva (upper limit n.d. – 401.48µg a.i./larva), while the EC_{50} was 3892.83 mg a.i./kg diet (95% confidence intervals are upper limit n.d. – 2609.61mg a.i./kg diet).

CONCLUSION

All study validity criteria were met.

Table KCP 10.3.1.4-5: Results of chronic toxicity to bees

Endpoints	µL f.p./larva	µg f.p./larva	µg a.i./larva
ED_{50}/LD_{50} [95% confidence intervals]	1.20 [U.L. n.d. – 0.80]	1430 [U.L. n.d. – 0.96]	598.90 [U.L. n.d. – 401.48]
NOED	< 0.048	< 57.31	< 20.48
LOED	0.048	57.31	20.48

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not relevant. No studies submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not relevant. No studies submitted.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods

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

Comments of zRMS:	The study is considered valid. All validity criteria were met: Mortality in control check: 1. Mortality in the water control to be ≤ 20% on day 7 (actual mortality was 6.00%, so the validity criterion was met). 2. Reproduction in control check Mean cumulative number of eggs per female in the water control to be ≥ 4 (actual value was 9.83, so this validity criterion was met). 3. Mortality in reference Corrected mortality to be between 50% and 100% in the toxic reference treatment on day 7 (actual value was 78.72%, so the validity criterion was met). **Agreed toxicity endpoints:** Standard laboratory test – *Typhlodromus pyri* Mortality parameter: 7d LR_{50} > 3000 ml formulation/ha (>1500 g diflufenikan/ha) Fecundity parameter: 14d ER_{50} > 3000 ml formulation/ha (>1500 g diflufenikan/ha)

Reference:	KCP 10.3.2.1/01
Report	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on Typhlodromus pyri in the laboratory – Standard laboratory test; Mautino G.; 2023; Study Code: 1017.1H.SAG22
Guideline(s):	Yes, IOBC, BART, EPPO
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria:	Validity criteria of the test:: -mortality in the water control ≤ 20% on day 7; -mean cumulative number of eggs per female in the water control ≥ 4; -corrected mortality between 50% and 100% in the toxic reference treatment on day 7.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	not relevant

2. Vehicle and/or positive control:	vehicle: deionised water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L)

3. Test organism

Species:	*Typhlodromus pyri* Scheuten, Phytoseiid (Acari: Phytoseiidae)
Source:	Katz Biotech AG, Baruth, Germany
Stage at delivery:	eggs
Age at test start:	protonymphs ≤24 hours old
Acclimation period:	1 day under test conditions in an incubator
Sex:	Females and males
Diet:	pollen (100% from apple, provided by the same supplier) *ad libitum*
Test units:	One glass disc (45-mm Ø) placed in a glass petri dish lid (54-mm Ø), with a central hole (6-mm Ø), located on a grid immersed in water. All systems were contained within a plastic container (250 × 250 × 80 mm3)

4. Environmental conditions:

Temperature:	24.75 ± 0.067 °C (24.68 – 25.52 °C)
Relative humidity:	68.5 ± 4.5% (65.5 – 76.1%)
Photoperiod:	daily cycle 16 h day/8 h night

STUDY DESIGN AND METHOD

The aim of the study was to determine the 7-day LR50 of test item DIFLUFENIKAN 500 SC (diflufenican 500 g/L) by assessing Typhlodromus pyri mortality and reproduction (fecundity), subsequent to their exposure to the test item applied once on glass discs. Test item will be compared to a control group (deionized water only) and to a reference item.
The study consisted of 7 treatments (5 rates of the test item, 1 control group, 1 reference item), with 5 replicates, each containing 20 individuals. The mites were exposed on glass discs previously treated with test item. Mortality was assessed after 3 and 7 days of exposure.
Mites were exposed on glass discs previously treated and observed for 3 and 7 days. At the end of this period, the observations consisted in percent mortality; the survived adults were counted, sex ratio defined and transferred to fresh glass discs, while eggs and nymphs removed. The test units were maintained for 7 additional days, after which, the number of juveniles and eggs was assessed three times from day 7 to day 14 with a maximum interval of 3 days, as well as the adult mite's vitality.
To verify the sensitivity of the test system, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Method used:	"Island method" by Joisten 2000, as described in Blumel et al (2000)
Test design:	tested concentrations, reference item and control in 5 replications, number of mites: 20 /replicate for test and reference item
Introduction of Individuals:	After drying of the test units, 30-40 minutes after the application.
Introduction Procedure:	With a fine brush, selection of the mites was impartially performed, following the spray scheme.
Exposure time:	7 days for mortality assessments + 7 days for fecundity assessments.
Tested concentrations, definitive test:	37.04 mL/ha (18.52 g a.i./ha) 111.11 mL/ha (55.56 g a.i./ha) 333.33 mL/ha (166.67 g a.i./ha) 1000 mL/ha (500 g a.i./ha) 3000 mL/ha (1500 g a.i./ha) (volume of application was 200 L/ha)
Dates:	start of the study: 04.11.2022 start of the experimental part: 21.11.2022 end of the experimental part: 05.12.2022 end of the study: 01.06.2023

Statistic:

Software used for statistical analysis was "ToxRatPro", version 3.3.0
Mortality data were processed using the Chi2 2×2 Table test with Bonferroni correction, α≤0.05 and at least the LR50 was calculated where possible. Mortality was corrected by the control mortality, using the Schneider-Orelli formula.
Reproduction data were analysed by the Dunnett's t-test, α≤0.05 and at least the ER50 value calculated where possible.
The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were determined where possible.

RESULTS

All study validity criteria were met.
Concerning mortality, no significant differences were noticed for all the treatments in comparison to the control group.
The 7-d NOER (mortality) value was estimated to be ≥ 3000 ml f.p./ha (≥ 1500 g a.i./ha) and the 7-d LOER (mortality) value estimated to be >3000 ml f.p./ha (> 1500 g a.i./ha). The LR10 was estimated to be >3000 mL/ha (1500 g a.i./ha) with 95% confidence intervals 674.37 – Upper Limit not determined, while the LR20 and LR50 values for DIFLUFENIKAN 500 SC could not be calculated due to the low mortality level. Therefore, it can be assumed a 48-h LR50 value >3000 mL/ha (1500 g a.i./ha).
For reproduction, no significant differences were noticed for all the treatments in comparison to the control group. The calculated 14-d NOER (reproduction) value was ≥3000 mL test item/ha and the 14-d LOER (reproduction) value calculated to be >3000 mL test item/ha. The 14-d ER50 value for DIFLUFENIKAN 500 SC could not be calculated, therefore, it can be assumed a 14-d LR50 value >3000 mL/ha (1500 g a.i./ha).

Table KCP 10.3.2.1-1 Mortality endpoints for predatory mite *Typhlodromus pyri*.

	DIFLUFENIKAN 500 SC						**ROGOR L40 ST**
	T1 Control	**T2 37.04 mL f.p./ha**	**T3 111.11 mL f.p./ha**	**T4 333.33 mL f.p./ha**	**T5 1000 mL f.p./ha**	**T6 3000 mL f.p./ha**	**T7 ROGOR L 40 ST at 15 mL test item/ha**
	Deionised water	**18.52 g a.i./ha**	**55.56 g a.i./ha**	**166.67 a.i./ha**	**500 g a.i./ha**	**1500 g a.i./ha**	**6 g a.i./ha**
Mortality (3 day) [mean %]	3.00	4.00	3.00	4.00	4.00	4.00	42.00
Mortality (7 day) [mean %]	6.00	6.00	7.00	10.00	10.00	13.00	80.00
Corrected mortality [a] (7 days) [%]	-	0.00	1.06	4.26	4.26	7.45	78.72
Significance [b]	-	n.s.	n.s.	n.s.	n.s.	n.s.	***

Endpoints	**mL f.p./ha**	**g a.i./ha**
7-day LR_{10} [95% confidence intervals]	>3000 [1348.73 – U.L. n.d.]	>1500 [674.37 – U.L. n.d.]

7-day LR_{20} [95% confidence intervals]	n.d. [95%-CLs n.d.]	n.d. [95%-CLs n.d.]
7-day LR_{50} [95% confidence intervals]	>3000	>1500
7-day NOER (Mortality)	≥3000	≥1500
7-day LOER (Mortality)	>3000	>1500

f.p.: formulated product
a.i.: active ingredient
-, not applicable
n.s., not significantly different compared to the control
a, mean mortality corrected by Schneider-Orelli's formula
b, Step-down Chi^2 2×2 Table test, α≤0.001 ***, 0.01 **, 0.05 *
95%-CLs, Confidence Limits
n.d.: not determined due to mathematical reasons

Table KCP 10.3.2.1-2 Fecundity endpoints for predatory mite *Typhlodromus pyri.*

	DIFLUFENIKAN 500 SC					
	T1 Control	**T2 37.04 mL f.p./ha**	**T3 111.11 mL f.p./ha**	**T4 333.33 mL f.p./ha**	**T5 1000 mL f.p./ha**	**T6 3000 mL f.p./ha**
	Deionised water	**18.52 g a.i./ha**	**55.56 g a.i./ha**	**166.67 a.i./ha**	**500 g a.i./ha**	**1500 g a.i./ha**
Reproduction [mean eggs/female]	9.83	9.41	9.81	8.55	9.38	9.73
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Effect on reproduc-tion [%Pr]	-	4.25	0.23	13.03	4.59	1.05

Endpoints	**mL f.p./ha**	**g a.i./ha**
14-day ER_{50}	>3000	>1500
14-day NOER (Reproduction)	≥3000	≥1500
14-day LOER (Reproduction)	>3000	>1500

f.p.: formulated product
a.i.: active ingredient
-, not applicable
n.s., not significantly different compared to the control
a, Dunnett's t-test α≤0.05

Comments of zRMS:	The study is considered valid. All validity criteria were met: 1. Mortality in control group Mortality should not exceed 5 out of 40 wasps (≤13%) after 48 hours (actual value was 0.00%, therefore, the validity criterion was met). 2. Parasitisation in control group Mean number of parasitized aphids (mummies) per female to be ≥ 5 (actual value was 30.53, therefore, the validity criterion was met). No more than two wasps producing zero mummies (actual value was zero, therefore, the validity criterion was met). **Agreed toxicity endpoints:** Standard laboratory test – *Aphidius rhopalosiphi* Mortality parameter: 48h LR_{50} > 3000 ml formulation/ha (>1500 g diflufenikan/ha) Reproduction parameter: 12d ER_{50} > 3000 ml formulation/ha (>1500 g diflufenikan/ha)

Reference:	KCP 10.3.2.1/02
Report	Effects of DIFLUFENICAN 500 SC (diflufenican 500 g/L) on *Aphidius rhopalosiphi* in the laboratory– Standard laboratory test*;* Mautino G.; 2023; Study Code: 1016.1H.SAG22
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	not relevant

2. Vehicle and/or positive control:	vehicle: deionized water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L)

3. Test organism

Species:	parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	2 days under test conditions
Stage at delivery:	Aphid mummies
Age at test start:	adults less than 48-hour old
Sex:	minimum 5 females per replicate
Diet:	During the acclimation period and the test, a solution of 30% of honey in 100 mL of water was prepared and put on a cotton wool pad and given ad libitum to the insects, during the mortality assessment it was put on a small plastic tube that was connected with the exposure units at the beginning of the experiment; for the reproduction assessment a solution of 30% (by volume) of honey in water was put on a cotton wool.
Hatching chambers	Cardboard cube (about 35 × 35 cm) with a frontal removable plastic tube.

Test units for mortality assessment:	Two treated round glass plates (11.4 cm Ø) fitted onto a round stainless-steel frame (12.5 cm Ø) which had four ventilated holes (1.5 cm outer Ø). Three holes were covered with fine stainless-steel mesh and one was left open to introduce the wasps. A plastic tubing system connected to a pulling pump was set up for the air circulation in the test units.
Test units for reproduction assessment:	Untreated pots (15.0 cm Ø) with barley seedlings (Hordeum vulgare; 30 seeds per pot) infested with ≥100 host aphids of all development stages (*Rhopalosiphum padi*; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (22 cm high and 12.5 cm Ø). The cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction. After the introduction of the insects, this hole was plugged up with a cotton wool. After the adult wasps were removed, the polyacrylic cylinders were left on the pots.
Plant:	Poaceae, Barley (*Hordeum vulgare* L), Calanque

4. Environmental conditions:

Temperature:	19.46 ± 0.362 °C (19.06 – 20.25 °C)
Relative humidity:	74.1 ± 4.1% (63.5 – 79.4% RH)
Photoperiod:	16 h light:8 h dark
Light Intensity:	Mortality: 890 – 1200, Reproduction: 10000 – 12000 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the 48-hour LR50 for test item DIFLUFENIKAN 500 SC (diflufenican 500 g/L) by assessing *Aphidius rhopalosiphi* mortality reproduction (fecundity), subsequent to their exposure to the test item applied once on glass discs. Test item will be compared to a control group (deionized water only) and to a reference item.
The study consisted of 7 treatments (5 rates of the test item, 1 control group, 1 reference item) with 4 replicates, each containing 10 parasitoids. The parasitoids were exposed on glass discs previously treated with test item and observed for 2, 24 and 48 hours.
At 48 hours the observations consisted of an evaluation of percent mortality. A minimum of 15 survived females per treatment, except the reference treatment, were removed and their reproductive capacity assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult females were removed after 24 hours and the aphid-infested plants left for a further 12 days before the number of aphid mummies that had developed was assessed.
To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Test design:	tested concentrations, reference and control in 4 replications, number of insects: 10 females/replicate

Introduction of Individuals:	After drying of the test units, 30-40 minutes after the application. For the mortality assessment wasp females were identified by observing their pointed abdomens. Transfer was done using an aspirator, following the spraying scheme; only live (alive and apparently unaffected) wasps were introduced. For the reproduction assessment wasp females were selected impartially and transferred using a mouth aspirator. Moribund insects were not included in this assessment.
Mortality exposure time:	48 hours
Fecundity exposure time:	parasitisation period was 24 hours, all treatment groups were evaluated 12 days after parasitisation
Tested concentrations, definitive test:	37.04 mL/ha (18.52 g a.i./ha) 111.11 mL/ha (55.56 g a.i./ha) 333.33 mL/ha (166.67 g a.i./ha) 1000 mL/ha (500 g a.i./ha) 3000 mL/ha (1500 g a.i./ha) (dilution ratio: 3; volume of application was 200 L/ha)
Dates:	start of the study: 04.11.2022 start of the experimental part: 08.11.2022 end of the experimental part: 13.12.2022 end of the study: 26.05.2023
Statistic:	Software used for statistical analysis was “ToxRatPro”, version 3.3.0 Mortality data were processed using the Fishers' Exact test with Bonferroni correction, $\alpha \leq 0.05$ and at least the LR50 value calculated where possible. Reproduction data were analysed by the Williams' t-test, $\alpha \leq 0.05$ and at least the ER50 value calculated where possible. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were determined where possible.
Validity criteria:	All validity criteria were met: -mortality should not exceed 5 out of 40 wasps (≤13%) after 48 hours; -mean number of parasitized aphids (mummies) per female ≥ 5; -no more than two wasps producing zero values of mummies.

RESULTS

All study validity criteria were met.

Concerning mortality, no significant differences were noticed for test item DIFLUFENIKAN 500 SC in comparison to the control group.

The 48-h NOER (mortality) value was estimated to be ≥ 3000 mL/ha (1500 g a.i./ha) and 48-h LOER (mortality) value was estimated to be > 3000 mL/ha (1500 g a.i./ha).

The 48-h LR_{10} value was 2698.56 mL test item/ha (95% confidence intervals: 800.59 – U.L. not determined mL test item/ha), while the LR_{20} and LR_{50} values for DIFLUFENIKAN 500 SC could not be calculated due to the low mortality level. Therefore, it can be assumed a 48-h LR_{50} value >3000 mL/ha (1500 g a.i./ha). The LR_{10} corresponding to 1349.28 g a.i./ha (95% confidence intervals: 400.30 g a.i./ha – Upper Limit not determined).

For reproduction, significant difference was observed for treatment T6 (3000 mL test item/ha), in comparison to the control group.

The 12-d NOER (reproduction) value was 1000 mL test item/ha (treatment T5 – 500 g a.i./ha) and the 12-d LOER (reproduction) value was 3000 mL test item/ha (treatment T6 – 1500 g a.i./ha).

The calculated 12-d ER_{10} value for DIFLUFENIKAN 500 SC was 86.66 mL test item/ha (95% confidence intervals not determined), the calculated 12-d ER_{20} value was 296.37 mL test item/ha (95% confidence intervals: Lower Limit not determined – 94.32 mL test item/ha) and the calculated 12-d ER_{50} value was estimated to be >3000 mL test item/ha (95% confidence intervals: 1016.28 mL test item/ha – Upper Limit not determined), corresponding to 43.33 g a.i./ha (95% confidence intervals not determined), 148.19 g a.i./ha (95% confidence intervals: Lower Limit not determined – 47.16 g a.i./ha) and >1500 g a.i./ha (95% confidence intervals: 508.14 g a.i./ha – Upper Limit not determined), respectively.

Table KCP 10.3.2.1-3 Mortality endpoints for *Aphidius rhopalosiphi*

	DIFLUFENIKAN 500 SC						**ROGOR L40 ST**
	T1 Control	**T2 37.04 mL f.p./ha**	**T3 111.11 mL f.p./ha**	**T4 333.33 mL f.p./ha**	**T5 1000 mL f.p./ha**	**T6 3000 mL f.p./ha**	**T7 0.3 mL f.p./ha**
	Deionised water	**18.52 g a.i./ha**	**55.56 g a.i./ha**	**166.67 a.i./ha**	**500 g a.i./ha**	**1500 g a.i./ha**	**0.12 g a.i./ha**
Mortality (2 hours) [mean %]	0.00	0.00	0.00	0.00	5.00	5.00	17.50
Mortality (24 hours) [mean %]	0.00	0.00	0.00	7.50	7.50	7.50	80.00
Mortality (48 hours) [mean %]	0.00	0.00	0.00	7.50	7.50	7.50	92.50
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.	***

Endpoints	**mL f.p./ha**	**g a.i./ha**
48-h LR_{10} [95% confidence intervals]	2698.56 [800.59 – U.L.n.d.]	1349.28 [400.30 – U.L.n.d.]
48-h LR_{20} [95% confidence intervals]	n.d. [95%-CLs n.d.]	n.d. [95%-CLs n.d.]
48-h LR_{50} [95% confidence intervals]	>3000	>1500
48-h NOER (Mortality)	≥3000	≥1500

48-h LOER (Mortality)	>3000	>1500

f.p.: formulated product
a.i.: active ingredient
-, not applicable;
n.s., not significantly different compared to the control
a, Fisher's Exact test with Bonferroni correction, α≤0.001 ***, 0.01 **, 0.05 *
U.L., Upper Limit
n.d., not determined due to mathematical reasons
95%-CLs, Confidence Limits

Table KCP 10.3.2.1-4 Reproduction endpoints for *Aphidius rhopalosiphi*

	DIFLUFENIKAN 500 SC					
	T1 Control	**T2 37.04 mL f.p./ha**	**T3 111.11 mL f.p./ha**	**T4 333.33 mL f.p./ha**	**T5 1000 mL f.p./ha**	**T6 3000 mL f.p./ha**
	Deionised water	**18.52 g a.i./ha**	**55.56 g a.i./ha**	**166.67 a.i./ha**	**500 g a.i./ha**	**1500 g a.i./ha**
Reproduction [mean mummies/female]	30.53	29.53	27.80	23.13	20.67	15.67
Significance [c]	-	n.s.	n.s.	n.s.	n.s.	*
Effect on reproduction [%R]	-	3.28	8.95	24.24	32.31	48.69

Endpoint	**mL f.p./ha**	**g a.i./ha**
12-d ER_{10} [95% confidence intervals]	86.66 [95%-CLs n.d.]	43.33 [95%-CLs n.d.]
12-d ER_{20} [95% confidence intervals]	296.37 [L.L. n.d. – 94.32]	148.19 [L.L. n.d. – 47.16]
12-d ER_{50} [95% confidence intervals]	>3000 [1016.28 – U.L. n.d.]	>1500 [508.14 – U.L. n.d.]
12-d NOER (Reproduction)	1000	500
12-d LOER (Reproduction)	3000	1500

f.p.: formulated product
a.i.: active ingredient
-, not applicable;
n.s., not significantly different compared to the control
a, Williams t-test α≤0.05
95%-CLs, Confidence Limits
n.d.: not determined due to mathematical reasons
L.L., Lower Limit
U.L., Upper Limit

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

Not relevant. No studies 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

Comments of zRMS:	The study is considered valid. All validity criteria were met: The results are considered valid because the following criteria were satisfied in the controls: ❖ each replicate produced from 86 to 148 juveniles (116.9 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment), ❖ the coefficient of variation of reproduction was 19.1% (criterion: ≤ 30%), ❖ adult mortality over the initial 4 weeks of the experiment was 1.3% (criterion: ≤ 10%). No deviations from OECD Guideline No. 222 (2016), and the study plan were noticed. **Agreed toxicity endpoints:** Reproduction/Survival parameter NOEC ≥ 1000 mg formulation/kg dw (≥ 419.3 mg diflufenikan/kg dw)

Reference:	KCP 10.4.1.1/01
Report	Diflufenikan 500 SC Earthworm reproduction test (*Eisenia andrei*); Pieczka P.; 2022; Study Code: G-89-21
Guideline(s):	Yes, OECD 222
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024

2. Vehicle and/or positive control: vehicle: deionized water
positive control: carbendazim

3. Test organism

Species:	earthworm *Eisenia andrei*
Source:	cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology
Age:	about 4 months old
Acclimation period:	1-day acclimatization
Diet:	air-dried finely ground cow manure
Test units:	boxes are 35 cm x 50 cm x 30 cm

4. Environmental conditions:

Temperature:	19.8 – 22°C
Soil:	artificial soil 10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
pH:	pH at the beginning of the experiment: 6.28 – 6.33 pH at the end of the experiment: 5.95 – 6.06
Soil moisture content:	at the beginning of the experiment: 23.6 – 24.2% (48.3 – 49.5% of the maximum water holding capacity) at the end of the experiment: 24.4 – 26.3% (49.9 – 53.8% of the maximum water holding capacity)
Photoperiod:	light-dark cycle: 16h : 8h light intensity at the beginning of the experiment: 526.4 – 588.3 lux light intensity at the end of the experiment: 569.7 – 583.3 lux

STUDY DESIGN AND METHOD

The aims of the study were to assess the impact of Diflufenikan 500 SC on reproduction of the earthworm, *Eisenia andrei* and to determine EC_{10}, EC_{20}, EC_{50} and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. The concentrations of the test item were: 18, 32, 56, 100, 180, 320, 560 and 1000 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Test design:	control in 8 replicates with 10 earthworms for each replication; tested concentrations in 4 replicates with 10 earthworms for each replication

Exposure time:	8 weeks
Tested concentrations, definitive test:	18, 32, 56, 100, 180, 320, 560 and 1000 mg/kg dry weight of soil
Dates:	start of the study: 25.02.2022 start of the experimental part: 15.03.2022 end of the experimental part: 12.05.2022 end of the study: 26.07.2022
Statistic:	Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Fisher's Exact Binomial Test with Bonferroni Correction , ToxRat Professional 2.10 statistical computer software

CONCLUSION

At concentrations ranging from 18 to 1000 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 2.5%. As for the control group, mortality of the adult earthworms was equal to 1.3%. The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 1000 mg/kg dry weight of the artificial soil (above 419.3 mg of diflufenican/kg dry weight of the artificial soil). No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed. After 4 weeks of the exposure period of the test item at the concentrations ranging from 18 to 1000 mg/kg dry weight of artificial soil, the body weight increase was between 24.7 and 38.3%. As for the control group, the body weight increase was equal to 26.9%.
After 8 weeks of the experiment, the mean number of juveniles was between 105.5 and 117.5 per replicate. The mean number of juveniles in the control group was equal to 116.9 per replicate. After 8 weeks of the experiment, it was concluded that Diflufenican 500 SC had no statistically significant impact on reproduction of the earthworms at the concentrations ranging from 18 to 1000 mg/kg dry weight of the artificial soil. The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

Table KCP 10.4.1.1-1: Earthworm reproduction test – final results

Parameter	Reproduction			Survival		
	EC_{50}	LOEC	NOEC	LC_{50}	LOEC	NOEC
Value [mg test item/kg dry weight of artificial soil]	> 1000.0	> 1000.0	> 1000.0	> 1000.0	> 1000.0	> 1000.0
Value [mg of diflufenican/kg dry weight of artificial soil]	> 419.3	> 419.3	≥ 419.3	> 419.3	> 419.3	≥ 419.3

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not relevant. No studies submitted.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Not relevant. No studies submitted. The risk assessment for non-target arthropods is acceptable at Tier I so testing on *Folsomia candida* and *Hypoaspis aculeifer* is not required.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not relevant. No studies submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered valid. All validity criteria were met: The coefficients of variation (CV) in the control group were 3.2, 1.6, 7.4 and 5.6%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%. **Deviation from the OECD Guideline No. 216 (2000):** According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. These deviation did not affect the results of the study. **Agreed toxicity endpoints:** The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 2.4 mg formulation/kg dry weight of soil (1.0 mg of diflufenican/kg dry weight of soil) and 5 x PEC: 12.0 mg formulation/kg dry weight of soil (5.0 mg of diflufenican/kg dry weight of soil) did not exceed 25% on 28 day of analysis.

Reference:	KCP 10.5/01
Report	Diflufenikan 500 SC Soil Microorganisms: Nitrogen Transformation Test; Pieczka P.; 2022; Study Code: G-90-21
Guideline(s):	Yes, OECD 216
Deviations:	According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. These deviation did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	Diflufenikan 500 SC
Formulation:	SC (diflufenican 500 g/L)
Description (physical state):	white liquid
Batch no.:	1/DIF/2022
Production date:	01.2022
Expiration date:	01.2024

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

3. Test organism

Soil:	the site chosen for soil collection was covered with grass, it had not been treated with any plant protection products or organic and inorganic fertilizers for at least 5 years, soil samples were taken from a depth of 20 cm, they were collected from different parts of the field to obtain a common laboratory sample, collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna
Source:	collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna
Soil preparation:	the collected soil was manually cleared of large objects, sieved to a particle size equal to 2 mm and thus the laboratory soil sample was obtained, the soil, prepared in that way, was thoroughly mixed and divided into three equal portions, the test item at two concentrations: PEC and 5 x PEC was added into two portions of the soil, the test item in the form of aqueous suspensions was introduced to the soil, the control artificial soil was mixed with deionized water alone, at the beginning of the experiment, the soil moisture content was adjusted with deionized water to obtain value between 40 – 60% (about 50%) of the maximum water holding capacity
Test units:	plastic containers covered with perforated aluminium foil

4. Environmental conditions:

Temperature:	20.1 – 22.0 °C
Soil moisture:	44.6 – 48.9% of the maximum water holding capacity
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The aim of the study was to detect long-term adverse effects of Diflufenikan 500 SC on the processes of nitrogen transformation in aerobic surface soils. The freshly collected agricultural soil was used in the experiment. It was manually cleared of large objects and sieved to a particle size of 2 mm. Two concentrations of the test item were used, i.e.: PEC and 5 x PEC. The treated and the control soils were divided into three replicates. On days 0, 7, 14 and 28 of incubation, soil samples were collected to determine the quantities of nitrate. The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using deionised water. The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

Test design:	concentrations and control in 3 replicates
Exposure time:	28 days
Tested concentrations, definitive test:	1PEC – 2.4 mg test item/kg soil (1 mg of diflufenican/kg soil), 5PEC - 12 mg test item/kg soil (5 mg of diflufenican/kg soil)
Dates:	start of the study 30.03.2022 start of the experimental part: 12.04.2022 end of the experimental part: 11.05.2022 end of the study: 26.07.2022
Statistic:	Shapiro-Wilk's test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, ToxRat 2.10. computer software

CONCLUSION

The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 2.4 mg of the test item/kg dry weight of soil (1.0 mg of diflufenican/kg dry weight of soil) and 5 x PEC: 12.0 mg test item/kg dry weight of soil (5.0 mg of diflufenican/kg dry weight of soil) did not exceed 25% on 28 day of analysis.

On the basis of the results, it was concluded that Diflufenikan 500 SC at the concentrations corresponding to the PEC: 2.4 mg of the test item/kg dry weight of soil (1.0 mg of diflufenican/kg dry weight of soil) and 5 x PEC: 12.0 mg test item/kg dry weight of soil (5.0 mg of diflufenican/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Table KCP 10.5.-1: Nitrogen transformation (deviation from the control) – final results

Time interval [d]	1PEC 2.4 mg test item/kg soil (1 mg of diflufenican/kg soil),	5PEC 12 mg test item/kg soil (5 mg of diflufenican/kg soil)
0-7	73.8	52.9
0-14	9.8	4.0
0-28	-17.4	-21.9

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:

The study is considered valid. All validity criteria were met:
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Diflufenikan 500 SC on seedling emergence and seedling growth of terrestrial plants were met:
- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
100.0% – sunflower,
100.0% – pea,
100.0% – cabbage,
95.0% – carrot,
95.0% – onion,
100.0% – perennial ryegrass,
- the mean survival of the emerged control seedlings was 100.0% for sunflower, pea, cabbage, carrot and perennial ryegrass and 95.0% for onion (validity criterion: 90.0%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.
Deviation from OECD Guideline No. 208:
According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 110.2 and 298.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. This deviation did not affect results of the experiment.

Agreed toxicity endpoints expressed as mL formulation/ha:

	Sunflower *Helianthus annuus*	Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	>300.0	>300.0	>300.0	>300.0	20.5	>300.0
NOER	≥300.0	≥300.0	120.0	≥300.0	7.7	≥300.0
Shoot length						
ER_{50}	>300.0	>300.0	>300.0	>300.0	25.4	179.3
NOER	48.0	120.0	19.2	≥300.0	3.1	48.0
Plant dry weight						
ER_{50}	>300.0	>300.0	88.5	>300.0	18.6	53.9
NOER	1.2	≥300.0	1.2	≥300.0	3.1	0.5
Plant Damage						
ER_{50}	>300.0	>300.0	66.0	>300.0	17.5	129.8

Agreed toxicity endpoints expressed as g diflufenikan/ha:

	Sunflower *Helianthus annuus*	Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	>150.2	>150.2	>150.2	>150.2	10.3	>150.2
NOER	≥150.2	≥150.2	60.1	≥150.2	3.9	≥150.2
Shoot length						
ER_{50}	>150.2	>150.2	>150.2	>150.2	12.7	89.8
NOER	24.0	60.1	9.6	≥150.2	1.6	24.0
Plant dry weight						
ER_{50}	>150.2	>150.2	44.3	>150.2	9.3	27.0
NOER	0.6	≥150.2	0.6	≥150.2	1.6	0.3
Plant Damage						
ER_{50}	>150.2	>150.2	33.0	>150.2	8.8	65.0

ER_{50} phytotoxicity parameter = 17.5 ml formulation/ha for *Allium cepa* for the most sensitive species

Reference: KCP 10.6.2/01

Report: Diflufenikan 500 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Pieczka P.; 2022; Study Code: G-92-21

Guideline(s): Yes, OECD 208

Deviations: According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 110.2 and 298.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. This deviation did not affect results of the experiment.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): Diflufenikan 500 SC

Formulation: SC (diflufenican 500 g/L)

Description (physical state): white liquid

Batch no.: 1/DIF/2022

Production date: 01.2022

Expiration date: 01.2024

Stability of test compound: stability test of the test item have not been performed

2. Vehicle and/or positive control:	vehicle control: water positive control: not relevant
3. Test plants:	sunflower (*Helianthus annuus*), pea (*Pisum sativum*), cabbage (Brassica oleracea var. capitata), carrot (*Daucus carota*), onion (*Allium cepa*) perennial ryegrass (*Lolium perenne*)
Soil:	sandy loam
Test containers:	plastic pots, each pot contained about 705 g of the soil (i.e. 600 g dry weight)
4. Environmental conditions:	
Temperature:	16-26.9°C
Relative humidity:	49.3 – 79.8%
Photoperiod:	lighting: 16 h light : 8 h dark; light intensity: 110.2 – 298.7 µE/m2/s
CO_2 concentration:	348 – 386 ppm

STUDY DESIGN AND METHODS

The study, aimed at evaluating the effect Diflufenikan 500 SC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 5 (carrot, onion and perennial ryegrass) or 3 (sunflower, pea, cabbage) seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day to the emergence of 50% of the control seedlings and after then every 1 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The exposure period finished 14 days after the emergence of 50% of the control seedlings. At the end of the exposure, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed. The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER_{10}, ER_{25}, ER_{50}, and NOER. Additionally, the ER_{50} was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period.

Test design:	number of rates: 8 + control; number of replicates/rate: 7 (sunflower, pea, cabbage), 4 (carrot, onion and perennial ryegrass). The total number of seeds per application rate: 21 (sunflower, pea, cabbage) or 20 (carrot, onion and perennial ryegrass)
Exposure time:	14 days since emergence of 50% seeds in control
Tested concentrations, definitive test:	0.5, 1.2, 3.1, 7.7, 19.2, 48, 120 and 300 mL of the test item /ha (0.3, 0.6, 1.6, 3.9, 9.6, 24,60.1 and 150.2 g of diflufenican/ha), dilution in 300 L water/ha

Dates: start of the study 21.03.2022
start of the experimental part: 15.04.2022
end of the experimental part: 06.05.2022
end of the study: 26.07.2022

Statistic: ToxRat Professional 3.3.0 computer software , ER10, ER_{25}, ER_{50} - probit analysis using linear max. likelihood regression or 3-param. Normal CDF, NOER for the plant number - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm, Qualitative Trend analysis by Contrasts (Monotonicity of Rate/Response), Chi2 2x2 Table Test with Bonferroni Correction, Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure, Fisher`s Exact Binomial Test with Bonferroni Correction; NOER for the shoot length - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure, Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, Dunnett`s Multiple t-test Procedure;
NOER for the plant dry weight - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Dunnett's Multiple t-test Procedure, Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure.

CONCLUSION

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements and ER_{50} values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

Table KCP 10.6.2-1: Seedling emergence and seedling growth test – final results (g of test item/ha)

	Sunflower *Helianthus annuus*	**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	> 300	> 300	> 300	> 300	20.5	> 300
NOER	≥ 300	≥ 300	120	≥ 300	7.7	≥ 300
Shoot length						
ER_{50}	> 300	> 300	> 300	> 300	25.4	179.3
NOER	48	120	19.2	≥ 300	3.1	48
Plant dry weight						
ER_{50}	> 300	> 300	88.5	> 300	18.6	53.9
NOER	1.2	≥ 300	1.2	≥ 300	3.1	0.5

Plant damage - phytotoxicity						
ER_{50}	> 300	> 300	66	> 300	17.5	129.8

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements and ER_{50} values for plant damages at the end of the exposure period expressed as g of diflufenican/ha for all test species are given below.

Table KCP 10.6.2-2: Seedling emergence and seedling growth test – final results (g of diflufenican/ha)

	Sunflower *Helianthus annuus*	**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	> 150.2	> 150.2	> 150.2	> 150.2	10.3	> 150.2
NOER	≥ 150.2	≥ 150.2	60.1	≥ 150.2	3.9	≥ 150.2
Shoot length						
ER_{50}	> 150.2	> 150.2	> 150.2	> 150.2	12.7	89.8
NOER	24	60.1	9.6	≥ 150.2	1.6	24
Plant dry weight						
ER_{50}	> 150.2	> 150.2	44.3	> 150.2	9.3	27
NOER	0.6	≥ 150.2	0.6	≥ 150.2	1.6	0.3
Plant damage - phytotoxicity						
ER_{50}	> 150.2	> 150.2	33	> 150.2	8.8	65

On the basis of the obtained results it was proved that the test item i.e. Diflufenikan 500 SC had varied impact on seedling emergence and seedling growth of the tested plant species. For the tested range of application rates, seedling emergence of plants was not delayed when compared with the controls. The death of onion and perennial ryegrass was observed during the experiment. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant number it was proved that the test item inhibited the seedling emergence and the process of growth of onion and cabbage.

No influence was observed in cultivation of sunflower, pea and carrot and perennial ryegrass. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of sunflower, pea, cabbage, onion, perennial ryegrass. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of sunflower, cabbage, onion and perennial ryegrass. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of pea, cabbage, onion and perennial ryegrass. Slight phytotoxic effect was observed in cultivation of sunflower. In the study, the lowest endpoints were observed for onion. The most resistant species for influence of the test item was carrot.

Comments of zRMS:	The study is considered valid. All validity criteria were met: On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Diflufenikan 500 SC on vegetative vigour of terrestrial plants were met: - the seedling emergence of plants (validity criterion: at least 70%) was as follows: 83.3 – 92.9 – sunflower, 83.3 – 88.1 – pea, 81.0 – 95.2 –cabbage, 87.5 – 95.0 – carrot, 82.5 – 95.0 – onion, 77.5 – 87.5 – perennial ryegrass, - the mean plant survival of the control was 100% for all tested species (validity

criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Deviation from OECD Guideline No. 227:
According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 105.7 – 249.3 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the experiment.

Agreed toxicity endpoints expressed as mL formulation/ha:

	Sunflower *Helianthus annuus*	Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	>300.0	>300.0	>300.0	>300.0	>300.0	>300.0
NOER	>300.0	>300.0	>300.0	>300.0	≥300.0	>300.0
Shoot length						
ER_{50}	>300.0	>300.0	>300.0	>300.0	>300.0	>300.0
NOER	≥300.0	≥300.0	>300.0	≥300.0	3.1	19.2
Plant dry weight						
ER_{50}	>300.0	>300.0	>300.0	>300.0	148.4	>300.0
NOER	120.0	≥300.0	120.0	120.0	19.2	≥300.0
Plant Damage						
ER_{50}	207.5	>300.0	>300.0	>300.0	83.5	>300.0

Agreed toxicity endpoints expressed as g diflufenican/ha:

	Sunflower *Helianthus annuus*	Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	>150.2	>150.2	>150.2	>150.2	>150.2	>150.2
NOER	>150.2	>150.2	>150.2	>150.2	≥150.2	>150.2
Shoot length						
ER_{50}	>150.2	>150.2	>150.2	>150.2	>150.2	>150.2
NOER	≥150.2	≥150.2	>150.2	≥150.2	1.6	9.6
Plant dry weight						
ER_{50}	>150.2	>150.2	>150.2	>150.2	74.3	>150.2
NOER	60.1	≥150.2	60.1	60.1	9.6	≥150.2
Plant Damage						
ER_{50}	>103.9	>150.2	>150.2	>150.2	41.8	>150.2

ER_{50} phytotoxicity parameter = 83.5 ml formulation/ha for *Allium cepa* for the most sensitive species

Reference: KCP 10.6.2/02

Report: Diflufenikan 500 SC Terrestrial Plant Test: Vegetative Vigour Test; Pieczka P.; 2022; Study Code: G-91-21

Guideline(s): Yes, OECD 227

Deviations: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 105.7 – 249.3 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the experiment.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): Diflufenikan 500 SC

Formulation: SC (diflufenican 500 g/L)

Description (physical state): white liquid

Batch no.: 1/DIF/2022

Production date:	01.2022
Expiration date:	01.2024
Stability of test compound:	stability test of the test item have not been performed
2. Vehicle and/or positive control:	vehicle control: water positive control: not relevant
3. Test plants:	sunflower (*Helianthus annuus*), pea (*Pisum sativum*), cabbage (Brassica oleracea var. capitata), carrot (*Daucus carota*), onion (*Allium cepa*) perennial ryegrass (*Lolium perenne*)
Soil:	sandy loam
Test containers:	plastic pots (pot's diameter – 15 cm, pot's surface area – about 177 cm^2)
4. Environmental conditions:	
Temperature:	16-26.9°C
Relative humidity:	47.6-79.8%
Photoperiod:	16h light and 8h dark, light intensity: 105.7 – 249.3 µE/m2/s
CO_2 concentration:	348 – 386 ppm

STUDY DESIGN AND METHODS

The study, aimed at evaluating the effect of Diflufenikan 500 SC on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for sunflower, pea, and cabbage; 10 seeds/pot for carrot, onion and perennial ryegrass). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. As a result, the number of plants per pot as well as the total number of plants per rate were:
- sunflower: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- pea: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- cabbage: 3 plants/pot – 21 plants/ application rate (7 pots/ application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- perennial ryegrass: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate).

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

Test design:	number of rates: 8 + control; number of replicates/rate: 7 (sunflower, pea, cabbage), 4 (carrot, onion, perennial ryegrass), the total number of plants per application rate: 21 (sunflower, pea, cabbage) or 20 (carrot, onion, perennial ryegrass)
Exposure time:	21 days after the spraying
Tested concentrations, definitive test:	0.5, 1.2, 3.1, 7.7, 19.2, 48, 120 and 300 mL of the test item /ha (0.3, 0.6, 1.6, 3.9, 9.6, 24,60.1 and 150.2 g of diflufenican/ha), dilution in 300 L water/ha
Dates:	start of the study 16.03.2022 start of the experimental part: 15.04.2022 end of the experimental part: 08.05.2022 end of the study: 03.08.2022
Statistic:	Probit analysis using linear max. likelihood regression, 3-param. Normal CDF, ToxRat Professional 3.3.0 computer software

CONCLUSION

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements and ER_{50} values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

Table KCP 10.6.2-3: Vegetative Vigour Test – final results (g of test item/ha)

	Sunflower *Helianthus annuus*	**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	> 300	> 300	> 300	> 300	> 300	> 300
NOEC	> 300	> 300	> 300	> 300	≥ 300	> 300
Shoot length						
ER_{50}	> 300	> 300	> 300	> 300	> 300	> 300
NOEC	≥ 300	≥ 300	> 300	≥ 300	3.1	19.2
Plant dry weight						
ER_{50}	> 300	> 300	> 300	> 300	148.4	> 300
NOEC	120	≥ 300	120	120	19.2	≥ 300
Plant damage - phytotoxicity						
ER_{50}	207.5	> 300	> 300	> 300	83.5	> 300

Table KCP 10.6.2-4: Vegetative Vigour Test – final results (g of diflufenican/ha)

	Sunflower *Helianthus annuus*	**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*
Plant number at the end of the experiment						
ER_{50}	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2
NOEC	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2
Shoot length						
ER_{50}	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2	> 150.2
NOEC	> 150.2	> 150.2	> 150.2	> 150.2	1.6	9.6
Plant dry weight						

ER_{50}	> 150.2	> 150.2	> 150.2	> 150.2	74.3	> 150.2
NOEC	60.1	> 150.2	60.1	60.1	9.6	> 150.2
Plant damage - phytotoxicity						
ER_{50}	103.9	> 150.2	> 150.2	> 150.2	41.8	> 150.2

On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant numer at the end of the experiment it was proved that the test item did not inhibit the process of growth of all tested plant species. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of onion. Slight effect was observed in cultivation of sunflower and perennial ryegrass.

On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of onion. Slight effect was observed in cultivation of sunflower, cabbage, carrot and perennial ryegrass.

During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of sunflower, pea, cabbage and onion. Slight effect was observed in cultivation of carrot and perennial ryegrass. In the study, the lowest endpoints were observed for onion.

The most resistant species for influence of the test item was pea.

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

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.8 KCP 10.8 Monitoring data

Not relevant. No studies submitted.