

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: AKO 600 SC

Product name(s): Tanger 600 SC, Libretto 600 SC

Chemical active substance:

Aclonifen, 600 g/L

Central Zone

Zonal Rapporteur Member State: Poland

Co-Rapporteur Member State: Hungary, Slovakia, Germany,
Romania

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 18.11.2024

zRMS Assessment : 28/06/2025

After commenting period : 01/10/2025

Final version : 29/06/2026

Version history

When	What
January 2025	Applicant's Update
May 2025	Applicant's Update
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June 2025	zRMS assessment
October 2025	After commenting period
June 2026	Final version

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9 Ecotoxicology (KCP 10)

According to Regulation 1107/2009 data protection for data at European level lasts for 10 years. Due to the fact that the aclonifen annex I was approved on 1/08/2009, data protection has expired 1/08/2019. Therefore, Innvigo can use the European data for aclonifen for the registration of the product AKO 600 SC [Libretto 600 SC, Tanger 600 SC]

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

GAP ver. 1.4 date: 22.05.2024

PPP (product name/code): AKO 600 SC / Tanger 600 SC, Libretto 600 SC
Active substance 1: Aclonifen
Safener:
Synergist:
Applicant: Innvigo sp. z o.o.
Zone(s): Central Zone
Verified by MS:
Field of use: Herbicide

Formulation type: SC
Conc. of as 1: 600 g/L
Conc. of as 2: (c)
Conc. of as: (c)
Conc. of safener: (c)
Conc. of synergist: (c)
Professional use:
Non professional use:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. (e)	Member state(s)	Crop and/ or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safen- er/synergist per ha (f)	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L prod- uct / 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	PL, DE	Potato <i>Solanum tuberosum</i> SOLTU	F	Mono and dicots weeds	Spray	BBCH 01-09	a) 1 b) 1	n/a	a) 1.95 – 3.0 L/ha b) 1.95 – 3.0 L/ha	a) 1.17 – 1.8 kg as/ha b) 1.17 – 1.8 kg as/ha	200- 400	40				Without R4 stram				
																	R4 stream at 1.8 kg a.s./ha			

[illegible]

6	PL	Sunflower <i>Helianthus annuus</i> HELAN	F	Mono and dicots weeds	Spray	BBCH 00-08	a) 1 b) 1	n/a	a) 1.95 – 3.0 L/ha b) 1.95 – 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 – 1.8 kg as/ha b) 1.17 – 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a							
7	PL, DE, SK	Spring rapeseed BRSNN	F	Mono and dicots weeds	Spray	BBCH 00-08	a) 1 b) 1	n/a	a) 1.95 – 3.0 L/ha b) 1.95 – 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 – 1.8 kg as/ha b) 1.17 – 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a		Without R3 stream and R4 stram	R3 stream and R4 stream at 1.8 kg a.s./ha	R3 stream and R4 stream at lower dose rate			
8	PL, HU, RO, SK	Flax LIUUT	F	Mono and dicots weeds	Spray	BBCH 00-08	a) 1 b) 1	n/a	a) 1.95 – 3.0 L/ha b) 1.95 – 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 – 1.8 kg as/ha b) 1.17 – 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a		Without R3 stream and R4 stram	R3 stream and R4 stream at 1.8 kg a.s./ha	R3 stream and R4 stream at lower dose rate			
9	PL, DE, HU, RO, SK	Opium poppy <i>Papaver somniferum</i> PAPSO	F	Mono and dicots weeds	Spray	BBCH 00- 08	a) 1 b) 1	n/a	a) 1.95 – 3.0 L/ha b) 1.95 – 3.0 L/ha a) 1.95 - 2.0 L/ha	a) 1.17 – 1.8 kg as/ha b) 1.17 – 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha	200- 300	n/a		Without R3 stream and R4 stram	R3 stream and R4 stream at 1.8 kg a.s./ha				

									b) 1.95 - 2.0 L/ha (***)	b) 1.17 - 1.2 kg as/ha (***)						R3 stream and R4 stream at lower dose rate				
10	PL, RO, SK	Mustard 3MUSC	F	Mono and dicots weeds	Spray	BBCH 00- 08	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a				Without R3 stream and R4 stram				
																R3 stream and R4 stream at 1.8 kg a.s./ha				
																R3 stream and R4 stream at lower dose rate				
11	PL	Soybeans GLXMA	F	Mono and dicots weeds	Spray	BBCH 00- 08	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a				Without R3 stream and R4 stream				
																R3 stream and R4 stream unresolved risk at 1.8 kg a.s./ha				
																R3 stream and R4 stream at lower dose rate than 1.8 kg a.s./ha				
12	PL, DE, HU, RO, SK	Pumpkin CUUPE	F	Mono and dicots weeds	Spray	BBCH 00-08	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a				Without R4 stream				
																R4 stream unresolved risk at 1.8 kg a.s./ha				
																R4 stream at lower dose rate than 1.8 kg a.s./ha				

13	PL, DE, HU, RO, SK	Hemp CNISA	F	Mono and dicots weeds	Spray	BBCH 00- 08	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a			Without R4 stream					
																R4 stream unresolved risk at 1.8 kg a.s./ha				
																R4 stream at lower dose rate than 1.8 kg a.s./ha				
14	PL, HU, RO, SK	Tobacco <i>Nicotiana tabacum</i> NIOTA	F	Mono and dicots weeds	Spray	Before planting	a) 1 b) 1	n/a	a) 1.8 - 2.5 L/ha b) 1.8 - 2.5 L/ha a) 1.8 - 2.0 L/ha b) 1.8 - 2.0 L/ha (***)	a) 1.08 - 1.50 kg as/ha b) 1.08 - 1.50 kg as/ha a) 1.08 - 1.2 kg as/ha b) 1.08 - 1.2 kg as/ha (***)	150- 400	n/a			Without R3 stream and R4 stream					
																R3 stream and R4 stream at 1.50 kg a.s./ha				
																R3 stream and R4 stream at lower dose rate				
15	PL, HU, RO, SK	Forest nurseries plants (pine tree, spruce, fir tree, larch) YPFLG	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a			Without R4 stream					
																R4 stream unresolved risk at 1.8 kg a.s./ha				
																R4 stream at lower dose rate than 1.8 kg a.s./ha				
16	PL, HU, RO, SK	Ornamental shrubs NNNZG	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2	200- 300	n/a			Without R4 stream					
																R4 stream unresolved risk at 1.5 kg a.s./ha				

									L/ha b) 1.95 - 2.0 L/ha (***)	kg as/ha b) 1.17 - 1.2 kg as/ha (***)					R4 stream at lower dose rate than 1.5 kg a.s./ha				
17	PL, HU, RO, SK	Basket willow <i>Salix</i> sp. SAXSS	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200-300	n/a			Without R4 stream R4 stream unresolved risk at 1.5 kg a.s./ha R4 stream at lower dose rate than 1.5 kg a.s./ha				
18	PL, HU, RO, SK	Purple willow SAXPU	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200-300	n/a			Without R4 stream R4 stream unresolved risk at 1.5 kg a.s./ha R4 stream at lower dose rate than 1.5 kg a.s./ha				
19	PL, HU, RO, SK	Forest tree 3FORC	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200-300	n/a			Without R4 stream R4 stream unresolved risk at 1.5 kg a.s./ha R4 stream unresolved risk at 1.5 kg a.s./ha				
20	PL, HU, RO, SK	Christmas tree PIEAB	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2	200-300	n/a			Without R4 stream R4 stream unresolved risk at 1.5 kg a.s./ha				

									L/ha b) 1.95 - 2.0 L/ha (***)	kg as/ha b) 1.17 - 1.2 kg as/ha (***)						R4 stream unresolved risk at 1.5 kg a.s./ha				
21	PL, HU, RO, SK	Energetic willow SAXSS	F	Mono and dicots weeds	Spray	Immediately after planting, when trees are dormant	a) 1 b) 1	n/a	a) 1.95 - 3.0 L/ha b) 1.95 - 3.0 L/ha a) 1.95 - 2.0 L/ha b) 1.95 - 2.0 L/ha (***)	a) 1.17 - 1.8 kg as/ha b) 1.17 - 1.8 kg as/ha a) 1.17 - 1.2 kg as/ha b) 1.17 - 1.2 kg as/ha (***)	200- 300	n/a				Without R4 stream R4 stream unresolved risk at 1.5 kg a.s./ha R4 stream unresolved risk at 1.5 kg a.s./ha				
Minor uses according to Article 51 (interzonal uses)																				
22																				

* 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

*** as a result of unacceptable risk to mammals for the proposed uses, the dose was limited to maximum of 2 L/ha (1.2 kg as/ha)

Explanation for column 15 – 21 “Conclusion”

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

**Remarks
table:**

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, *e.g.*: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- (12) If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
- (13) PHI - minimum pre-harvest interval
- (14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

General comment

On the request DE, received during the comment period, the following general comment was added:
According to regulation (EC) No 1107/2009, article 4, unacceptable impact on non-target species as well as on biodiversity and the ecosystem should be avoided. Neither in the regulation (EC) No. 1107/2009 nor in the current data requirements (regulations (EU) 283/2013 and 284/2013) nor in the uniform principles (regulation No. 546/2011) a distinction is made between non-target species in-field and off-field. As the product has a broad-spectrum herbicidal activity non-target plants occurring in-field will be affected in the same way as the intended target plants. Therefore, an overall reduction of the whole arable flora is inevitably linked to the unspecific herbicidal activity of the product and will also affect other arable species due to the interruption of the food web. These risks are not covered by the risk assessment according to the current guidance and MSs should decide themselves whether under their conditions further management is regarded necessary.

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)

AKO 600 SC pose no unacceptable risk to birds and mammals used according to the label.

The mammalian assessment due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) indicates an acceptable risk only at a dose of 1.2 kg a.s./ha therefore exposure calculations for birds and mammals for higher doses were omitted and the assessment was performed for a dose of 1.2 kg a.s./ha by the zRMS.

AKO 600 SC poses no unacceptable risk to birds used according to the label.

AKO 600 SC poses no unacceptable acute and chronic risk to mammals if used according to the new proposed dose – ~~2.32L~~ 2.0 L of AKO 600 SC/ha.

There is also low risk of secondary poisoning of earthworm eating mammals if new proposed dose of the product is used accordingly.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of AKO 600 SC according to EFSA/2009/1438 criteria and therefore the risk to birds and mammals via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating birds and mammals are expected following application of AKO 600 SC (Tanger) according to the proposed use pattern (2.0 L of AKO 600 SC/ha). No risk mitigation measures are required.

Tank mix AKO 600 SC with BOA PRO 480 EC pose no unacceptable risk to mammals.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

AKO 600 SC pose no unacceptable risk to aquatic organisms according to the label with appropriate risk mitigation measures.

AKO 600 SC poses no unacceptable risk to aquatic organism if used as a tank mix with BOA PRO 480 EC. Use of 20 meters vegetative strip, 20 meters no-spray buffer zone and 90% of nozzle reduction is required.

9.1.1.3 Effects on bees (KCP 10.3.1)

AKO 600 SC pose no unacceptable risk to bees according to the label.

There is currently no EU agreed chronic risk assessment scheme for bees. However, as agreed in the Central Zone a risk assessment based on the EFSA bee GD is presented below for illustrative purposes.

The ETR values are less than the Tier 1 trigger values for downward sprays indicating that the chronic risk to honeybee larvae and bees is acceptable following use of AKO 600 SC according to the proposed use pattern excluding weeds scenario.

In the Tier 1 assessment, an unacceptable chronic risk was indicated for adult bees foraging on weeds in the treated fields. As no further studies were submitted to solve the chronic risk indicated for adult bees, the risk assessment could not be finalized. For bee larvae an unacceptable risk was indicated for weeds scenario. As no further studies were submitted to solve the chronic risk indicated for adult bees and larvae the risk assessment could not be finalized..

Final decision of this issue and its consequence on the registration process will have to be dealt with at the product authorization by the cMS that consider indications of EFSA (2013) at the national level, since at the zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level.

An acceptable risk to bees of the formulation AKO 600 SC can be concluded, based on the risk assessment scheme of the Guidance Document on Terrestrial Eco-toxicology (SANCO/10329/2002 rev 2).

As the EFSA bee guidance applied for the risk assessment is not noted yet, decisions should be taken on member state level whether the indicated chronic risk for adult bees and larvae foraging on weeds in the treated field is relevant.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

AKO 600 SC pose no unacceptable risk to NTA according to the label

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

AKO 600 SC pose no unacceptable risk to non-target soil meso- and macrofauna and microbial activity according to the label.

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

AKO 600 SC pose no unacceptable risk to non-target terrestrial plants according to label with appropriate buffer zone and drift reducing techniques.

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

Not relevant

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk enve-

lope approach (according to SANCO/11244/2011).

Table 9.1-2: Critical use pattern of AKO 600 SC grouped according to environmental risk assessment section

Grouping according to environmental risk assessment				
Group No.	Group	Intended uses	Relevant use parameters for grouping	Relevant parameter or value for sorting
1.	Bare soil	Potato, sunflower, spring rapeseed, flax, breadseed poppy, mustard. Soybeans, pumpkin, hemp,	BBCH, application rate, number of application	BBCH < 10 Application rate: 1.8 kg/ha (worse case scenario) No. of applications: 1
2.	Bare soil	Forest nurseries, ornamental shrubs, basket willow, purple willow, tobacco, forest tree, christmas tree, energetic willow	BBCH, application rate, number of application	BBCH < 10 (stages: immediately after planting, before planting and trees are dormant, are considered as the earliest stage of growth – BBCH 00) Application rate: 1.8 kg/ha (worse case scenario) No. of applications: 1

9.1.3 Consideration of metabolites

Aclonifen doesn't have metabolites.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with aclonifen and its metabolites (not relevant). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of AKO 600 SC were evaluated as part of the EU assessment of aclonifen.

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Aclonifen	acute	LD50 > 2000 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,
Coturnix coturnix japonica	Aclonifen	acute	LD50 > 15000 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,
Serinus canarius	Aclonifen	acute	LD50 > 15000 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Aclonifen	Short-term, 5 d dietary	LD50 > 1027 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,
Coturnix coturnix japonica	Aclonifen	long-term, 6 weeks dietary	NOEL reproduction > 141 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,

Risk assessment of clomazone is not a main subject of this document. However, it's required for combined exposure assessment of a mixture: AKO 600 SC and BOA PRO 480 EC. Therefore, for details of assessment of Clomazone please refer to core dossier of BOA PRO 480 EC.

9.2.1.1 Justification for new endpoints

No new endpoints. All endpoints were assessment on EU level.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the risk assessment for the 2 main crop groups (see table 9.1.2) covers the risk for birds from all other intended uses described in GAP.

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.

Evaluator comment:

The mammalian assessment due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) indicates an acceptable risk only at a dose of 1.2 kg a.s./ha therefore exposure calculations for birds and mammals for higher doses were omitted and the assessment was performed for a dose of 1.2 kg a.s./ha by the evaluator.

Selection of the endpoints for acute risk assessment

According to EFSA Journal 2009;7(12):1438, where the dietary LC₅₀ is lower than the acute LD₅₀, the dietary value should be used in the acute risk assessment. Therefore, LD₅₀ > 1027 mg a.s./kg bw/d will be used in the acute risk assessment as worst-case.

Table 9.2-1: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of AKO 600 SC in Bare soil (group 1 and 2) (BCCH <10; application rate: 1.2kg/ha, number of applications: 1)

Acute Dietary Risk Assessment for Birds - Aclonifen

Intended use	group 1 and 2				
Active substance/product	Aclonifen				
Application rate (kg/ha)	1.2				
Acute toxicity (mg/kg bw)	>1027				
TER criterion	10				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small granivorous bird	25.3	30.36	1.0	30.36	33.8
Reproductive Risk Assessment for Birds- Aclonifen					
Intended use	group 1 and 2				
Active substance/product	Aclonifen				
Application rate (kg/ha)	1.2				
Reproductive toxicity (mg/kg bw)	102.7				
TER criterion	5				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small granivorous bird	11.4	13.68	1.0	7.25	14.2

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

Table 9.2-1: ~~First tier assessment of the acute and long-term/reproductive risk for birds due to the use of AKO 600 SC in Group No. 1 (see Table 9.1-2) (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)~~

Acute Dietary Risk Assessment for Birds – Aclonifen					
Intended use	Group No. 1 Aclonifen 1.8 kg/ha 2000 1027 10				
Active substance/product					
Application rate (g/ha)					
Acute toxicity (mg/kg bw)					
TER criterion					
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	158.8	285.84	1.0	285.84	7.0 3.6
First Tier Risk Assessment					
Indicator species	Short cut value		TER		
Small granivorous bird “finch”	24.7		45 23.1		
Small insectivorous bird “wagtail”	10.9		101.9 52.3		
Small omnivorous bird “lark”	17.4		63.9 32.8		
Reproductive Risk Assessment for Birds – Aclonifen					
Intended use	Group No. 1 Aclonifen 1.8 kg/ha 141 5 10				
Active substance/product					
Application rate (g/ha)					
Reproductive toxicity (mg/kg bw)					
TER criterion					
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	64.8	116.64	1.0	61.82	2.3
First Tier Risk Assessment					
Small granivorous bird “finch”	11.4		13		
Small insectivorous bird “wagtail”	5.9		25.1		
Small omnivorous bird “lark”	8.2		18.0		

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

Table 9.2 2: First tier assessment of the acute and long-term/reproductive risk for birds due to the use of AKO 600 SC in Group No. 2 (see Table 9.1 2) (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)

Acute Dietary Risk Assessment for Birds– Aclonifen					
Intended use	Group No. 2 Aclonifen 1.8 kg/ha				
Active substance/product					
Application rate (g/ha)					
Acute toxicity (mg/kg bw)	2000				
TER criterion	1027				
	10				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	46.8	84.24	1	84.24	23.7 12.2
First Tier Risk Assessment					
Indicator species		Short cut value		TER	
Small granivorous bird “finch”		24.7		45.0 23.1	
Small insectivorous bird “wagtail”		10.9		101.9 52.3	
Small omnivorous bird “lark”		17.4		63.9 32.8	
Reproductive Risk Assessment for Birds– Aclonifen					
Intended use	Group No. 2 Aclonifen 1.8 kg/ha				
Active substance/product					
Application rate (g/ha)					
Reproductive toxicity (mg/kg bw)	141				
TER criterion	5				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	18.2	32.76	1	17.36	8.1
First Tier Risk Assessment					
Small granivorous bird “finch”		11.4		13	
Small insectivorous bird “wagtail”		5.9		25.1	
Small omnivor		8.2		18.0	

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

Combined risk assessment for a mixture of 2.0L of AKO 600 SC and 0.2L of BOA PRO 480 EC (worst case)

Table 9.2.3: First tier assessment of the acute and long-term/reproductive risk for birds due to the use of a mixture of AKO 600 SC and BOA PRO 480 EC in Group No. 1 (potatoes) (see Table 9.1.2) (BCCH <10; application rate: 1.2 kg of AKO 600 SC and 0.096 kg of BOA PRO 480 EC/ha, number of applications: 1)

	Acute Dietary Risk Assessment for Birds – Aclonifen					Acute Dietary Risk Assessment for Birds – Clomazone				
Intended use	Group no. 1 Aclonifen 1.2 kg/ha					Group no. 1 Clomazone 0.096 kg/ha				
Active substance/product										
Application rate (g/ha)										
Acute toxicity (mg/kg bw)	2000 1027					1671				
TER criterion	10					10				
	Screening step									
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	158.8	190.56	1.0	190.56	10.5 5.4	158.8	15.24	1.0	15.24	109.6
	First Tier Risk Assessment									
Indicator species	Short cut value			TER		Short cut value			TER	
Small granivorous bird “finch”	24.7			67.5 34.6		24.7			704.7	
Small insectivorous bird “wagtail”	10.9			152.9 78.5		10.9			1596.9	
Small omnivorous bird “lark”	17.4			95.8 49.2		17.4			1000.4	
		Reproductive Risk Assessment for Birds – Aclonifen					Reproductive Risk Assessment for Birds – Clomazone			
Intended use		Group no. 1								

Active substance/product	Aclonifen					Group no. 1 Clomazone				
Application rate (g/ha)	1.2 kg/ha					0.096 kg/ha				
Acute toxicity (mg/kg bw)	141					94				
TER criterion	5					5				
	Screening step									
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small omnivorous bird	64.8	77.76	1.0	41.21	3.4	64.8	6.22	1.0	3.30	28.5
	First Tier Risk Assessment									
Indicator species	Short cut value		TER			Short cut value		TER		
Small granivorous bird “finch”	11.4		19.4			11.4		162.1		
Small insectivorous bird “wagtail”	5.9		37.6			5.9		313.1		
Small omnivorous bird “lark”	8.2		27.0			8.2		225.3		

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

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of a mixture of AKO 600 SC and BOA PRO 480 EC in Group No. 1 and 2 (see Table 9.1-2) (BCCH <10; application rate: 1.2 kg of AKO 600 SC and 0.096 kg of BOA PRO 480 EC/ha, number of applications: 1)

	Acute Dietary Risk Assessment for Birds - Aclonifen					Acute Dietary Risk Assessment for Birds – Clomazone				
Intended use	Group no. 1 Aclonifen					Group no. 1 Clomazone				
Active sub- stance/product										
Application rate (kg/ha)	1.2					0.096				
Acute toxicity (mg/kg bw)	2000 1027					1671				
TER criterion	10					10				
	Screening step									
Indicator species	Short cut value	Daily Dieta- ry Dose (single)	MAF (90)	Daily Die- tary Dose (Multiple)	TE R	Short cut value	Daily Dieta- ry Dose (single)	MAF (90)	Daily Die- tary Dose (Multiple)	TER
Small granivorous bird	25.3	30.36	1.0	30.36	33.8	25.3	2.43	1.0	2.43	687
	Reproductive Risk Assessment for Birds - Aclonifen					Reproductive Risk Assessment for Birds - Clom- azone				
Intended use	Group no. 1 Aclonifen					Group no. 1 Clomazone				
Active sub- stance/product										
Application rate (kg/ha)	1.2 kg/ha					0.096 kg/ha				
Acute toxicity (mg/kg bw)	102.7					94				
TER criterion	5					5				
	Screening step									
Indicator spe- cies	Shor t cut val- ue	Daily Dieta- ry Dose (sin- gle)	MAF (90)	Daily Dietary Dose (Multi- ple)	TER	Shor t cut val- ue	Daily Dieta- ry Dose (sin- gle)	MAF (90)	Daily Dietary Dose (Multi- ple)	TER
Small omnivo- rous bird	11.4	13.68	1.0	7.25	14.2	11.4	1.09	1.0	0.58	162.1

First Tier Risk Assessment				
Indicator species	Short cut value	TER	Short cut value	TER
Small granivorous bird "finch"	11.4	19.4	11.4	162.1
Small insectivorous bird "wagtail"	5.9	37.6	5.9	313.1
Small omnivorous bird "lark"	8.2	27.0	8.2	225.3

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.

Acute risk

For the assessment of the acute risk to birds, the following formula is used to derive a surrogate LD₅₀ for the mixture of active substances with known toxicity assuming additivity:

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

With:

X(a.s._i) = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

LD₅₀(a.s._i) = acute toxicity value for active substance [i]

For mixture of AKO 600 SC and BOA PRO 480 EC, the LD₅₀ (mix) amounts to 1057 mg/kg bw = ((0.926/1027) + (0.074/1671))⁻¹

Intended use	group 1 and 2				
Active substance/product	mixture of AKO 600 SC and BOA PRO 480 EC				
Application rate (g/ha)	1.296 g/ha				
Acute toxicity (mg/kg bw)	>1057				
TER criterion					
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small granivorous bird	25.3	32.8	1.0	32.8	32

Combined screening step:

All TER > trigger value x n (where: n = no. of active substances)

~~Combined toxicity~~ using the TER values for active substances: Aclonifen and Clomazone use: bare soil (BBCH <10)

	TER value for Aclonifen	TER value for Clomazone	Criteria
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Acute	10.5 5.4	109.6	Criteria: 20
Chronic	3.4	28.5	Criteria: 10

All TER > trigger value x n (where: n = no. of active substances)

Combined toxicity - using the TER values for active substances: Aclonifen and Clomazone – use: bare soil (BBCH < 10)

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

where:

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

	TER value for Aclonifen	TER value for Clomazone	Criteria
Acute	33.8	687	Criteria: 20
Chronic	14.2	162.1	Criteria: 10

Combined first tier:

A $TER_{(mix)}$ is calculated with the following formula:

Combined toxicity – using the TER values for active substances: Aclonifen and Clomazone – use: bare soil (BBCH < 10)

		$TER_{(mix)}$	Criteria
Acute	Aclonifen (worst case first tier): $TER = 67.5$ Clomazone (worst case first tier): $TER = 704.7$ $TER_{(mix)}: 1/((1/67.5) + (1/704.7)) = 61.6$	61.6	Criteria: 10
Chronic	Aclonifen (worst case first tier): $TER = 14.2$ Clomazone (worst case first tier): $TER = 162.1$ $TER_{(mix)}: 1/((1/14.2) + (1/162.1)) = 17.3$	17.3	Criteria: 5

Combined toxicity - using the TER values for active substances: Aclonifen and Clomazone – use: bare soil (BBCH < 10)

		$TER_{(mix)}$	Criteria
Acute	Aclonifen (worst case first tier): $TER = 33.8$ Clomazone (worst case first tier): $TER = 687$ $TER_{(mix)}: 1/((1/33.8) + (1/687)) = 32$	32	Criteria: 10

Chronic	Aclonifen (worst case first tier): TER = 19.4 Clomazone (worst case first tier): TER = 162.1 TER(mix): $1/((1/19.4) + (1/162.1)) = 17.33$	17.3	Criteria: 5
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Conclusion

The calculated worst case TER_(mix) values are higher than the trigger value, indicating the mixture of AKO 600 SC and BOA PRO 480 EC poses no unacceptable acute and chronic risk for birds. No further risk refinement is needed

9.2.2.1 Higher-tier risk assessment

Not necessary

9.2.2.2 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 AKO 600 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a K(f)_{oc} of ~~6749~~ 7126 (Arith. Mean, n= 3), aclonifen belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment covers the risk for birds from all intended uses described in the GAP.

Effective application rate (g/ha)	=	1800		
Acute toxicity (mg/kg bw)	=	2000 1027	quotient =	0.90 1.75
Reprod. toxicity (mg/kg bw/d)	=	141	quotient =	12.77

9.2.2.3 Effects of secondary poisoning

The log P_{ow} of aclonifen is 4.37 thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the group no. 1 (worst case scenario – application rate: 1.8 kg/ha; BCCH 00) also covers the risk for birds from all other intended uses in group 2 (see 9.1.2).

Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	Aclonifen	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	2.5382 2.6256	PEC _{soil,accu} (twa=21d) for potatoes, application rate: 1.8kg/ha, Escape ver. 2
log P _{ow} / P _{ow}	4.37 (Log P _{ow}) / 23442.3 (P _{ow})	EFSA Scientific Report (2008) 149, 1-80
Koc	6749 7126	Geometric Mean (n = 3) Arith. Mean (n = 3), EFSA Scientific Report (2008) 149, 1-80
foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BCF _{worm}	2.090 1.98	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) = (0.84 + 0.012 × P _{ow}) / foc × Koc
PEC _{worm}	5.305 5.2	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	5.57 5.46	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	141	EFSA Scientific Report (2008) 149, 1-80
TER _{lt}	25.32 25.83	TER _{lt} = NOEL _{longterm} / DDD

TER_{lt} > 5 therefore no further refinement is required

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 Aclonifen in water.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the group no. 1 (worst case scenario – application rate: 1.8 kg/ha, BCCH 00) also covers the risk for birds from all other intended uses in group no. 2 (see 9.1.2).

Table 9.2-7: Assessment of the risk for fish-eating birds due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in potatoes

Parameter	Aclonifen	comments
PEC _{sw} (initial) (mg/L)	0.01655 0.02085	FOCUS STEP 2 Worst case assumption taking into account FOCUS

Parameter	Aclonifen	comments
		step 2 (tobacco/ pome fruits)
BCF _{fish}	2896	EFSA Scientific Report (2008) 149, 1-80
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	47.93 60.4	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	7.62 9.6	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	>141	EFSA Scientific Report (2008) 149, 1-80
TER _{lt}	18.50 14.7	TER _{lt} = NOEL _{longterm} / DDD

TER_{lt} > 5 therefore no further refinement is required

9.2.2.4 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

The acute, short term and long term risk on birds from the proposed uses of Aclonifen was found acceptable. AKO 600 SC do not pose unacceptable risk to birds according to the label.

zRMS comments:

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

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the application rate of 1.2 kg a.s./ha exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substance following use of AKO 600 SC in compliance with modified GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of AKO 600 SC according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating birds are expected following application of AKO 600 SC (Tanger) according to the proposed use pattern.

No risk mitigation measures are required.

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 aclonifen. Full details of these studies are provided in the respective EU DAR and related document.

Effects on mammals of AKO 600 SC were not evaluated as part of the EU assessment of aclonifen.

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

Species	Substance	Exposure System	Results	Reference
Rattus spec. and mouse	Aclonifen	acute, oral	LD50 > 5000 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,
Rattus spec.	BANDUR SC 600 g/L	acute, oral	LD50 5596 = 2770 mg as	EFSA Scientific Report (2008) 149, 1-80,
Rattus spec.	Aclonifen	long-term, 2-generation, reproduction	NOEL reproduction: 8 (mg/kg bw/day)	EFSA Scientific Report (2008) 149, 1-80,
Rattus spec.	Aclonifen	2-generation study	NOEL reproduction: 35 (mg/kg bw/day)	DAR Aclonifen Annex B, B-9 Ecotoxicology Ref. No: Becker, 1985, TOX2005-113

9.3.1.1 Justification for new endpoints

No new endpoints were established

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 Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the risk assessment for the 2 main crop groups covers the risk for mammals from all other intended uses described in GAP (see 9.1.2).

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

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

Evaluator comment:

The mammalian assessment due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) indicates an acceptable risk only at a dose of 1.2 therefore exposure calculations for birds and mammals for higher doses were omitted and the assessment was performed for a dose of 1.2 kg a.s./ha by the evaluator.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AKO 600 SC in Bare soil (group 1 and 2) (BCCH <10; application rate: 1.2kg/ha, number of applications: 1)

Acute Dietary Risk Assessment for Mammals - Aclonifen					
Intended use	Group 1 and 2 Aclonifen 1.2				
Active substance/product					
Application rate (kg/ha)					
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small granivorous mammal	14.4	17.28	1.0	17.28	289.4
Reproductive Risk Assessment for Mammals Birds Aclonifen					
Intended use	Group 1 and 2 Aclonifen 1.2				
Active substance/product					
Application rate (kg/ha)					
Reproductive toxicity (mg/kg bw)	8				
TER criterion	5				
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small granivorous mammal	6.6	7.92	1.0	4.2	1.91

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AKO 600 SC in group no. 1 (see Table 9.1.2) (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)

Acute Dietary Risk Assessment for Mamals – Aclonifen						
Intended use			Group no. 1 Aclonifen 1.8 kg/ha			
Active substance/product						
Application rate (g/ha)						
Acute toxicity (mg/kg bw)			2770			
TER criterion			10			
Screening step						
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	
Small herbivorous mammal	118.4	213.12	1.0	213.12	13.0	
First Tier Risk Assessment						
Indicator species		Short cut value	TER			
Small omnivorous mammal “mouse”		14.3	107.6			
Reproductive Risk Assessment for Mamals – Aclonifen						
Intended use		Group no. 1 Aclonifen 1.8 kg/ha				
Active substance/product						
Application rate (g/ha)						
Reproductive toxicity (mg/kg bw)		8.0				
TER criterion		5				
Screening step						
Indicator species	Short cut value		Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small herbivorous mammal	48.3		86.94	1.0	46.08	0.17
First Tier Risk Assessment						
Indicator species		Short cut value		TER		
Small omnivorous mammal “mouse”		5.7		1.5		

Due to TER value (TER < 5) higher tier risk assessment was necessary.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AKO 600 SC in group no. 2 (see Table 9.1.2) (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)

Acute Dietary Risk Assessment for Mamals – Aclonifen						
Intended-use			Group no. 2 Aclonifen 1.8 kg/ha			
Active substance/product						
Application rate (g/ha)						
Acute toxicity (mg/kg bw)			2770			
TER criterion			10			
Screening step						
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	
Small herbivorous mammal	136.4	245.52	1.0	245.52	11.3	
First Tier Risk Assessment						
Indicator species		Short cut value	TER			
Small omnivorous mammal “mouse”		14.3	107.6			
Reproductive Risk Assessment for Mamals– Aclonifen						
Intended-use		Group no. 2 Aclonifen 1.8 kg/ha				
Active substance/product						
Application rate (g/ha)						
Reproductive toxicity (mg/kg bw)		8.0				
TER criterion		5				
Screening step						
Indicator species	Short cut value		Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
Small herbivorous mammal	72.3		130.14	1.0	68.97	0.12
First Tier Risk Assessment						
Indicator species		Short cut value		TER		
Small omnivorous mammal “mouse”		5.7		1.5		

Due to TER value (TER < 5) higher tier risk assessment was necessary.

9.3.2.2 Higher-tier risk assessment

Table 9.3 2.1: Higher tier assessment of the reproductive risk for mammals due to the use of AKO 600 SC in group no. 1 (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)

Reproductive Risk Assessment for Mamals Aelonifen					
Intended use			Group no. 1		
Active substance/product			Aelonifen		
Application rate (g/ha)			1.8 kg/ha		
Reproductive toxicity (mg/kg bw)			35		
TER criterion			5		
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small herbivorous mammal	48.	86.94	1.0	46.08	0.76
First Tier Risk Assessment					
Indicator species	Short cut value			TER	
Small omnivorous mammal “mouse”	5.7			6.4	

Table 9.3 3.1: Higher tier assessment of the reproductive risk for mammals due to the use of AKO 600 SC in group no. 2 (BCCH <10; application rate: 1.8kg/ha, number of applications: 1)

Reproductive Risk Assessment for Mamals—Aelonifen					
Intended use			Group no. 2		
Active substance/product			Aelonifen		
Application rate (g/ha)			1.8 kg/ha		
Reproductive toxicity (mg/kg bw)			35		
TER criterion			5		
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small	72	130.14	1.0	68.97	0.51

herbivorous mammal					
First Tier Risk Assessment					
Indicator species	Short cut value			TER	

Refinement of PT according to **Addendum 4 to the Draft Assessment Report** of 31 August 2006 (relating to Volume 1 + 3 + Addendum 3 of 13 May 2008)

“Generic data on focal species in sunflower are not available. However, maize fields can be considered as a surrogate for drilled sunflower fields. A generic study on maize and sugar beet (Wolf, 2005, see Chapter B.9.3.2) is available. The data on sugar beet fields are not appropriate since individual wood-mice are well known digging out beet seed pills and by this damaging the culture which is not given in sunflowers. Therefore the focus should be mainly on maize. In this study of Wolf (2005), re-evaluated for mammals by Barfknecht (2010) mean proportion of active time within the field were 0 % and 2 % (90th perc. 0 % and 4 %) for maize and 38 % and 32 % (90th perc. 81 % and 93 %) for sugar beet (freshly drilled andgerminated).

To provide a conservative value for **PT**, the mean value of 38 % for sugar beet is used as surrogate for sunflower knowing that this is significantly overestimating the real portion of time due to the above mentioned special preference. The RMS is of the opinion that this value can be supported due to the number of field studies presented by the notifier”

ZRMS agrees with the use PT value of 0.38 in the risk assessment.

The zRMS accepts the application of a PT value of 0.38 in the risk assessment. It is agreed that pre-emergence use in sunflowers can serve as a proxy for pre-emergence applications intended for AKO 600 SC, given that aclonifen is applied to bare soil in all situations. Consequently, a PT value of 0.38 was used with the wood mouse as the focal species in the higher-tier risk assessment presented below.

zRMS agrees with the DE comment that the refinement of the PT (0.38) to bare soil scenarios (BBCH < 10) that the refinement of the PT is not suitable for applications in crops where sowing is carried out on overgrown soils or as mulch sowing. Therefore the risk assessment only refers to applications that take place exclusively on bare soil.

Table 9.3-3.1: Higher-tier risk assessment of the long-term/reproductive risk for mammals due to the use of AKO 600 SC in group no. 1 and 2 – refined parameters: PT = 0.38 (BBCH <10; application rate: 1.8 kg/ha, number of applications: 1)

Intended use	Group 1 and 2						
Active substance/product	Aclonifen						
Application rate (g/ha)	1 x 1.8 kg of aclonifen/ha						
Reprod. toxicity (mg/kg bw/d)	8						
TER criterion	5						
Focal species	Food category, % in diet	FIR/bw	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Small omnivorous mammal “mouse”	50% weed seeds, 50% ground arthropods*	0.24	23.8	0.53	0.38	2.07	3.86

Since TER value obtained for the proposed dose of aclonifen (1.8 kg of a.s/ha) is below criterion value calculation for lower dose has been made:

Since

Since $TER < 5$ new dose of AKO 600 SC (1.39kg of a.s/ha) has been proposed. New calculations have been made.

Table 9.3-3.2: Higher-tier risk assessment of the long-term/reproductive risk for mammals due to the use of AKO 600 SC in group no. 1 and 2 – refined parameters: PT = 0.38 (BBCH <10; application rate: 1.39 kg of a.s/ha, number of applications: 1)

Intended use		Group 1 and 2					
Active substance/product		Aclonifen					
Application rate (g/ha)		1 x 1.39kg of aclonifen/ha (2.32L of AKO 600 SC/ha)					
Reprod. toxicity (mg/kg bw/d)		8					
TER criterion		5					
Focal species	Food category, % in diet	FIR/bw	$RUD_m \times DF$ (mg/kg food)	$MAF_m \times TWA$	PT	DDD_m (mg/kg bw/d)	TER_{it}
Small omnivorous mammal "mouse"	50% weed seeds, 50% ground arthropods*	0.24	23.8	0.53	0.38	1.599	5.003

$TER \geq 5$ therefore new maximum proposed dose of AKO 600 SC – 1.39 kg of a.s/ha - poses no unacceptable chronic risk for mammals. No further risk refinement is needed

Combined risk assessment for a mixture of 2.0L of AKO 600 SC and 0.2L of BOA PRO 480 EC (worst case)

Table 9.3 3.2: ~~First tier assessment of the acute and long term/reproductive risk for mammals due to the use of a mixture of AKO 600 SC and BOA PRO 480 EC in Group No. 1 (potatoes) (see Table 9.1 2) (BCCH <10; application rate: 1.2 kg of AKO 600 SC and 0.096 kg of BOA PRO 480 EC/ha, number of applications: 1)~~

	Acute Dietary Risk Assessment for mammals—Aclonifen					Acute Dietary Risk Assessment for mammals—Clomazone				
Intended use	Group no. 1 Aclonifen 1.2 kg/ha					Group no. 1 Clomazone 0.096 kg/ha				
Active substance/product										
Application rate (g/ha)										
Reproductive Acute toxicity (mg/kg bw)	2770 10					1369 10				
TER criterion										
	Screening step									
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small herbivorous mammal	118.4	142.08	1.0	142.08	19.5	118.4	11.37	1.0	11.37	120.4
	First Tier Risk Assessment									
Indicator species	Short cut value		TER		Short cut value		TER			
Small omnivorous mammal “mouse”	14.3		161.4		14.3		997.2			

	Reproductive Risk Assessment for mammals—Aclonifen					Reproductive Risk Assessment for mammals—Clomazone				
Intended use	Group no. 1 Aclonifen 1.2 kg/ha					Group no. 1 Clomazone 0.096 kg/ha				
Active substance/product										
Application rate (g/ha)										
Reproductive toxicity (mg/kg bw)	35 (higher tier value) 8					100				
TER criterion	5					5				
	Screening step									
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small herbivorous mammal	48.3	57.96	1.0	30.72	1.14	48.3	4.64	1.0	2.46	40.69
	First Tier Risk Assessment									
Indicator species	Short cut value		TER		Short cut value		TER			
Small omnivorous mammal “mouse”	5.7		9.7 2.2		5.7		344.8			

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.

Combined risk assessment for a mixture of 2.0L of AKO 600 SC and 0.2L of BOA PRO 480 EC (worst case)

Table 9.3-3.2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of a mixture of AKO 600 SC and BOA PRO 480 EC (BCCH <10; application rate: 1.2 kg of AKO 600 SC and 0.096 kg of BOA PRO 480 EC/ha, number of applications: 1)

	Acute Dietary Risk Assessment for mammals - Aclonifen					Acute Dietary Risk Assessment for mammals – Clomazone				
Intended use	Group no. 1 and 2 Aclonifen					Group no. 1 and 2 Clomazone				
Active substance/product										
Application rate (kg/ha)	1.2					0.096				
Reproductive Acute toxicity (mg/kg bw)	5000					1369				
TER criterion	10					10				
	Screening step									
Indicator species	Short cut	Daily Dietary	MAF (90)	Daily Dietary	TER	Short cut	Daily Dietary	MAF (90)	Daily Dietary	TER

	value	Dose (single)		Dose (Multiple)		value	Dose (single)		Dose (Multiple)	
Small granivorous mammal	14.4	17.28	1.0	17.28	289.4	14.4	1.38	1.0	1.38	990.3

	Reproductive Risk Assessment for mammals- Aclonifen					Reproductive Risk Assessment for mammals- Clomazone				
Intended use	Group no. 1 Aclonifen					Group no. 1 Clomazone				
Active substance/product										
Application rate (kg/ha)	1.2					0.096				
Reproductive toxicity (mg/kg bw)	8					100				
TER criterion										
	Screening step									
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small granivorous mammal	6.6	7.92	1.0	4.2	1.91	6.6	0.63	1.0	0.34	297.79

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.

Due to TER value (TER < 5) higher tier risk assessment was necessary.

Table 9.3-3.3 Higher-tier risk assessment of the long-term/reproductive risk for mammals due to the dose of AKO 600 SC in tank mix with BOA PRO 480 EC – refined parameters: PT = 0.38 (BBCH <10; application rate: 1.2kg of a.s/ha, number of applications: 1)

Intended use	Potato - In the tank mix with BOA PRO 480 EC						
Active substance/product	Aclonifen						
Application rate (g/ha)	1 x 1.2 kg of aclonifen/ha (2L of AKO 600 SC/ha)						
Reprod. toxicity (mg/kg bw/d)	8						
TER criterion	5						
Focal species	Food category, % in diet	FIR/bw	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Small omnivorous mammal "mouse"	50% weed seeds, 50% ground	0.24	23.8	0.53	0.38	1.38	5.79

	arthropods*						

Combined screening step:

Acute risk

For the assessment of the acute risk to birds, the following formula is used to derive a surrogate LD₅₀ for the mixture of active substances with known toxicity assuming additivity:

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

With:

X(a.s._i) = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

LD₅₀(a.s._i) = acute toxicity value for active substance [i]

For mixture of AKO 600 SC and BOA PRO 480 EC, the LD₅₀ (mix) amounts to 4179 mg/kg bw = ((0.926/5000) + (0.074/1369))⁻¹

Intended use	group 1 and 2				
Active substance/product	mixture of AKO 600 SC and BOA PRO 480 EC				
Application rate (g/ha)	1.296 g/ha				
Acute toxicity (mg/kg bw)	>4179				
TER criterion					
Screening step					
Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
Small granivorous mammal	14.4	18.66	1.0	18.66	223.9

All TER > trigger value x n (where: n = no. of active substances)

~~Combined toxicity~~ using the TER values for active substances: Aclonifen and Clomazone use: bare soil (BBCH <10)

	TER value for Aclonifen	TER value for Clomazone	Criteria
Acute	19.5 289.4	120 990.3	Criteria: 20
Chronic	1.14 5.79	40.69 297.79	Criteria: 10

Combined first tier:

A TER_(mix) is calculated with the following formula:

Combined toxicity - using the TER values for active substances: Aclonifen and Clomazone – use: bare soil (BBCH < 10)			
		TER _(mix)	Criteria
Acute:	Aclonifen (worst case first tier): TER = 161.4 289.4 Clomazone (worst case first tier): TER = 997.2 990.3 TER(mix): $1/((1/161.4\ 289.4) + (1/997.2\ 990.3)) = 138.92\ 224$	138.92 224	Criteria: 10
Chronic:	Aclonifen (worst case first tier): TER = 9.7 5.79 Clomazone (worst case first tier): TER = 344.8 297.79 TER(mix): $1/((1/9.7\ 5.79) + (1/344.8\ 297.79)) = 9.43$	9.43 5.69 5.68	Criteria: 5

Conclusion

The calculated worst case trigger value, indicating the PRO 480 EC poses no unacceptably high risk for mammals. No further risk

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

where:

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

TER_(mix) values are higher than the mixture of AKO 600 SC and BOA ceptable acute and chronic risk for refinement is needed

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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 6749, Aclonifen belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use potatoes also covers the risk for mammals from all other intended uses in groups described in GAP (see 9.1.2).

Effective application rate (g/ha)	=	1800		
Acute toxicity (mg/kg bw)	=	2770	quotient =	0.65
Reprod. toxicity (mg/kg bw/d)	=	8	quotient =	225
Reprod. toxicity (mg/kg bw/d)	=	35 (higher tier)	quotient =	51.43

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Aclonifen amounts to 4.37 and thus exceeds 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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the group no. 1 (worst case scenario – application rate: 1.8 kg/ha, BCCH 00) also covers the risk for mammals from all other intended uses in group no. 2 (see Table 9.1.2).

Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in potatoes (dose – 1.8 kg of aclonifen/ha)

Parameter	Aclonifen	comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	2.5382	PEC _{soil,accu} (twa=21d) for potatoes, application rate: 1.8kg/ha, Escape ver. 2
log P _{ow} / P _{ow}	4.37	EFSA Scientific Report (2008) 149, 1-80
K _{oc}	6749	Geometric Mean (n = 3), EFSA Scientific Report (2008) 149, 1-80
f _{oc}	Organic carbon content of soil (0.02 taken as a default value)	Default
BCF _{worm}	2.090	$BCF_{worm/soil} = (PEC_{worm,ww} / PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	5.305	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	6.79	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	8 / 35*	EFSA Scientific Report (2008) 149, 1-80; Becker, H., 1985
TER _{lt}	1.18	For NOEL = 8 (mg/kg bw/d)
TER _{lt} *	5.16	For NOEL = 35 (mg/kg bw/d)

According to endpoint proposed by the applicant, higher tier $TER_{lt}^* > 5$ therefore no further refinement is required

According to Addendum 4 to the Draft Assessment Report of 31 August 2006 (relating to Volume 1 + 3 + Addendum 3 of 13 May 2008) in cases where the relevant model species for the risk assessment is a mouse or a vole, the TER acceptability criterion according to Annex VI of Directive 91/414/EEC may be modified. In terms of size and potential exposure, mice and voles already represent the 'worst-case' for agricultural areas in Europe's middle zone. Furthermore, the toxicological endpoints and effect values for the assessment are determined on phylogenetically closely related species. Hence, a $TER \geq 5$ in the acute exposure scenario and a $TER \geq 2$ in the long term exposure scenario may be accepted as sufficient.

Since $TER_{lt} < 2$ further refinement is required. Therefore calculations for the new maximum proposed dose – 1.39 kg of aclonifen/ha – have been presented below.

ZRMS comment: The zRMS is aware that a reduction of the trigger values for mouse and vole was accepted by the RMS Germany in Addendum 4 to the Draft Assessment Report of aclonifen. However, this approach is not in line with the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA and will therefore not be applied by the zRMS PL.

Since TER_{lt} does not meet acceptance criteria, further refinement is necessary. A new, lower proposed dose of the active substance has been submitted by the applicant. Calculations for the dose of AKO 600 SC which poses an acceptable risk to earthworm-eating mammals via bioaccumulation (secondary poisoning) are presented below:

Table 9.3-5.1: Assessment of the risk for earthworm-eating mammals due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in potatoes (dose – 1.39 1.2 kg of aclonifen/ha)

Parameter	Aclonifen	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	1.7859 1.5417 1.7504	$PEC_{soil,accu}$ (twa=21d) for potatoes, application rate: 1.39 1.2 kg of a.s/ha, Escape ver. 2
$\log P_{ow} / P_{ow}$	4.37 / 23442	EFSA Scientific Report (2008) 149, 1-80
Koc	6749 7126	Geometric Mean (n = 3), EFSA Scientific Report (2008) 149, 1-80
$PT_{redefined}$	0.38	Refinement of PT according to Addendum 4 to the Draft Assessment Report of 31 August 2006 (relating to Volume 1 + 3 + Addendum 3 of 13 May 2008)
foc	Organic carbon content of soil (0.02 taken as a default value)	Default
BCF_{worm}	2.090 1.98	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	3.733 3.222 3.465	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	1.82 1.567 4.44	$DDD = PEC_{worm} \times 1.28 \times PT_{redefined}$
NOEL (mg/kg bw/d)	8	EFSA Scientific Report (2008) 149, 1-80; Becker, H., 1985
TER_{lt}	4.39 5.11 1.8	For NOEL = 8 (mg/kg bw/d)

Daily dietary dose (mg/kg bw/d)	1.685	$DDD = PEC_{worm} \times 1.28 \times 0.38$
NOEL (mg/kg bw/d)	8	EFSA Scientific Report (2008) 149, 1-80; Becker, H., 1985
TER _{it}	4.75	For NOEL = 8 (mg/kg bw/d)

The use of a 100% earthworm diet as a worst-case; the associated FIR/bw of wood mouse is calculated according to Appendix G & L of the B&M guidance:

- Energy content (kJ/g dry) of 19.3 (appendix L, table 1)
- Moisture (%) of 84.6 (appendix L, table 1)
- Assimilation efficiency of 0.87 (appendix G, table 3)

The resulting FIR/bw of the wood mouse (21.7 g) is approximately **1.05 g wet food/g bw/day**.

$$DDD_{tier2} = PEC_{worm} \times FIR/bw_{(woodmouse)} \times PT = 3.465 \times 1.05 \times 0.38$$

$$TER = 8 / 1.38 = 5.8$$

Proposed use of AKO 600 SC meets the criterion of $TER \geq 2$ therefore it can be assumed that proposed dose of the active substance poses no unacceptable risk to mammals via bioaccumulation in earthworms (secondary poisoning).

The newly proposed use of AKO 600 SC meets the criterion of $TER \geq 5$; therefore it is concluded that the proposed dose of the active substance poses no unacceptable risk to mammals via bioaccumulation in earthworms.

Risk assessment for fish-eating mammals via secondary poisoning

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

Table 9.3-6: Assessment of the risk for fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in potatoes (dose 1.8 kg of aclonifen/ha)

Parameter	Aclonifen	Comments
PEC _{sw} (initial) (mg/L)	0.01655	FOCUS STEP 2
BCF _{fish}	2896	EFSA Scientific Report (2008) 149, 1-80
BMF	Not relevant	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	47.93	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	6.81	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	8/35*	EFSA Scientific Report (2008) 149, 1-80; Becker, H., 1985
TER _{it}	1.17	For NOEL = 8 (mg/kg bw/d)
TER _{it} *	5.14	For NOEL = 35 (mg/kg bw/d)

According to endpoint proposed by the applicant, higher tier TER_{it}* > 5 therefore no further refinement is required

Since $TER_{lt} < 5$ further refinement is required. Therefore calculations for the new proposed dose — 1.2 kg of aclonifen/ha — have been made.

Table 9.3-6.1: Assessment of the risk for fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in potatoes – dose: 1.8 kg of aclonifen/ha

Parameter	Aclonifen	comments
PEC_{sw} (initial) (mg/L)	0.0004938	FOCUS SWASH Step 3 scenario D3 - $PEC_{sw, twa}$ 21 days worst case scenario for surfacewater
BCF_{fish}	2896	EFSA Scientific Report (2008) 149, 1-80
BMF	Not relevant	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	1.43	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.2031	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	8	EFSA Scientific Report (2008) 149, 1-80; Becker, H., 1985
TER_{lt}	39.389	For NOEL = 8 (mg/kg bw/d)

$TER_{lt} \geq 5$ therefore no further refinement is required. Use of the maximum dose of AKO 600 SC – 1.8kg of a.s/ha – pose no unacceptable risk to mammals via bioaccumulation in fish (secondary poisoning)

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

~~The acute, short term and long term risk on mammals from the proposed uses of Aclonifen was found acceptable. AKO 600 SC do not pose unacceptable risk to mammals with according to the label.~~

Due to the possibility of excessive exposure of mammals to the product, new dose of the product has been proposed and the calculations were carried out. The long-term risk to mammals was found to be acceptable at the new proposed dose of ~~2.32 L~~ 2.0 L of AKO 600 SC/ha. Calculations also prove that proposed dose of the product poses no unacceptable risk for earthworm-eating mammals via bioaccumulation either. For fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) maximum intended dose from the GAP of the product is safe to use.

Tank mix AKO 600 SC with BOA PRO 480 EC pose no unacceptable risk to mammals.

zRMS comments:

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

The results of the ‘screening phase’ acute dietary risk assessment and Tier-1 long term dietary

risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the application rate 1.2 kg a.s./ha (2.0 L of AKO 600 SC/ha) in modified GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for mammals resulting from acute and long-term exposure to active substance following use of AKO 600 SC in modified GAP – for one application of 1.2 kg a.s./ha.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of AKO 600 SC according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating mammals are expected following application of AKO 600 SC (Tanger) according to the proposed use pattern (1.2 kg a.s./ha).

No risk mitigation measures are required when maximum application rater in GAP for all uses are modified to 1x 1.2 kg a.s./ha ((1 x 2.0 L of AKO 600 SC/ha).

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

Birds and mammals are regarded as adequate surrogates for terrestrial stages of amphibians and reptiles. For the aquatic stages of amphibians, please refer to the risk assessment for fish presented in KCP 10.2.

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

Effects on aquatic organisms of AKO 600 SC were not evaluated as part of the EU assessment of Aclonifen. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Justifications are provided below.

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

Species	Substance	Exposure System	Results (Toxicity) (mg/L)	Reference
Fish				
Oncorhynchus mykiss	Aclonifen	96 h (static)	Mortality, LC ₅₀ = 0.67 nom	EFSA Scientific Report (2008) 149, 1-80

Pimephales promelas	Aclonifen	35 d ELS (flow-through)	Growth, NOEC: 0.005 nom Hatch, NOEC: 0.011 nom	EFSA Scientific Report (2008) 149, 1-80
Aquatic invertebrate				
Daphnia magna	Aclonifen	48 h (static)	Mortality, EC ₅₀ = 1.2 nom (0.952 mm)	EFSA Scientific Report (2008) 149, 1-80
Daphnia magna	Aclonifen	21 d, (semi-static)	Reproduction, NOEC: 0.016 mm	EFSA Scientific Report (2008) 149, 1-80
Sediment dwelling organisms				
Chironomus riparius	Aclonifen	28 d, spiked water (static)	Emergence/development, NOEC: 0.472 initial measured conc.	EFSA Scientific Report (2008) 149, 1-80
Chironomus riparius	Aclonifen	28 d, spiked sediment (static)	Emergence/development, NOEC: 32 mg/kg nom	EFSA Scientific Report (2008) 149, 1-80
Algae				
Desmodesmus subspicatus	Aclonifen	96 h (static)	Biomass: E _b C ₅₀ : 0.0067 nom Growth rate: E _r C ₅₀ : 0.0069 nom	EFSA Scientific Report (2008) 149, 1-80
Desmodesmus subspicatus	Aclonifen	96 h (static) water / sediment system	Biomass: E_bC₅₀: not valid Growth rate: E_rC₅₀: not valid	EFSA Scientific Report (2008) 149, 1-80
Navicula pelliculosa	Aclonifen	72 h (static)	Biomass: E _b C ₅₀ : 0.47 mm Growth rate: E _r C ₅₀ : 1.2 mm	EFSA Scientific Report (2008) 149, 1-80
<i>Chlorella vulgaris</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.0866 mg a.i./L E _r C ₅₀ = 0.450 mg a.i./L	DE, Addendum 4 to DAR, 2011
<i>Chlamydomonas reinhardtii</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.0158 mg a.i./L E _r C ₅₀ = 0.0753 mg a.i./L	Addendum 4 to DAR, 2011
<i>Xanthonema debile</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.0987 mg a.i./L E _r C ₅₀ = 0.319 mg a.i./L	DE, Addendum 4 to DAR, 2011
<i>Closterium cornu</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.0682 mg a.i./L E _r C ₅₀ = 0.112 mg a.i./L	Addendum 4 to DAR, 2011
<i>Synechococcus leopoliensis</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.037 mg a.i./L E _r C ₅₀ = 0.0749 mg a.i./L	DE, Addendum 4 to DAR, 2011
<i>Nannochloropsis limnetica</i> *	Aclonifen	72h, s	E _b C ₅₀ = 0.461 mg a.i./L E _r C ₅₀ = 0.513 mg a.i./L	Addendum 4 to DAR, 2011

HC5*	Aclonifen	-	HC5= 5,3 µg a.s./L	DE, Addendum 4 to DAR, 2011
Higher plant				
Lemna gibba	Aclonifen	14 d (static)	Biomass: E _b C ₅₀ : 0.006 mm Growth rate fronds E _r C ₅₀ : 0.012 mm	EFSA Scientific Report (2008) 149, 1-80

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

* With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011), a HC₅ based on E_bC₅₀ values was recalculated by zRMS. The value of HC₅ = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment.

Table 9.5-24: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Clomazone – endpoints necessary for the tank mix risk assessment

Species	Substance	Exposure System	Results (Toxicity) (mg/L)	Reference
Fish				
Oncorhynchus mykiss	Clomazone	96 h (static)	LC ₅₀ = 15.5	EFSA Scientific Report (2007) 109, 1-73
Aquatic invertebrate				
Daphnia magna	Clomazone	48 hours (static)	EC ₅₀ (immobility) = 12.7	EFSA Scientific Report (2007) 109, 1-73
Mysidopsis bahia	Clomazone		EC ₅₀ = 0.57 mg a.s./L nom	EFSA Scientific Report (2007) 109, 1-73
Algae				
Navicula pelliculosa	Aclonifen	120 hours (static)	E _b C ₅₀ = 0.136 E _r C ₅₀ > 0.185 mg/L mm	EFSA Scientific Report (2007) 109, 1-73
Higher plant				
Lemna gibba	Clomazone	7 d (static)	EC ₅₀ , growth rate = 34	EFSA Scientific Report (2007) 109, 1-73

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – AKO 600 SC

Species	Substance	Exposure System	Results (Toxicity) (mg/L)	Reference
Daphnia magna	AKO 600 SC	48 h (static)	EC ₅₀ = 15.46mg test	Czarnecka M.

Species	Substance	Exposure System	Results (Toxicity) (mg/L)	Reference
			$\text{item/L}_{\text{nom}}$ $\text{EC}_{50} = 7.71 \text{ mg aclonifen/L}_{\text{nom}}$	Study code: W-19-23 Date: 01.2024
Pseudokirchneriella subcapitata	AKO 600 SC	72 h	$\text{E}_{\text{r}}\text{C}_{50} = 0.025 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{r}}\text{C}_{50} = 12.801 \text{ } \mu\text{g aclonifen/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} = 0.008 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} = 4.154 \text{ } \mu\text{g aclonifen/L}_{\text{nom}}$	Czarnecka M. Study code: W-20-23 Date: 01.2024
Lemna gibba	AKO 600 SC	7 d (semi-static)	$\text{E}_{\text{r}}\text{C}_{50} \text{ frond number} = 0.0135 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{r}}\text{C}_{50} \text{ frond number} = 0.006748 \text{ mg aclonifen/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} \text{ frond number} = 0.0036 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} \text{ frond number} = 0.001803 \text{ mg aclonifen/L}_{\text{nom}}$ $\text{E}_{\text{r}}\text{C}_{50} \text{ dry weight} = 0.01702 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{r}}\text{C}_{50} \text{ dry weight} = 0.008493 \text{ mg/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} \text{ dry weight} = 0.002942 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} \text{ dry weight} = 0.001468 \text{ mg aclonifen/L}_{\text{nom}}$	Czarnecka M. Study code: W-21-23 Date: 04.2024
Anabaena flos-aquae UTEX B 1444	AKO 600 SC	72h	$\text{E}_{\text{r}}\text{C}_{50} = 2.81 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{r}}\text{C}_{50} = 1.40 \text{ mg aclonifen/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} = 0.55 \text{ mg test item/L}_{\text{nom}}$ $\text{E}_{\text{y}}\text{C}_{50} = 0.27 \text{ mg aclonifen/L}_{\text{nom}}$	Czarnecka M. Study code: W-22-23 Date: 03.2024
Higher-tier studies (micro- or mesocosm studies)				
n/a				

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

9.5.1.1 Justification for new endpoints

No new endpoints for Aclonifen were established.

9.5.2 Risk assessment for aclonifen and AKO 600 SC to aquatic organisms

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 - 4 PEC_{sw} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Use in Potatoes (also the surrogate crop for: pumpkin for D3, D4, R1 and R3 scenarios)

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in potatoes (dose: 1.8 kg; 1.5 kg; 1.4 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged		
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmodesmus subspicatus</i>	<i>Anabea</i>	<i>Pseudokirchnerella</i>	<i>HC5</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>C. riparius</i>		
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level mm/nom	ErC50 – study code	NOEC – EU level	ErC50/ErC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50/ErC50 – EU level	ErC50 – study code		NOEC		
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000		
AF			100	10	100	100	10	10	10	10	5	10	10	10		10		
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200		
FOCUS Scenario		PEC ^{gl-} _{max} (µg/L)													PEC _{sed} _{max} (µg/kg)*			
			Step 1															
		76.56	11.4268657	153.12	8.042017	0.992996	47.85	114.2687/110.9565	0.54685714	59.80783	72.23	1.622034	127.6/63.8	113.4558	4050	1.27		
			Step 2															
N-Europe		16.55	2.47014925	33.1	1.738445	0.214656	10.34375	24.70149/23.9855	0.11821429	12.92868	15.61	0.350636	27.58333/13.79	24.52579	881.61	0.28		
			Step 3															
D3	ditch	9.372	1.39880597	18.744	0.984454	0.121556	5.8575	13.98806/13.5826	0.06694286	7.321303	8.84	0.198559	15.62/7.81	13.88856	6.973	0.002		
D4	pond	0.3777	0.05637313	0.7554	0.039674	0.004899	0.2360625	0.563731/0.5474	0.00269786	0.295055	0.36	0.008002	0.6295/0.315	0.559721	2.538	0.001		
D4	stream	7.735	1.15447761	15.47	0.8125	0.100324	4.834375	11.54478/11.210	0.05525	6.042497	7.30	0.163877	12.89167/6.446	11.46266	0.358	0.0001		
D6	ditch	9.268	1.38328358	18.536	0.973529	0.120208	5.7925	13.83284/13.1319	0.0662	7.240059	8.74	0.196356	15.44667/7.723	13.73444	3.23	0.001		

R1	pond	0.3928	0.05862687	0.7856	0.04126 1	0.00509 5	0.2455	0.586269 /0.5693	0.0028057 1	0.306851	0.37	0.008322	0.654667 /0.327	0.58209 8	7.472	0.002
R1	stream	6.475	0.96641791	12.95	0.68014 7	0.08398 2	4.046875	9.664179 /9.3841	0.04625	5.058199	6.11	0.137182	10.79167 /5.396	9.59543 6	36.22	0.01
R2	stream	8.575	1.27985075	17.15	0.90073 5	0.11121 9	5.359375	12.79851 /12.4275	0.06125	6.698695	8.09	0.181674	14.29167 /7.146	12.7074 7	240.7	0.08
R3	stream	9.144	1.36477612	18.288	0.96050 4	0.11859 9	5.715	13.64776 /13.2522	0.0653142 9	7.143192	8.63	0.193729	15.24 /7.62	13.5506 8	28.44	0.009
VFS Mode - STEP 4 - 1800 g																
Vegetative strip: 20 m, No spray buffer: 20 m, Nozzle reduction: 90 %																
D3	ditch	0.0843 2	0.01258507	0.16864	0.00885 7	0.00109 4	0.0527	0.125851 /0.122	0.0006022 9	0.06587	0.08	0.001786	0.140533 /0.070	0.12495 6	-	-
D4	pond	0.0203 1	0.00303134	0.04062	0.00213 3	0.00026 3	0.012693 8	0.030313 /0.029	0.0001450 7	0.015866	0.02	0.00043	0.03385 /0.017	0.03009 8	-	-
D4	stream	0.1427	0.02129851	0.2854	0.01498 9	0.00185 1	0.089187 5	0.212985 /0.207	0.0010192 9	0.111476	0.13	0.003023	0.237833 /0.012	0.21147	-	-
D6	ditch	0.1132	0.01689552	0.2264	0.01189 1	0.00146 8	0.07075	0.168955 /0.164	0.0008085 7	0.088431	0.11	0.002398	0.188667 /0.009	0.16775 3	-	-
R1	pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.010106 3	0.024134 /0.023	0.0001155	0.012632	0.02	0.000343	0.02695 /0.001	0.02396 3	-	-
R1	stream	0.0748 9	0.01117761	0.14978	0.00786 7	0.00097 1	0.046806 3	0.111776 /0.109	0.0005349 3	0.058503	0.07	0.001587	0.124817 /0.006	0.11098 1	-	-
R2	stream	0.0991 9	0.01480448	0.19838	0.01041 9	0.00128 7	0.061993 8	0.148045 /0.144	0.0007085	0.077486	0.09	0.002101	0.165317 /0.008	0.14699 2	-	-
R3	stream	0.1058	0.01579104	0.2116	0.01111 3	0.00137 2	0.066125	0.15791 /0.153	0.0007557 1	0.08265	0.10	0.002242	0.176333 /0.009	0.15678 7	-	-
STEP 4 - 1500 g																
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %						
D3	ditch	0.0703 1	0.01049403	0.14062	0.00738 6	0.00091 2	0.043943 8	0.10494 /0.010	0.0005022 1	0.054925	0.007	0.00149	0.117183 /0.06	0.10419 4	-	-
D4	pond	0.0163 1	0.00243433	0.03262	0.00171 3	0.00021 2	0.010193 8	0.024343 /0.0024	0.0001165	0.012741	0.01	0.000346	0.027183 /0.01	0.02417	-	-
D4	stream	0.1154	0.01722388	0.2308	0.01212 2	0.00149 7	0.072125	0.172239 /0.0167	0.0008242 9	0.090149	0.109	0.002445	0.192333 /0.10	0.17101 4	-	-
D6	ditch	0.0912 9	0.01362537	0.18258	0.00958 9	0.00118 4	0.057056 3	0.136254 /0.0132	0.0006520 7	0.071315	0.086	0.001934	0.15215 /0.08	0.13528 5	-	-
R1	pond	0.0438 1	0.00653881	0.08762	0.00460 2	0.00056 8	0.027381 3	0.065388 /0.0063	0.0003129 3	0.034224	0.041	0.000928	0.073017 /0.04	0.06492 3	-	-
R1	stream	0.4682	0.0698806	0.9364	0.04918 1	0.00607 3	0.292625	0.698806 /0.068	0.0033442 9	0.365753	0.442	0.009919	0.780333 /0.39	0.69383 5	-	-
R2	stream	0.1694	0.02528358	0.3388	0.01779 4	0.00219 7	0.105875	0.252836 /0.025	0.00121	0.132333	0.160	0.003589	0.282333 /0.14	0.25103 7	-	-
R3	stream	0.5365	0.08007463	1.073	0.05635 5	0.00695 8	0.335312 5	0.800746 /0.078	0.0038321 4	0.419108	0.506	0.011367	0.894167 /0.45	0.79505	-	-

		STEP 4 - 1400 g														
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %						
D3	ditch	0.0657 5	0.00981343	0.1315	0.00690 7	0.00085 3	0.041093 8	0.098134 /0.0095	0.0004696 4	0.051363	0.062	0.001393	0.109583 0.055	0.09743 6	-	-
D4	pond	0.0150 1	0.0022403	0.03002	0.00157 7	0.00019 5	0.009381 3	0.022403 /0.0022	0.0001072 1	0.011726	0.014	0.000318	0.025017 /0.013	0.02224 4	-	-
D4	stre- am	0.1064	0.0158806	0.2128	0.01117 6	0.00138	0.0665	0.158806 /0.0154	0.00076	0.083119	0.100	0.002254	0.177333 /0.089	0.15767 6	-	-
D6	ditch	0.0841 6	0.01256119	0.16832	0.00884	0.00109 2	0.0526	0.125612 /0.0122	0.0006011 4	0.065745	0.079	0.001783	0.140267 /0.07	0.12471 8	-	-
R1	pond	0.0407	0.00607463	0.0814	0.00427 5	0.00052 8	0.025437 5	0.060746 /0.0059	0.0002907 1	0.031794	0.038	0.000862	0.067833 /0.034	0.06031 4	-	-
R1	stre- am	0.4347	0.0648806	0.8694	0.04566 2	0.00563 8	0.271687 5	0.648806 /0.063	0.003105	0.339583	0.410	0.00921	0.7245 /0.36	0.64419 1	-	-
R2	stre- am	0.1555	0.02320896	0.311	0.01633 4	0.00201 7	0.097187 5	0.23209 /0.0225	0.0011107 1	0.121475	0.147	0.003294	0.259167 /0.13	0.23043 9	-	-
R3	stre- am	0.4979	0.07431343	0.9958	0.0523	0.00645 8	0.311187 5	0.743134 /0.0722	0.0035564 3	0.388954	0.470	0.010549	0.829833 /0.12415	0.73784 8	-	-

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

* PECsed values from Part B8

zRMS comments:

Daphnia magna:

In EFSA Scientific Report (2008) 149, 1-80 for *Daphnia magna* the EU agreed endpoints 48h EC₅₀ at 1.2 mg/L_{nom} and 0.952 mg/L_{mm} were provided.

According to DAR Aclonifen – Annex B.9: Ecotoxicology: “Results are based on nominal concentrations since measured values (except the highest 3.2 mg/L) ranged between 81 - 97 % of nominal values throughout the study. At 3.2 mg/L settlement of undissolved material may have been the reason for the lower measured value after 48 hours (74 % of nominal). The PEC/RAC calculations was performed with the worst-case value. The use of EC₅₀ = 1.2 mg/L will not affect the assessment results.

Algae:

With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011) a HC₅ based on E_bC₅₀ values was recalculated by RMS. The value of HC₅ = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment. PEC/RAC calculations with HC₅ was added by zRMS.

Algae and higher plants:

According to AGD (EFSA Journal 2013;11(7):3290) “Growth rate (r) is the preferred endpoint. Other, usually more sensitive, endpoints such as yield may also be used if growth rate endpoints are not provided.” Since growth rate endpoints are available they should be used in risk assessment. PEC/RAC calculations with these endpoints were added by zRMS for completeness.

Following the recommendations of the EFSA “Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting

publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic. The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance aclonifen.

Test species	Measured toxicity of formulation	Measured toxicity of a.s.	Formulation endpoint recalculated for a.s.	Ratio a.s. endpoint/recalculated formulation endpoint
Daphnia magna	15.46 mg/L	1.2 mg/L	7.71 mg/L	0.16
Algae	0.025 mg/L	6.9 µg/L	12.801 µg/L	0.54
Lemna	0.0135 mg/L	12 µg/L	6.7498 µg/L	1.78

The endpoint of the formulation, expressed in terms of aclonifen, is not more than three times lower for daphnia, algae and Lemna. Therefore, the formulation endpoints expressed in terms of aclonifen need not be used in the risk assessment for the active substance.

However, these endpoints were used in the risk assessment for the active substance in the table above by the Applicant and were assessed by zRMS.

For Step 3 calculations the PEC_{sw} values for application rate at 1.8 kg were used as the worst-case scenario by Applicant.

According to Part B8, due to the lack of two relevant scenarios for Central Zone D5 and R4 the surrogate crop (Legumes) was used. The PEC/RAC ratios for Legumes for application rate at 1.8 kg/ha and 0.8 kg/ha are presented in Table 9.5 8.

The application rates for potato are at 1.17 – 1.8 kg a.s./ha and 0.48 – 1.2 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower dose rates. However the risk assessment for the following applications rates was assessed by Applicant:

- Potatoes: 1.8 kg/ha, 1.5 kg/ha, 1.4 kg/ha
- Legumes as surrogate crop for scenarios D5 and R4: 1.8 kg/ha, 0.8 kg/ha

The following risk mitigation measures are required:

Application rate 1.8 kg a.s./ha (**VFS mode** – Step 4):

- D3 ditch, D4 pond, D4 stream, D6 ditch, R1 pond, R1 stream, R2 stream R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.5 kg a.s./ha (Step 4):

- D3 ditch, D4 pond, D4 stream, D6 ditch, R1 pond, R1 stream, R2 stream– 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.
- For R3 stream scenario the risk is unresolved.

Application rate 1.4 kg a.s./ha (Step 4):

- D3 ditch, D4 pond, D4 stream, D6 ditch, R1 pond, R1 stream, R2 stream R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop Legumes, **VFS mode** – Step 4):

- D5 pond, D5 stream - – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.
- For R4 stream scenario the risk is unacceptable.

Application rate 0.8 kg a.s./ha (surrogate crop Legume, Step 4):

- D5 pond, D5 stream, R4 stream - – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

The use of VFS mode should be considered on the MS level.

Surrogate crop:

According to Part B8 potatoes is the surrogate crop for:

- pumpkin for D3, D4, R1 and R3 scenarios.

Use in sunflower

Table 9.5-4-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in sunflower (dose: 1.8 kg; 1.5 kg; 0.9 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Desmodesmus subspicatus</i>	<i>Anabea</i>	<i>Pseudokirchnerella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>C. riparius</i>
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50 – study code	ErC50 – study code		NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748	32000
AF			100	10	100	100	10	10	10	10	5	10	10	10	10
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748	3200

FOCUS Scenario		PEC _{gl-max} (µg/L)													PEC _{sed} max (µg/kg) *		
		Step 1															
		76.56	11.4268657	153.12	8.04201 7	0.99299 6	47.85	114.2687/ 110.95 7	0.5468571 4	59.80783	72.23	1.622034	127.6 /63.8	113.455 8	4050	1.266	
		Step 2															
N-Europe		16.55	2.47014925	33.1	1.73844 5	0.21465 6	10.34375	24.70149 23.99	0.1182142 9	12.92868	15.61	0.350636	27.5833 3 /13.79	24.5257 9	881.61	0.276	
		Step 3															
D5	Pond	0.3779	0.05640299	0.7558	0.03969 5	0.00490 1	0.2361875	0.56403/ 0.55	0.0026992 9	0.295211	0.36	0.008006	0.62983 3 /0.3149	0.56001 8	2.600	0.0008	
D5	Stream	8.469	1.26402985	16.938	0.88960 1	0.10984 4	5.293125	12.6403/ 12.27	0.0604928 6	6.615889	7.99	0.179428	14.115 /7.0575	12.5503 9	0.4693	0.0001	
R1	Pond	0.3952	0.05898507	0.7904	0.04151 3	0.00512 6	0.247	0.589851/ 0.57	0.0028228 6	0.308726	0.37	0.008373	0.65866 7 /0.3293	0.58565 5	8.083	0.003	
R1	Stream	6.469	0.96552239	12.938	0.67951 7	0.08390 4	4.043125	9.655224/ 9.38	0.0462071 4	5.053511	6.10	0.137055	10.7816 7 /5.391	9.58654 4	45.71	0.014	
R3	Stream	9.138	1.3638806	18.276	0.95987 4	0.11852 1	5.71125	13.63881/ 13.24	0.0652714 3	7.138505	8.62	0.193602	15.23 /7.615	13.5417 9	33.48	0.01	
R4	Stream	6.487	0.96820896	12.974	0.68140 8	0.08413 7	4.054375	9.68209/ 9.40	0.0463357 1	5.067573	6.12	0.137436	10.8116 7 /5.406	9.61321 9	44.077	0.014	
		VFS Mode - STEP 4 - 1800 g															
Vegetative strip: 20 m				No spray buffer: 20 m							Nozzle reduction: 90 %						
D5	Pond	0.0162 9	0.00243134	0.03258	0.00171 1	0.00021 1	0.0101813	0.024313/ 0.236	0.0001163 6	0.012726	0.02	0.000345	0.02715 /0.0136	0.02414	-	-	
D5	Stream	0.0979 6	0.0146209	0.19592	0.01029	0.00127 1	0.061225	0.146209/ 0.142	0.0006997 1	0.076525	0.09	0.002075	0.16326 7 /0.082	0.14516 9	-	-	
R1	Pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.0101063	0.024134/ 0.023	0.0001155	0.012632	0.02	0.000343	0.02695 /0.0135	0.02396 3	-	-	
R1	Stream	0.3663	0.05467164	0.7326	0.03847 7	0.00475 1	0.2289375	0.546716/ 0.531	0.0026164 3	0.28615	0.35	0.007761	0.6105 /0.305	0.54282 8	-	-	
R3	Stream	0.1057	0.01577612	0.2114	0.01110 3	0.00137 1	0.0660625	0.157761/ 0.015	0.000755	0.082572	0.10	0.002239	0.17616 7 /0.088	0.15663 9	-	-	
R4	Stream	0.0766 9	0.01144627	0.15338	0.00805 6	0.00099 5	0.0479313	0.114463/ 0.111	0.0005477 9	0.059909	0.07	0.001625	0.12781 7 /0.064	0.11364 8	-	-	

		STEP 4 - 1500 g															
Vegetative strip: 20 m				No spray buffer: 20 m							Nozzle reduction: 90 %						
D5	Pond	0.0135 1	0.00201642	0.02702	0.00141 9	0.00017 5	0.0084438	0.020164/0.02	0.0000965	0.010554	0.013	0.000286	0.02251 7 /0.0113	0.02002 1	-	-	
D5	Stream	0.0815 7	0.01217463	0.16314	0.00856 8	0.00105 8	0.0509813	0.121746/0.118	0.0005826 4	0.063722	0.08	0.001728	0.13595 7 /0.068	0.12088	-	-	
R1	Pond	0.0470 2	0.00701791	0.09404	0.00493 9	0.00061	0.0293875	0.070179/0.068	0.0003358 6	0.036732	0.04	0.000996	0.07836 7 /0.039	0.06968	-	-	
R1	Stream	0.4783	0.07138806	0.9566	0.05024 2	0.00620 4	0.2989375	0.713881/0.693	0.0034164 3	0.373643	0.45	0.010133	0.79716 7 /0.399	0.70880 3	-	-	
R3	Stream	0.4648	0.06937313	0.9296	0.04882 4	0.00602 9	0.2905	0.693731/0.674	0.00332	0.363097	0.44	0.009847	0.77466 7 /0.387	0.68879 7	-	-	
R4	Stream	0.8253	0.1231791	1.6506	0.08669 1	0.01070 4	0.5158125	1.231791/1.196	0.005895	0.644715	0.79	0.017485	1.3755 7 /0.688	1.22302 9	-	-	
		STEP 4 - 900 g															
Vegetative strip: 20 m				No spray buffer: 20 m							Nozzle reduction: 90 %						
D5	Pond	0.0081 3	0.00121343	0.01626	0.00085 4	0.00010 5	0.0050813	0.012134/0.012	5.8071E-05	0.006351	0.01	0.000172	0.01355 7 /0.007	0.01204 8		-	
D5	Stream	0.0487 9	0.00728209	0.09758	0.00512 5	0.00063 3	0.0304938	0.072821/0.070	0.0003485	0.038114	0.05	0.001034	0.08131 7 /0.041	0.07230 3		-	
R1	Pond	0.0272 1	0.00406119	0.05442	0.00285 8	0.00035 3	0.0170063	0.040612/0.039	0.0001943 6	0.021256	0.03	0.000576	0.04535 7 /0.0227	0.04032 3		-	
R1	Stream	0.2758	0.04116418	0.5516	0.02897 1	0.00357 7	0.172375	0.411642/0.400	0.00197	0.215452	0.26	0.005843	0.45966 7 /0.230	0.40871 4		-	
R3	Stream	0.3247	0.04846269	0.6494	0.03410 7	0.00421 1	0.2029375	0.484627/0.471	0.0023192 9	0.253652	0.31	0.006879	0.54116 7 /0.271	0.48118		-	
R4	Stream	0.475	0.07089552	0.95	0.04989	0.00616 1	0.296875	0.708955/0.688	0.0033928 6	0.371065	0.45	0.010064	0.79166 7 /0.396	0.70391 2		-	

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

zRMS comments:

Daphnia magna:

In EFSA Scientific Report (2008) 149, 1-80 for *Daphnia magna* the EU agreed endpoints 48h EC₅₀ at 1.2 mg/L_{nom} and 0.952 mg/L_{mm} were provided.

According to DAR Aclonifen – Annex B.9: Ecotoxicology: “Results are based on nominal concentrations since measured values (except the highest 3.2 mg/L) ranged between 81 - 97 % of nominal values throughout the study. At 3.2 mg/L settlement of undissolved material may have been the reason for the lower meas-

ured value after 48 hours (74 % of nominal). The PEC/RAC calculations was performed with the worst-case value. The use of $EC_{50} = 1.2$ mg/L will not affect the assessment results.

Algae:

With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011) a HC_5 based on E_bC_{50} values was recalculated by RMS. The value of $HC_5 = 5.3$ µg/L with the assessment factor of 5 should be used in the risk assessment. PEC/RAC calculations with HC_5 was added by zRMS.

Algae and higher plants:

According to AGD (EFSA Journal 2013;11(7):3290) “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive, endpoints such as yield may also be used if growth rate endpoints are not provided.” Since growth rate endpoints are available they should be used in risk assessment. PEC/RAC calculations with these endpoints were added by zRMS for completeness.

Following the recommendations of the EFSA “Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic. The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance aclonifen.

Test species	Measured toxicity of formulation	Measured toxicity of a.s.	Formulation end-point recalculated for a.s.	Ratio a.s. end-point/recalculated formulation endpoint
Daphnia magna	15.46 mg/L	1.2 mg/L	7.71 mg/L	0.16
Algae	0.025 mg/L	6.9 µg/L	12.801 µg/L	0.54
Lemna	0.0135 mg/L	12 µg/L	6.7498 µg/L	1.78

The endpoint of the formulation expressed in terms of aclonifen is not more than three times lower for daphnia, algae and Lemna. Therefore, the formulation endpoints expressed in terms of aclonifen need not be used in the risk assessment for the active substance.

However, these endpoints were used in the risk assessment for the active substance in the table above by the Applicant and were assessed by zRMS.

For Step 3 calculations the PEC_{sw} values for application rate at 1.8 kg were used as the worst-case scenario by Applicant.

According to Part B8, due to the lack of two relevant scenarios for Central Zone D3 and D4 the surrogate crop (Maize) was used. The PEC/RAC ratios for application rate at 1.8 kg/ha, 1.5 kg/ha and 0.8 kg/ha in maize are presented in Table 9.5 5 below. Only D3 and D4 scenarios were assessed by zRMS.

The application rates for Sunflower are from 1.17 to 1.8 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower doses. However the risk assessment for the following application rates was assessed by Applicant:

- Sunflower: 1.8 kg/ha, 1.5 kg/ha, 0.9 kg/ha
- Maize as surrogate crop for scenarios D3 and D4: 1.8 kg/ha, 1.5 kg/ha, 0.8 kg/ha. In Part B8 only PEC_{sw} for maize as surrogate crop at application rate 1.8 was assessed by zRMS, therefore PEC/RAC ratios for this application was assessed below.

The following risk mitigation measures are required:

Application rate 1.8 kg a.s./ha (VFS mode – Step 4):

- D5 stream, R1 stream, R3 stream, R4 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.5 kg a.s./ha (Step 4):

- D5 stream, R1 stream, R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;
- For R4 stream scenario the risk is unresolved.

Application rate 0.9 kg a.s./ha (Step 4):

- D5 stream, R1 stream, R3 stream, R4 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (VFS mode – surrogate crop Maize, Step 4):

- D3 ditch, D4 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Use in maize as surrogate crop for: sunflower for D3 and D4 scenarios

Table 9.5-5 6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in maize (dose: 1.8 kg; 1.5 kg; 0.8 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Desmodesmus subspicatus</i>	<i>Anabea</i>	<i>Pseudokirchnerella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>C. riparius</i>
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50 – study code	ErC50 – study code	EbC50	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748	32000
AF			100	10	100	100	10	10	10	5	10	10	10		10

RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200	
FOCUS Scenario		PEC _{gl-max} (µg/L)													PEC _{sed} max (µg/kg) *		
	Step 1																
		76.56	11.4268657	153.12	8.04201 7	0.99299 6	47.85	114.2687	0.5468571 4	59.80783		1.622034	127.6	113.455 8			
	Step 2																
	N-Europe	16.55	2.47014925	33.1	1.73844 5	0.21465 6	10.34375	24.70149	0.1182142 9	12.92868		0.350636	27.5833 3	24.5257 9			
	Step 3																
D3	Ditch	0.0843 3 9.3740 *	0.01258657 1.399	0.16866 18.748	0.00885 8 0.984	0.00109 4 0.122	0.0527063 5.859	0.125866 13.99/13.586	0.0006023 6 0.0670	0.065878 7.323	8.84	0.001787 0.198	0.14055 15.623 /7.812	0.12497	7.0580	0.0022	
D4	Pond	0.0161 7 0.3777 *	0.00241343 0.056	0.03234 0.755	0.00169 9 0.040	0.00021 0.0049	0.0101063 0.236	0.024134 0.564/0.547	0.0001155 0.0027	0.012632 0.295	0.356	0.000343 0.008	0.02695 /0.315	0.02396 3 0.630	2.4350	0.0008	
D4	Stream	0.0928 4 8.0260 *	0.01385672 1.20	0.18568 16.052	0.00975 2 0.843	0.00120 4 0.104	0.058025 5.016	0.138567 11.979/11.632	0.0006631 4 0.057	0.072526 6.270	7.57	0.001967 0.170	0.15473 3 /6.688	0.13758 2 13.377	0.5521	0.0002	
D5	Pond	0.0162 6	0.00242687	0.03252	0.00170 8	0.00021 1	0.0101625	0.024269	0.0001161 4	0.012702		0.000344	0.0271	0.02409 6			
D5	Stream	0.0925 1	0.01380746	0.18502	0.00971 7	0.0012	0.0578188	0.138075	0.0006607 9	0.072268		0.00196	0.15418 3	0.13709 2			
D6	Ditch	0.0931 4	0.01390149	0.18628	0.00978 4	0.00120 8	0.0582125	0.139015	0.0006652 9	0.07276		0.001973	0.15523 3	0.13802 6			
R1	Pond	0.0540 3	0.00806418	0.10806	0.00567 5	0.00070 1	0.0337688	0.080642	0.0003859 3	0.042208		0.001145	0.09005	0.08006 8			
R1	Stream	0.5821	0.0868806	1.1642	0.06114 5	0.00755	0.3638125	0.868806	0.0041578 6	0.45473		0.012333	0.97016 7	0.86262 6			
R2	Stream	0.1726	0.02576119	0.3452	0.01813	0.00223 9	0.107875	0.257612	0.0012328 6	0.134833		0.003657	0.28766 7	0.25577 9			
R3	Stream	0.5273	0.07870149	1.0546	0.05538 9	0.00683 9	0.3295625	0.787015	0.0037664 3	0.411921		0.011172	0.87883 3	0.78141 7			
R4	Stream	1.105	0.16492537	2.21	0.11607 1	0.01433 2	0.690625	1.649254	0.0078928 6	0.863214		0.023411	1.84166 7	1.63752 2			
	VFS Mode - STEP 4 - 1800 g																
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %							
D3	Ditch	0.0843	0.01258657	0.16866	0.00885	0.00109	0.0527063	0.125866/0.12	0.0006023	0.065878	0.080	0.001787	0.14055	0.12497	-	-	

		3			8	4		2	6				/0.070			
D4	Pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.0101063	0.024134/0.02 3	0.0001155	0.012632	0.015	0.000343	0.02695 /0.013	0.02396 3	-	-
D4	Stream	0.0928 4	0.01385672	0.18568	0.00975	0.00120 4	0.058025	0.138567/0.13 5	0.0006631 4	0.072526	0.088	0.001967	0.15473 3 /0.093	0.13758 2	-	-
D5	Pond	0.0162 6	0.00242687	0.03252	0.00170 8	0.00021 4	0.0101625	0.024269	0.0001161 4	0.012702		0.000344	0.0271	0.02409 6		
D5	Stream	0.0925 1	0.01380746	0.18502	0.00971 7	0.0012	0.0578188	0.138075	0.0006607 9	0.072268		0.00196	0.15418 3	0.13709 2		
D6	Ditch	0.0931 4	0.01390149	0.18628	0.00978 4	0.00120 8	0.0582125	0.139015	0.0006652 9	0.07276		0.001973	0.15523 3	0.13802 6		
R1	Pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.0101063	0.024134	0.0001155	0.012632		0.000343	0.02695	0.02396 3		
R1	Stream	0.0748 9	0.01117761	0.14978	0.00786 7	0.00097 1	0.0468063	0.111776	0.0005349 3	0.058503		0.001587	0.12481 7	0.11098 1		
R2	Stream	0.0995 8	0.01486269	0.19916	0.01046	0.00129 2	0.0622375	0.148627	0.0007112 9	0.077791		0.00211	0.16596 7	0.14757		
R3	Stream	0.106	0.0158209	0.212	0.01113 4	0.00137 5	0.06625	0.158209	0.0007571 4	0.082806		0.002246	0.17666 7	0.15708 4		
R4	Stream	0.0821 3	0.01225821	0.16426	0.00862 7	0.00106 5	0.0513313	0.122582	0.0005866 4	0.064159		0.00174	0.13688 3	0.12171		
STEP 4 - 1500 g																
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %						
D3	Ditch	0.0703 2	0.01049552	0.14064	0.00738 7	0.00091 2	0.04395	0.104955	0.0005022 9	0.054933		0.00149	0.1172	0.10420 9		
D4	Pond	0.0134 1	0.00200149	0.02682	0.00140 9	0.00017 4	0.0083813	0.020015	9.5786E-05	0.010476		0.000284	0.02235	0.01987 3		
D4	Stream	0.0773	0.01153731	0.1546	0.00812	0.00100 3	0.0483125	0.115373	0.0005521 4	0.060386		0.001638	0.12883 3	0.11455 2		
D5	Pond	0.0134 8	0.00201194	0.02696	0.00141 6	0.00017 5	0.008425	0.020119	9.6286E-05	0.01053		0.000286	0.02246 7	0.01997 6		
D5	Stream	0.0770 3	0.01149701	0.15406	0.00809 1	0.00099 9	0.0481438	0.11497	0.0005502 1	0.060175		0.001632	0.12838 3	0.11415 2		
D6	Ditch	0.0750 4	0.0112	0.15008	0.00788 2	0.00097 3	0.0469	0.112	0.000536	0.05862		0.00159	0.12506 7	0.11120 3		
R1	Pond	0.0444 3	0.00663134	0.08886	0.00466 7	0.00057 6	0.0277688	0.066313	0.0003173 6	0.034708		0.000941	0.07405	0.06584 2		
R1	Stream	0.4783	0.07138806	0.9566	0.05024 2	0.00620 4	0.2989375	0.713881	0.0034164 3	0.373643		0.010133	0.79716 7	0.70880 3		
R2	Stream	0.1416	0.02113433	0.2832	0.01487 4	0.00183 7	0.0885	0.211343	0.0010114 3	0.110616		0.003	0.236	0.20984		
R3	Stream	0.4331	0.06464179	0.8662	0.04549 4	0.00561 7	0.2706875	0.646418	0.0030935 7	0.338333		0.009176	0.72183 3	0.64182		
R4	Stream	0.9075	0.13544776	1.815	0.09532 6	0.01177	0.5671875	1.354478	0.0064821 4	0.708929		0.019227	1.5125	1.34484 3		

STEP 4 - 800 g													
Vegetative strip: 20 m				No spray buffer: 20 m				Nozzle reduction: 90 %					
D3	Ditch	0.0374 2	0.00558507	0.07484	0.00393 1	0.00048 5	0.0233875	0.055851	0.0002672 9	0.029232	0.000793	0.06236 7	0.05545 3
D4	Pond	0.0071 9	0.00107313	0.01438	0.00075 5	9.33E-05	0.0044938	0.010731	5.1357E-05	0.005617	0.000152	0.01198 3	0.01065 5
D4	Stream	0.0411 7	0.00614478	0.08234	0.00432 5	0.00053 4	0.0257313	0.061448	0.0002940 7	0.032162	0.000872	0.06861 7	0.06101 1
D5	Pond	0.0072 3	0.0010791	0.01446	0.00075 9	9.38E-05	0.0045188	0.010791	5.1643E-05	0.005648	0.000153	0.01205	0.01071 4
D5	Stream	0.0410 2	0.00612239	0.08204	0.00430 9	0.00053 2	0.0256375	0.061224	0.000293	0.032044	0.000869	0.06836 7	0.06078 8
D6	Ditch	0.0374 3	0.00558657	0.07486	0.00393 2	0.00048 5	0.0233938	0.055866	0.0002673 6	0.02924	0.000793	0.06238 3	0.05546 8
R1	Pond	0.0226 9	0.00338657	0.04538	0.00238 3	0.00029 4	0.0141813	0.033866	0.0001620 7	0.017725	0.000481	0.03781 7	0.03362 5
R1	Stream	0.2431	0.03628358	0.4862	0.02553 6	0.00315 3	0.1519375	0.362836	0.0017364 3	0.189907	0.00515	0.40516 7	0.36025 5
R2	Stream	0.0716	0.01068657	0.1432	0.00752 1	0.00092 9	0.04475	0.106866	0.0005114 3	0.055933	0.001517	0.11933 3	0.10610 6
R3	Stream	0.2197	0.03279104	0.4394	0.02307 8	0.00285	0.1373125	0.32791	0.0015692 9	0.171627	0.004655	0.36616 7	0.32557 8
R4	Stream	0.4599	0.06864179	0.9198	0.04830 9	0.00596 5	0.2874375	0.686418	0.003285	0.359269	0.009744	0.7665	0.68153 5

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

zRMS comments:

According to Part B8 maize is the surrogate crop for:
- sunflower for D3 and D4 scenarios.

Use in spring Oilseed

Table 9.5-67: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in spring oilseeds (dose: 1.8 kg; 1.7 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant		Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabaena</i>	<i>Pseudokirchnerella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		<i>Chironomus riparius</i>

Endpo- int			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/EyC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EbC5 0	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC		
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000		
AF			100	10	100	100	10	10	10	10	5	10	10	10		10		
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200		
FOCUS Scenario		PEC _{gl- max} (µg/L)													PEC _{sed} max (µg/kg) *			
		Step 1																
		76.56	11.4268657	153.12	8.04201 7	0.99299 6	47.85	114.2687/110.9	0.5468571 4	59.80783	72.23	1.622034	127.6 /63.8	113.455 8	4050	1.266		
		Step 2																
N-Europe		16.55	2.47014925	33.1	1.73844 5	0.21465 6	10.34375	24.70149/23.99	0.1182142 9	12.92868	15.61	0.350636	27.5833 3 /13.79	24.5257 9	881.61	0.276		
		Step 3																
D1	Ditch	11.38	1.69850746	22.76	1.19537 8	0.14760 1	7.1125	16.98507/16.50	0.0812857 1	8.88993	10.74	0.241102	18.9666 7 /9.483	16.8642 6	16.84	0.004		
D1	Strea m	9.11	1.35970149	18.22	0.95693 3	0.11815 8	5.69375	13.59701/13.20	0.0650714 3	7.116632	8.59	0.193008	15.1833 3 /7.591	13.5003	0.5167	0.0002		
D3	Ditch	11.32	1.68955224	22.64	1.18907 6	0.14682 2	7.075	16.89552/16.41	0.0808571 4	8.843059	10.68	0.239831	18.8666 7 /9.433	16.7753 4	7.756	0.0024		
D4	Pond	0.3899	0.05819403	0.7798	0.04095 6	0.00505 7	0.2436875	0.58194/0.565	0.002785	0.304586	0.368	0.008261	0.64983 3 /0.325	0.57780 1	2.860	0.8938		
D4	Strea m	8.693	1.29746269	17.386	0.91313	0.11275	5.433125	12.97463/12.60	0.0620928 6	6.790876	8.20	0.184174	14.4883 3 /7.244	12.8823 4	0.3282	0.0001		
D5	Pond	0.3901	0.05822388	0.7802	0.04097 7	0.00506	0.2438125	0.582239/0.564	0.0027864 3	0.304742	0.37	0.008265	0.65016 7 /0.325	0.57809 7	2.864	0.0009		
D5	Strea m	8.974	1.33940299	17.948	0.94264 7	0.11639 4	5.60875	13.39403/13.01	0.0641	7.01039	8.47	0.190127	14.9566 7 /7.478	13.2987 6	0.2495	0.0001		
R1	Pond	0.3996	0.05964179	0.7992	0.04197 5	0.00518 3	0.24975	0.596418/0.579	0.0028542 9	0.312163	0.38	0.008466	0.666 /0.333	0.59217 5	6.586	0.0021		
R1	Strea m	7.457	1.11298507	14.914	0.78329 8	0.09671 9	4.660625	11.12985/10.80 7	0.0532642 9	5.825326	7.03	0.157987	12.4283 3	11.0506 8	33.87	0.0106		

[illegible]

													0.071			
R1	Pond	0.0422 9	0.00631194	0.08458	0.00444 2	0.00054 9	0.0264313	0.063119/0.06	0.0003020 7	0.033036	0.040	0.000896	0.07048 3 0.035	0.06267	-	-
R1	Stream	0.4838	0.07220896	0.9676	0.05081 9	0.00627 5	0.302375	0.72209/0.701	0.0034557 1	0.377939	0.456	0.01025	0.80633 3 0.403	0.71695 3	-	-

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

zRMS comments:

Daphnia magna:

In EFSA Scientific Report (2008) 149, 1-80 for *Daphnia magna* the EU agreed endpoints 48h EC₅₀ at 1.2 mg/L_{nom} and 0.952 mg/L_{mm} were provided. According to DAR Aclonifen – Annex B.9: Ecotoxicology: “Results are based on nominal concentrations since measured values (except the highest 3.2 mg/L) ranged between 81 - 97 % of nominal values throughout the study. At 3.2 mg/L settlement of undissolved material may have been the reason for the lower measured value after 48 hours (74 % of nominal). The PEC/RAC calculations was performed with the worst-case value. The use of EC₅₀ = 1.2 mg/L will not affect the assessment results.

Algae:

With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011) a HC₅ based on E_bC₅₀ values was recalculated by RMS. The value of HC₅ = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment. PEC/RAC calculations with HC₅ was added by zRMS.

Algae and higher plants:

According to AGD (EFSA Journal 2013;11(7):3290) “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive, endpoints such as yield may also be used if growth rate endpoints are not provided.” Since growth rate endpoints are available they should be used in risk assessment. PEC/RAC calculations with these endpoints were added by zRMS for completeness.

Following the recommendations of the EFSA “Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic. The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance aclonifen.

Test species	Measured toxicity of formulation	Measured toxicity of a.s.	Formulation endpoint recalculated for a.s.	Ratio a.s. endpoint/recalculated formulation endpoint
Daphnia magna	15.46 mg/L	1.2 mg/L	7.71 mg/L	0.16
Algae	0.025 mg/L	6.9 µg/L	12.801 µg/L	0.54
Lemna	0.0135 mg/L	12 µg/L	6.7498 µg/L	1.78

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The endpoint of the formulation expressed in terms of aclonifen is not more than three times lower for daphnia, algae and Lemna. Therefore, the formulation endpoints expressed in terms of aclonifen need not be used in the risk assessment for the active substance. However, these endpoints were used in the risk assessment for the active substance in the table above by the Applicant and were assessed by zRMS.

For Step 3 calculations the PEC_{sw} values for application rate at 1.8 kg were used by Applicant as the worst-case scenario.

According to Part B8, due to the lack of two relevant scenarios for Central Zone R3 and R4 the surrogate crops (winter OSR for R3 scenario and Legumes for R4 scenario) were used.

The PEC/RAC ratio for R3 stream scenario for application rates at 1.8 kg/ha and 1.3 kg/ha in winter OSR is presented in Table 9.5-7 below.

The PEC/RAC ratio for R4 stream scenario for application rates at 1.8 kg/ha and 0.8 kg/ha in legumes is presented in Table 9.5-8 below.

The application rates for spring oilseed rape are from 1.17 to 1.8 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower doses. However the risk assessment for the following application rates was assessed by Applicant:

- Spring oilseed rape: 1.8 kg/ha and 1.7 kg/ha;
- Winter oilseed rape as surrogate crop for scenarios R3 stream: 1.8 kg/ha and 1.3 kg/ha.
- Legumes as surrogate crop for scenario R4 stream: 1.8 kg/ha and 0.8 kg/ha.

The following risk mitigation measures are required:

Application rate 1.8 kg a.s./ha (VFS mode – Step 4):

- D1 ditch, D1 stream, D3 ditch, D4 stream, D5 stream, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.7 kg a.s./ha (Step 4):

- D1 ditch, D1 stream, D3 ditch, D4 stream, D5 pond, D5 stream, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop winter OSR, Step 4):

- R3 stream – risk is unresolved.

Application rate 1.3 kg a.s./ha (surrogate crop winter OSR, Step 4):

- R3 stream - 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop legumes, Step 4):

- R4 stream – risk is unresolved.

Application rate 0.8 kg a.s./ha (surrogate crop legumes, Step 4):

- R4 stream - 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Spring oilseed rape is also surrogate crop for flax, breadssd poppy, mustard and hemp.

Use in winter oilseed as the surrogate crop for:

- spring oilseed rape for R3 scenario.

- flax, breadssd poppy, mustard and hemp.

Table 9.5-7 8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in winter oilseeds (dose: 1.8 kg; 1.3 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged	
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabea</i>	<i>Pseudokirchenella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>Chironomus riparius</i>	
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/EyC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC	
(µg/L)			670	5	952	7710	16	6.76.9	1400	12.801	5.3	472	6/12	6.748		32000	
AF			100	10	100	100	10	10	10	10	5	10	10	10		10	
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.670.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200	
FOCUS Scenario		PEC _{gl-max} (µg/L)														PEC _{sed max} (µg/kg) *	
Step 1																	
		76.56	11.4268657	153.12	8.042017	0.992996	47.85	114.2687	0.54685714	59.80783		1.622034	127.6	113.4558			
Step 2																	
N-Europe		16.55	2.47014925	33.1	1.738445	0.214656	10.34375	24.70149	0.11821429	12.92868		0.350636	27.58333	24.52579			

		Step 3															
D2	Ditch	11.47	1.7119403	22.94	1.204832	0.148768	7.16875	17.1194	0.08192857	8.960237		0.243008	19.11667	16.99763			
D2	Stream	10.2	1.52238806	20.4	1.071429	0.132296	6.375	15.22388	0.07285714	7.968127		0.216102	17	15.11559			
D3	Ditch	11.37	1.69701493	22.74	1.194328	0.147471	7.10625	16.97015	0.08121429	8.882119		0.24089	18.95	16.84944			
D4	Pond	0.3901	0.05822388	0.7802	0.040977	0.005061	0.2438125	0.582239	0.00278643	0.304742		0.008265	0.650167	0.578097			
D4	Stream	9.794	1.46179104	19.588	1.028782	0.12703	6.12125	14.61791	0.06995714	7.650965		0.2075	16.32333	14.51393			
D5	Pond	0.3902	0.05823881	0.7804	0.040987	0.005061	0.243875	0.582388	0.00278714	0.30482		0.008267	0.650333	0.578245			
D5	Stream	10.57	1.57761194	21.14	1.110294	0.137095	6.60625	15.77612	0.0755	8.257167		0.223941	17.61667	15.6639			
R1	Pond	0.4087	0.061	0.8174	0.042931	0.005301	0.2554375	0.61	0.00291929	0.319272		0.008659	0.681167	0.605661			
R1	Stream	7.487	1.11746269	14.974	0.786458	0.097108	4.679375	11.17463	0.05347857	5.848762		0.158623	12.47833	11.09514			
R3	Stream	10.47	1.56268657	20.94	1.09979	0.135798	6.54375	15.62687/15.17	0.07478571	8.179049	9.88	0.221822	17.45/8.725	15.51571	55.10	0.02	
		VFS Mode - STEP 4 - 1800 g															
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %							
D2	Ditch	0.08573	0.01279552	0.17146	0.009005	0.001112	0.0535813	0.127955	0.00061236	0.066971		0.001816	0.142883	0.127045			
D2	Stream	0.1025	0.01529851	0.205	0.010767	0.001329	0.0640625	0.152985	0.00073214	0.080072		0.002172	0.170833	0.151897			
D3	Ditch	0.08462	0.01262985	0.16924	0.008889	0.001098	0.0528875	0.126299	0.00060443	0.066104		0.001793	0.141033	0.1254			
D4	Pond	0.01751	0.00261343	0.03502	0.001839	0.000227	0.0109438	0.026134	0.00012507	0.013679		0.000371	0.029183	0.025948			
D4	Stream	0.1606	0.02397015	0.3212	0.01687	0.002083	0.100375	0.239701	0.00114714	0.125459		0.003403	0.267667	0.237996			
D5	Pond	0.01622	0.0024209	0.03244	0.001704	0.00021	0.0101375	0.024209	0.00011586	0.012671		0.000344	0.027033	0.024037			
D5	Stream	0.106	0.0158209	0.212	0.011134	0.001375	0.06625	0.158209	0.00075714	0.082806		0.002246	0.176667	0.157084			
R1	Pond	0.0687	0.01025373	0.1374	0.007216	0.000891	0.0429375	0.102537	0.00049071	0.053668		0.001456	0.1145	0.101808			
R1	Stream	0.4672	0.06973134	0.9344	0.049076	0.00606	0.292	0.697313	0.00333714	0.364971		0.009898	0.778667	0.692353			
R3	Stream	0.6532	0.09749254	1.3064	0.068613	0.008472	0.40825	0.974925/0.95	0.00466571	0.510273	0.62	0.013839	1.088667/0.544	0.967991	-	-	
		STEP 4 - 1300 g															

Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %						
D2	Ditch	0.0618 1	0.00922537	0.12362	0.00649 3	0.00080 2	0.0386313	0.092254	0.0004415	0.048285		0.00131	0.10301 7	0.09159 8		
D2	Stream	0.0741 4	0.01106567	0.14828	0.00778 8	0.00096 2	0.0463375	0.110657	0.0005295 7	0.057917		0.001571	0.12356 7	0.10987		
D3	Ditch	0.0610 8	0.00911642	0.12216	0.00641 6	0.00079 2	0.038175	0.091164	0.0004362 9	0.047715		0.001294	0.1018	0.09051 6		
D4	Pond	0.0119	0.00177612	0.0238	0.00125	0.00015 4	0.0074375	0.017761	0.000085	0.009296		0.000252	0.01983 3	0.01763 5		
D4	Stream	0.1096	0.01635821	0.2192	0.01151 3	0.00142 2	0.0685	0.163582	0.0007828 6	0.085618		0.002322	0.18266 7	0.16241 8		
D5	Pond	0.0116 6	0.0017403	0.02332	0.00122 5	0.00015 1	0.0072875	0.017403	8.3286E-05	0.009109		0.000247	0.01943 3	0.01727 9		
D5	Stream	0.0767	0.01144776	0.1534	0.00805 7	0.00099 5	0.0479375	0.114478	0.0005478 6	0.059917		0.001625	0.12783 3	0.11366 3		
R1	Pond	0.0485 9	0.00725224	0.09718	0.00510 4	0.00063	0.0303688	0.072522	0.0003470 7	0.037958		0.001029	0.08098 3	0.07200 7		
R1	Stream	0.3294	0.04916418	0.6588	0.03460 1	0.00427 2	0.205875	0.491642	0.0023528 6	0.257324		0.006979	0.549	0.48814 5		
R3	Stream	0.459	0.06850746	0.918	0.04821	0.00595 3	0.286875	0.685075/0.67	0.0032785 7	0.358566	0.43	0.009725	0.765 /0.383	0.68020 2		

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

zRMS comments:

According to Part B8 winter OSR is the surrogate crop for:

- spring oilseed rape for R3 scenario.
- flax, breadssd poppy, mustard and hemp.

Use in legumes as the surrogate crop for:

- potatoes for D5 and R4 scenarios,
- spring oilseed rape for R4 scenario,
- soybeans for D5 scenario,
- pumpkin for D5 and R4 scenarios,
- flax, breadssd poppy, mustard and hemp,
- pumpkin.

Table 9.5-8 9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in legumes (dose: 1.8 kg; 0.8 kg)

Group			Fish acute	Fish prolon- ged	Inverteb. acute		Inverteb. prolon- ged	Algae			Al- gae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolon- ged	
Test species			<i>Oncorhyn- chus mykiss</i>	<i>Oncorhyn- chus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmode- smus subpica- tus</i>	<i>Anabea</i>	<i>Pseudokirchenel- la</i>	HC5	<i>Chirono- mus ripa- rius</i>	<i>Lemna gibba</i>			<i>Chirono- mus ripa- rius</i>	
Endpo- int			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/ErC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50/ErC50 – EU level	ErC50 – study code		NOEC	
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000	
AF			100	10	100	100	10	10	10	10	5	10	10	10		10	
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200	
FOCUS Scena- rio		PEC ^{gl-max} (µg/L)													PEC ^{sed} max (µg/kg) *		
	Step 1																
		76.56	11.4268657	153.12	8.042017	0.992996	47.85	114.26874	0.54685714	59.80783		1.622034	127.6	113.4558			
	Step 2																
N-Europe		2.47014925	33.1	1.738445	0.214656	10.34375	24.70149	0.11821429	12.92868	0.350636		27.58333	24.52579	2.47014925			
	Step 3																
D3	Ditch	9.357	1.39656716	18.714	0.982878	0.121362	5.848125	13.96567	0.06683571	7.309585		0.198242	15.595	13.86633			
D4	Pond	0.3776	0.05635821	0.7552	0.039664	0.004898	0.236	0.563582	0.00269714	0.294977		0.008	0.629333	0.559573			
D4	Stream	7.497	1.11895522	14.994	0.7875	0.097237	4.685625	11.18955	0.05355	5.856574		0.158835	12.495	11.10996			
D5	Pond	0.3778	0.05638806	0.7556	0.039685	0.0049	0.236125	0.563881/0.548	0.00269857	0.295133	0.356	0.008004	0.629667/0.315	0.55987	2.776	0.0009	
D5	Stream	7.786	1.16208955	15.572	0.817857	0.100986	4.86625	11.6209/11.284	0.05561429	6.082337	7.345	0.164958	12.97667/6.488	11.53823	0.2165	0.0001	
D6	Ditch	9.376	1.39940299	18.752	0.984874	0.121608	5.86	13.99403	0.06697143	7.324428		0.198644	15.62667	13.89449			
R1	Pond	0.39	0.05820896	0.78	0.040966	0.005058	0.24375	0.58209	0.00278571	0.304664		0.008263	0.65	0.577949			

R1	Stream	6.476	0.96656716	12.952	0.68025 2	0.08399 5	4.0475	9.665672	0.0462571 4	5.05898		0.137203	10.79333	9.596918		
R2	Stream	8.61	1.28507463	17.22	0.90441 2	0.11167 3	5.38125	12.85075	0.0615	6.726037		0.182415	14.35	12.75934		
R3	Stream	9.162	1.36746269	18.324	0.96239 5	0.11883 3	5.72625	13.67463	0.0654428 6	7.157253		0.19411	15.27	13.57736		
R4	Stream	6.469	0.96552239	12.938	0.67951 7	0.08390 4	4.043125	9.655224/9.37 5	0.0462071 4	5.053511	6.103	0.137055	10.78167 /5.391	9.586544	172.3	0.054
VFS mode STEP 4 - 1800 g																
Vegetative strip: 20 m				No spray buffer: 20 m					Nozzle reduction: 90 %							
D3	Ditch	0.08419	0.01256567	0.16838	0.00884 3	0.00109 2	0.052618 8	0.125657	0.0006013 6	0.065768		0.001784	0.140317	0.124763		
D4	Pond	0.01617	0.00241343	0.03234	0.00169 9	0.00021	0.010106 3	0.024134	0.0001155	0.012632		0.000343	0.02695	0.023963		
D4	Stream	0.1107	0.01652239	0.2214	0.01162 8	0.00143 6	0.069187 5	0.165224	0.0007907 1	0.086478		0.002345	0.1845	0.164049		
D5	Pond	0.01636	0.00244179	0.03272	0.00171 8	0.00021 2	0.010225	0.024418/0.02 4	0.0001168 6	0.01278	0.015	0.000347	0.027267 /0.014	0.024244	-	-
D5	Stream	0.09006	0.01344179	0.18012	0.00946	0.00116 8	0.056287 5	0.134418/0.13 1	0.0006432 9	0.070354	0.085	0.001908	0.1501 /0.075	0.133462	-	-
D6	Ditch	0.08436	0.01259104	0.16872	0.00886 1	0.00109 4	0.052725	0.12591	0.0006025 7	0.065901		0.001787	0.1406	0.125015		
R1	Pond	0.03553	0.00530299	0.07106	0.00373 2	0.00046 1	0.022206 3	0.05303	0.0002537 9	0.027756		0.000753	0.059217	0.052653		
R1	Stream	0.498	0.07432836	0.996	0.05231 1	0.00645 9	0.31125	0.743284	0.0035571 4	0.389032		0.010551	0.83	0.737996		
R2	Stream	0.1391	0.02076119	0.2782	0.01461 1	0.00180 4	0.086937 5	0.207612	0.0009935 7	0.108663		0.002947	0.231833	0.206135		
R3	Stream	0.6872	0.10256716	1.3744	0.07218 5	0.00891 3	0.4295	1.025672	0.0049085 7	0.536833		0.014559	1.145333	1.018376		
R4	Stream	1.159	0.17298507	2.318	0.12174 4	0.01503 2	0.724375	1.729851/1.68 0	0.0082785 7	0.905398	1.09	0.024555	1.931667 /0.966	1.717546	-	-
STEP 4 - 800 g																
Vegetative strip: 20 m				No spray buffer: 20 m					Nozzle reduction: 90 %							
D3	Ditch	0.03735	0.00557463	0.0747	0.00392 3	0.00048 4	0.023343 8	0.055746	0.0002667 9	0.029177		0.000791	0.06225	0.05535		
D4	Pond	0.00719	0.00107313	0.01438	0.00075 5	9.33E- 05	0.004493 8	0.010731	5.1357E- 05	0.005617		0.000152	0.011983	0.010655		
D4	Stream	0.04309	0.00643134	0.08618	0.00452 6	0.00055 9	0.026931 3	0.064313	0.0003077 9	0.033661		0.000913	0.071817	0.063856		
D5	Pond	0.00726	0.00108358	0.01452	0.00076 3	9.42E- 05	0.004537 5	0.010836/0.01 1	5.1857E- 05	0.005671	0.002	0.000154	0.0121 /0.006	0.010759	-	-
D5	Stream	0.03993	0.0059597	0.07986	0.00419 4	0.00051 8	0.024956 3	0.059597/0.57 9	0.0002852 1	0.031193	0.04	0.000846	0.06655 /0.033	0.059173	-	-

D6	Ditch	0.03743	0.00558657	0.07486	0.00393 2	0.00048 5	0.023393 8	0.055866	0.0002673 6	0.02924		0.000793	0.062383	0.055468		
R1	Pond	0.01508	0.00225075	0.03016	0.00158 4	0.00019 6	0.009425	0.022507	0.0001077 1	0.01178		0.000319	0.025133	0.022347		
R1	Stream	0.2077	0.031	0.4154	0.02181 7	0.00269 4	0.129812 5	0.31	0.0014835 7	0.162253		0.0044	0.346167	0.307795		
R2	Stream	0.05781	0.00862836	0.11562	0.00607 2	0.00075	0.036131 3	0.086284	0.0004129 3	0.045161		0.001225	0.09635	0.08567		
R3	Stream	0.285	0.04253731	0.57	0.02993 7	0.00369 6	0.178125	0.425373	0.0020357 1	0.222639		0.006038	0.475	0.422347		
R4	Stream	0.4819	0.07192537	0.9638	0.05062	0.00625	0.301187 5	0.719254/0.69 8	0.0034421 4	0.376455	0.45	0.01021	0.803167 /0.402	0.714138	-	-

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

zRMS comments:

According to Part B8 Legumes are the surrogate crop for:

- potatoes for D5 and R4 scenarios,
- spring oilseed rape for R4 scenario,
- soybeans for D5 scenario,
- pumpkin for D5 and R4 scenarios,
- flax, breadseed poppy, mustard and hemp,
- pumpkin.

Use in Flax, Breadseed poppy, Mustard and Hemp

According to Part B8 spring oilseed rape, winter oilseed rape and legumes are the surrogate crops for flax, breadseed poppy, mustard and hemp due to lack of scenarios.

The application rates for flax, breadseed poppy, mustard and hemp are from 1.17 to 1.8 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower doses.

However the risk assessment for the following application rates was assessed by Applicant:

- Spring oilseed rape: 1.8 kg/ha and 1.7 kg/ha;
- Winter oilseed rape as surrogate crop for scenarios R3 stream: 1.8 kg/ha and 1.3 kg/ha.
- Legumes as surrogate crop for scenario R4 stream: 1.8 kg/ha and 0.8 kg/ha.

Therefore the following risk mitigation measures should be considered:

Application rate 1.8 kg a.s./ha (VFS mode – Step 4):

- D1 ditch, D1 stream, D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.7 kg a.s./ha (Step 4):

- D1 ditch, D1 stream, D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop winter OSR, Step 4):

- R3 stream – risk is unresolved.

Application rate 1.3 kg a.s./ha (surrogate crop winter OSR, Step 4):

- R3 stream - 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop legumes, Step 4):

- R4 stream – risk is unresolved.

Application rate 0.8 kg a.s./ha (surrogate crop legumes, Step 4):

- R4 stream - 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Use in Soybeans

Table 9.5-9 10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in soybeans (dose: 1.8 kg; 1.2 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute		Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabea</i>	<i>Pseudokirchenella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>Chironomus riparius</i>
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/ErC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC

(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000		
AF			100	10	100	100	10	10	10	10	5	10	10	10		10		
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200		
FOCUS Scenario		PEC gl-max (µg/L)														PECsed max (µg/kg)*		
		Step 1																
		76.56	11.4268657	153.12	8.042017	0.992996	47.85	114.2687/111	0.54685714	59.80783	72.23	1.622034	127.6 /63.8	113.4558	4050	1.27		
		Step 2																
N-Europe		16.55	2.47014925	33.1	1.738445	0.214656	10.34375	24.70149/24	0.11821429	12.92868	15.61	0.350636	27.58333 /13.79	24.52579	881.61	0.28		
		Step 3																
R3	Stream	9.112	1.36	18.224	0.957143	0.118184	5.695	13.61/13.2	0.06508571	7.118194	8.60	0.193051	15.18667 /7.59	13.50326	33.52	0.010		
R4	Stream	6.464	0.96477612	12.928	0.678992	0.083839	4.04	9.647761/9.37	0.04617143	5.049605	6.10	0.136949	10.77333 /5.39	9.579135	26.08	0.002		
		STEP 4 - 1800 g																
Vegetative strip: 20 m				No spray buffer: 20 m							Nozzle reduction: 90 %							
R3	Stream	0.6245	0.09320896	1.249	0.065599	0.0081	0.3903125	0.93209/0.91	0.00446071	0.487853	0.59	0.013231	1.040833 /0.520	0.925459	-	-		
R4	Stream	0.723	0.10791045	1.446	0.075945	0.009377	0.451875	1.079104/1.05	0.00516429	0.5648	0.68	0.015318	1.205 /0.603	1.071429	-	-		
		STEP 4 - 1200 g																
Vegetative strip: 20 m				No spray buffer: 20 m							Nozzle reduction: 50 %							
R3	Stream	0.4022	0.06002985	0.8044	0.042248	0.005217	0.251375	0.600299/0.58	0.00287286	0.314194	0.38	0.008521	0.670333 /0.335	0.596028	-	-		
R4	Stream	0.4684	0.06991045	0.9368	0.049202	0.006075	0.29275	0.699104/0.68	0.00334571	0.365909	0.44	0.009924	0.780667 /0.390	0.694132	-	-		

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

zRMS comments:

Daphnia magna:

In EFSA Scientific Report (2008) 149, 1-80 for *Daphnia magna* the EU agreed endpoints 48h EC₅₀ at 1.2 mg/L_{nom} and 0.952 mg/L_{mm} were provided. According to DAR Aclonifen – Annex B.9: Ecotoxicology: “Results are based on nominal concentrations since measured values (except the highest 3.2 mg/L) ranged between 81 - 97 % of nominal values throughout the study. At 3.2 mg/L settlement of undissolved material may have been the reason for the lower measured value after 48 hours (74 % of nominal). The PEC/RAC calculations was performed with the worst-case value. The use of EC₅₀ = 1.2 mg/L will not affect the

assessment results.

Algae:

With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011) a HC₅ based on E_bC₅₀ values was recalculated by RMS. The value of HC₅ = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment. PEC/RAC calculations with HC₅ was added by zRMS.

Algae and higher plants:

According to AGD (EFSA Journal 2013;11(7):3290) “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive, endpoints such as yield may also be used if growth rate endpoints are not provided.” Since growth rate endpoints are available they should be used in risk assessment. PEC/RAC calculations with these endpoints were added by zRMS for completeness.

Following the recommendations of the EFSA “Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic. The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance aclonifen.

Test species	Measured toxicity of formulation	Measured toxicity of a.s.	Formulation endpoint recalculated for a.s.	Ratio a.s. endpoint/recalculated formulation endpoint
Daphnia magna	15.46 mg/L	1.2 mg/L	7.71 mg/L	0.16
Algae	0.025 mg/L	6.9 µg/L	12.801 µg/L	0.54
Lemna	0.0135 mg/L	12 µg/L	6.7498 µg/L	1.78

The endpoint of the formulation expressed in terms of aclonifen is not more than three times lower for daphnia, algae and Lemna. Therefore, the formulation endpoints expressed in terms of aclonifen need not be used in the risk assessment for the active substance.

However, these endpoints were used in the risk assessment for the active substance in the table above by the Applicant and were assessed by zRMS.

For Step 3 calculations the PEC_{sw} values for application rate at 1.8 kg were used by Applicant as the worst-case scenario.

According to Part B8, due to the lack of two relevant scenarios for Central Zone D3, D4, D5 and R1 the surrogate crops:

- Field beans for D3, D4, R1 scenarios,
- Legumes for D5 scenario

were used.

The PEC/RAC ratios for D3, D4 and R1 scenarios for application rates at 1.8 kg/ha, 1.5 kg/ha and 0.9 kg/ha in field beans are presented in Table 9.5-12 below. The PEC/RAC ratio for D5 scenario for application rates at 1.8 kg/ha and 0.8 kg/ha in legumes is presented in Table 9.5-8 above.

The application rate for soybeans is from 1.17 to 1.8 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower doses. However the risk assessment for the following application rates was assessed by Applicant:

- Soybeans: 1.8 kg/ha and 1.2 kg/ha;
- Field beans as surrogate crop for scenarios D3, D4, R1: 1.8 kg/ha, 1.5 kg/ha and 0.9 kg/ha.
- Legumes as surrogate crop for scenario D5: 1.8 kg/ha and 0.8 kg/ha.

The following risk mitigation measures are required:

Application rate 1.8 kg a.s./ha (Step 4):

- R3 stream, R4 stream – risk is unresolved;

Application rate 1.2 kg a.s./ha (Step 4):

- R3 stream, R4 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 50% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop - Field beans, VFS Mode Step 4):

- D3 ditch, D4 stream, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Application rate 1.5 kg a.s./ha (surrogate crop - Field beans, Step 4):

- D3 ditch, D4 stream, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Application rate 0.9 kg a.s./ha (surrogate crop - Field beans, Step 4):

- D3 ditch, D4 stream, R1 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Application rate 1.8 kg a.s./ha (surrogate crop legumes, Step 4):

- D5 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Application rate 0.8 kg a.s./ha (surrogate crop legumes, Step 4):

- D5 stream - 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Use in tobacco

Table 9.5-10 11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in tobacco (dose: 1.5 kg; 1.4 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant			Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabea</i>	<i>Pseudokirchnerella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>Chironomus riparius</i>
Endpoint			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/EyC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50 – EU level	ErC50 – study code	NOEC
(µg/L)			670	5	952	7710	16	6.7	1400	12.801	5.3	472	6/12	6.748	32000
AF			100	10	100	100	10	10	10	10	5	10	10	10	10
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748	3200
FOCUS Scenario		PEC gl-max (µg/L)													PECsed max (µg/kg)*
Step 1															
		63.8	9.52238806	127.6	6.701681	0.827497	39.875	95.22388/92.47	0.45571429	49.83986	50.6	1.351695	106.3333/53.17	94.54653	3370 1.05
Step 2															
N-Europe		13.8	2.05970149	27.6	1.44958	0.178988	8.625	20.59701/20	0.09857143	10.78041	13.0	0.292373	23/11.5	20.4505	734.67 0.230
Step 3															
R3	Stream	7.004	1.04537313	14.008	0.735714	0.090843	4.3775	10.45373/10.15	0.05002857	5.471448	6.61	0.14839	11.67333/5.84	10.37937	36.09 0.01
STEP 4 - 1500 g															
Vegetative strip: 20 m				No spray buffer: 20 m				Nozzle reduction: 90 %							
R3	Stream	0.5357	0.07995522	1.0714	0.056271	0.006948	0.3348125	0.799552/0.776	0.00382643	0.418483	0.51	0.01135	0.892833/0.446	0.793865	
STEP 4 - 1400 g															
Vegetative strip: 20 m				No spray buffer: 20 m				Nozzle reduction: 90 %							
R3	Stream	0.4972	0.07420896	0.9944	0.052227	0.006449	0.31075	0.74209/0.7206	0.00355143	0.388407	0.47	0.010534	0.828667/0.414	0.736811	

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

zRMS comments:

Daphnia magna:

In EFSA Scientific Report (2008) 149, 1-80 for *Daphnia magna* the EU agreed endpoints 48h EC₅₀ at 1.2 mg/L_{nom} and 0.952 mg/L_{mm} were provided. According to DAR Aclonifen – Annex B.9: Ecotoxicology: “Results are based on nominal concentrations since measured values (except the highest 3.2 mg/L) ranged between 81 - 97 % of nominal values throughout the study. At 3.2 mg/L settlement of undissolved material may have been the reason for the lower measured value after 48 hours (74 % of nominal). The PEC/RAC calculations was performed with the worst-case value. The use of EC₅₀ = 1.2 mg/L will not affect the assessment results.

Algae:

With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011) a HC₅ based on E_bC₅₀ values was recalculated by RMS. The value of HC₅ = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment. PEC/RAC calculations with HC₅ was added by zRMS.

Algae and higher plants:

According to AGD (EFSA Journal 2013;11(7):3290) “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive, endpoints such as yield may also be used if growth rate endpoints are not provided.” Since growth rate endpoints are available they should be used in risk assessment. PEC/RAC calculations with these endpoints were added by zRMS for completeness.

Following the recommendations of the EFSA “Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic. The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance aclonifen.

Test species	Measured toxicity of formulation	Measured toxicity of a.s.	Formulation end-point recalculated for a.s.	Ratio a.s. end-point/recalculated formulation endpoint
Daphnia magna	15.46 mg/L	1.2 mg/L	7.71 mg/L	0.16
Algae	0.025 mg/L	6.9 µg/L	12.801 µg/L	0.54
Lemna	0.0135 mg/L	12 µg/L	6.7498 µg/L	1.78

The endpoint of the formulation expressed in terms of aclonifen is not more than three times lower for daphnia, algae and Lemna. Therefore, the formulation endpoints expressed in terms of aclonifen need not be used in the risk assessment for the active substance.

However, these endpoints were used in the risk assessment for the active substance in the table above by the Applicant and were assessed by zRMS.

For Step 3 calculations the PEC_{sw} values for application rate at 1.8 kg were used by Applicant as the worst-case scenario.

According to Part B8, due to the lack of two relevant scenarios for Central Zone D3, D4, D5, R1 and R4 the surrogate crop:

- Pome fruits for D3, D4, D5, R1 and R4 scenarios was used.

The PEC/RAC ratios for D3, D4, D5, R1 and R4 scenarios for application rates at 1.8 kg/ha and 1.4 kg/ha in pome fruits are presented in Table 9.5-12 below.

The application rate for tobacco is from 1.08 to 1.5 kg a.s./ha. PEC/RAC calculations at application rate 1.5 kg a.s./ha covers the lower doses. However the risk assessment for the following application rates was assessed by Applicant:

- Tobacco: 1.5 kg/ha and 1.4 kg/ha;
- Pome fruits as surrogate crop for scenarios D3, D4, D5, R1, R4: 1.8 kg/ha, 1.4 kg/ha.

PEC/RAC calculations at application rate 1.8 kg a.s./ha for pome fruits as surrogate crops for D3, D4, D5, R1, R4 covers the dose rate 1.5 kg a.s./ha for tobacco.

The following risk mitigation measures should be considered:

Application rate 1.5 kg a.s./ha (Step 4):

- R3 stream – risk is unresolved;

Application rate 1.4 kg a.s./ha (Step 4):

- R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop - Pome fruits, Step 4):

- D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream – 20 m vegetative filter strip and 90 m no spray buffer zone.
- R4 stream – risk is unresolved

Application rate 1.4 kg a.s./ha (surrogate crop - Pome fruits, Step 4):

- D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream, R4 stream – 20 m vegetative filter strip and 90 m no spray buffer zone.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetonifon for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in leafy vegetables (dose: 1.8 kg; 1.6 kg; 1 kg)

Group			Fish-acute	Fish-prolonged	Inverteb.-acute	Inverteb.-prolonged	Algae	Sed.-dwelling-prolonged	Higher-plant		Sed.-dwelling-prolonged
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Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabea</i>	<i>Pseudokirchenella</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>			<i>Chironomus riparius</i>
Endpoint			LC50—EU level	NOEC—EU level	EC50—EU level	ErC50—study code	NOEC—EU level	ErC50/EyC50—EU level	ErC50—study code	ErC50—study code	NOEC—EU level	EbC50—EU level	ErC50—study code		NOEC
(µg/L)			670	5	952	7710	16	6.7	1400	12.801	472	6	6.748		32000
AF			100	10	100	100	10	10	10	10	10	10	10		10
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67	140	1.2801	47.2	0.6	0.6748		3200
FOCUS Scenario		PEC _{gl-max} (µg/L)												PEC _{sed-max} (µg/kg)*	
Step 1															
		76.56	11.4268657	153.12	8.042017	0.992996	47.85	114.2687	0.54685714	59.80783	1.622034	127.6	113.4558	4050	
Step 2															
N-Europe		16.55	2.47014925	33.1	1.738445	0.214656	10.34375	24.70149	0.11821429	12.92868	0.350636	27.58333	24.52579	881.61	
Step 3															
D3	Ditch	11.33	1.69104478	22.66	1.190126	0.146952	7.08125	16.91045	0.08092857	8.850871	0.240042	18.88333	16.79016	8.362	
D4	Pond	0.3899	0.05819403	0.7798	0.040956	0.005057	0.2436875	0.58194	0.002785	0.304586	0.008261	0.649833	0.577801	2.626	
D4	Stream	8.881	1.32552239	17.762	0.932878	0.115188	5.550625	13.25522	0.06343571	6.937739	0.188157	14.80167	13.16094	0.3965	
D6	Ditch	11.09	1.65522388	22.18	1.164916	0.143839	6.93125	16.55224	0.07921429	8.663386	0.234958	18.48333	16.4345	2.291	
R1	Pond	0.4941	0.07374627	0.9882	0.051901	0.006409	0.3088125	0.737463	0.00352929	0.385985	0.010468	0.8235	0.732217	12.36	
R1	Stream	7.465	1.1141791	14.93	0.784139	0.096822	4.665625	11.14179	0.05332143	5.831576	0.158157	12.44167	11.06254	140.0	
R2	Stream	9.792	1.46149254	19.584	1.028571	0.127004	6.12	14.61493	0.06994286	7.649402	0.207458	16.32	14.51097	80.93	
R3	Stream	10.49	1.56567164	20.98	1.101891	0.136057	6.55625	15.65672	0.07492857	8.194672	0.222246	17.48333	15.54535	91.31	
R4	Stream	7.456	1.11283582	14.912	0.783193	0.096706	4.66	11.12836	0.05325714	5.824545	0.157966	12.42667	11.0492	34.32	
VFS Mode – STEP 4 – 1800 g															
Vegetative strip: 20 m				No spray buffer: 20 m					Nozzle reduction: 90 %						
D3	Ditch	0.08431	0.01258358	0.16862	0.008856	0.001094	0.0526938	0.125836	0.00060221	0.065862	0.001786	0.140517	0.124941		
D4	Pond	0.01889	0.0028194	0.03778	0.001984	0.000245	0.0118063	0.028194	0.00013493	0.014757	0.0004	0.031483	0.027993		
D4	Stream	0.1341	0.02001493	0.2682	0.014086	0.001739	0.0838125	0.200149	0.00095786	0.104757	0.002841	0.2235	0.198726		

D6	Ditch	0.5082	0.07585075	1.0164	0.053382	0.006591	0.317625	0.758507	0.00363	0.397	0.010767	0.847	0.753112		
R1	Pond	0.01617	0.00241343	0.03234	0.001699	0.00021	0.0101063	0.024134	0.0001155	0.012632	0.000343	0.02695	0.023963		
R1	Stream	0.07492	0.01118209	0.14984	0.00787	0.000972	0.046825	0.111821	0.00053514	0.058527	0.001587	0.124867	0.111025		
R2	Stream	0.09827	0.01466716	0.19654	0.010322	0.001275	0.0614188	0.146672	0.00070193	0.076767	0.002082	0.163783	0.145628		
R3	Stream	0.1053	0.01571642	0.2106	0.011061	0.001366	0.0658125	0.157164	0.00075214	0.082259	0.002231	0.1755	0.156046		
R4	Stream	0.07482	0.01116716	0.14964	0.007859	0.00097	0.0467625	0.111672	0.00053443	0.058449	0.001585	0.1247	0.110877		
STEP 4 - 1600 g															
Vegetative strip: 20 m				No spray buffer: 20 m				Nozzle reduction: 90 %							
D3	Ditch	0.07487	0.01117463	0.14974	0.007864	0.000971	0.0467938	0.111746	0.00053479	0.058488	0.001586	0.124783	0.110951		
D4	Pond	0.0164	0.00244776	0.0328	0.001723	0.000213	0.01025	0.024478	0.00011714	0.012811	0.000347	0.027333	0.024303		
D4	Stream	0.1169	0.01744776	0.2338	0.012279	0.001516	0.0730625	0.174478	0.000835	0.091321	0.002477	0.194833	0.173237		
D6	Ditch	0.4405	0.06574627	0.881	0.046271	0.005713	0.2753125	0.657463	0.00314643	0.344114	0.009333	0.734167	0.652786		
R1	Pond	0.08762	0.01307761	0.17524	0.009204	0.001136	0.0547625	0.130776	0.00062586	0.068448	0.001856	0.146033	0.129846		
R1	Stream	0.4658	0.06952239	0.9316	0.048929	0.006042	0.291125	0.695224	0.00332714	0.363878	0.009869	0.776333	0.690279		
R2	Stream	0.138	0.02059701	0.276	0.014496	0.00179	0.08625	0.20597	0.00098571	0.107804	0.002924	0.23	0.204505		
R3	Stream	0.5858	0.08743284	1.1716	0.061534	0.007598	0.366125	0.874328	0.00418429	0.45762	0.012411	0.976333	0.868109		
R4	Stream	0.7706	0.11501493	1.5412	0.080945	0.009995	0.481625	1.150149	0.00550429	0.601984	0.016326	1.284333	1.141968		
STEP 4 - 1000 g															
Vegetative strip: 20 m				No spray buffer: 20 m				Nozzle reduction: 90 %							
D3	Ditch	0.04685	0.00699254	0.0937	0.004921	0.000608	0.0292813	0.069925	0.00033464	0.036599	0.000993	0.078083	0.069428		
D4	Pond	0.00932	0.00139104	0.01864	0.000979	0.000121	0.005825	0.01391	6.6571E-05	0.007281	0.000197	0.015533	0.013811		
D4	Stream	0.06755	0.01008209	0.1351	0.007096	0.000876	0.0422188	0.100821	0.0004825	0.052769	0.001431	0.112583	0.100104		
D6	Ditch	0.2488	0.03713433	0.4976	0.026134	0.003227	0.1555	0.371343	0.00177714	0.19436	0.005271	0.414667	0.368702		
R1	Pond	0.05306	0.0079194	0.10612	0.005574	0.000688	0.0331625	0.079194	0.000379	0.04145	0.001124	0.088433	0.078631		
R1	Stream	0.2807	0.04189552	0.5614	0.029485	0.003641	0.1754375	0.418955	0.002005	0.21928	0.005947	0.467833	0.415975		
R2	Stream	0.08284	0.01236418	0.16568	0.008702	0.001074	0.051775	0.123642	0.00059171	0.064714	0.001755	0.138067	0.122762		
R3	Stream	0.3518	0.05250746	0.7036	0.036954	0.004563	0.219875	0.525075	0.00251286	0.274822	0.007453	0.586333	0.52134		
R4	Stream	0.465	0.06940299	0.93	0.048845	0.006031	0.290625	0.69403	0.00332143	0.363253	0.009852	0.775	0.689093		

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

Use in field beans as the surrogate crop for: soybeans for D3, D4 and R1 scenarios

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in field beans (dose: 1.8 kg; 1.5 kg; 0.9 kg)

Group			Fish acute	Fish pro- longed	Inverteb. acute		Inver- teb. prolon- ged	Algae			Al- gae	Sed. dwel. prolonged	Higher plant		Sed. dwel. prolonged		
Test species			<i>Oncorhyn- chus mykiss</i>	<i>Oncorhyn- chus mykiss</i>	<i>Daphnia magna</i>		<i>Daphnia magna</i>	<i>Desmodesmus subpicatus</i>	<i>Anabea</i>	<i>Pseudokirche- nella</i>	HC5	<i>Chirono- mus ripa- rius</i>	<i>Lemna gibba</i>			<i>Chiro- no-mus riparius</i>	
Endpo- int			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/EyC50 EbC50/ErC50 – EU level	ErC50 – study code	ErC50 – study code	EC50 0	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC	
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000	
AF			100	10	100	100	10	10	10	10	5	10	10	10		10	
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.06	47.2	0.6/1.2	0.6748		3200	
FOCUS Scena- rio		PEC gl-max (µg/L)													PECse d max (µg/kg) *		
Step 1																	
		76.56	11.4268657	153.12	8.04201 7	0.99299 6	47.85	114.2687/111	0.546857 14	59.80783		1.622034	127.6 /63.8	113.455 8	4040	1.263	
Step 2																	
N-Europe		16.55	2.47014925	33.1	1.73844 5	0.21465 6	10.34375	24.70149/23.9 9	0.118214 29	12.92868		0.350636	27.5833 3 /13.79	24.5257 9	881.61	0.276	
Step 3																	
D2	Ditch	9.451	1.41059701	18.902	0.99275 2	0.12258 1	5.906875	14.10597	0.067507 14	7.383017		0.200233	15.7516 7	14.0056 3			
D2	Stream	8.367	1.24880597	16.734	0.87888 7	0.10852 1	5.229375	12.48806	0.059764 29	6.536208		0.177267	13.945	12.3992 3			
D3	Ditch	9.367	1.3980597	18.734/ /	0.98392 9	0.12149 2	5.854375	13.9806/13.58	0.066907 14	7.317397	8.84	0.198453	15.6116 7 /7.806	13.8811 5	6.663	0.0222	

D4	Pond	0.3776	0.05635821	0.7552	0.03966 4	0.00489 8	0.236	0.563582/0.54	0.002697 14	0.294977	0.36	0.008	0.62933 3 /0.315	0.55957 3	2.802	0.0009
D4	Stream	7.497	1.11895522	14.994	0.7875	0.09723 7	4.685625	11.18955/10.8 7	0.05355	5.856574	7.07	0.158835	12.495 /6.248	11.1099 6	0.2716	0.0001
D6	Ditch	9.268	1.38328358	18.536	0.97352 9	0.12020 8	5.7925	13.83284	0.0662	7.240059		0.196356	15.4466 7	13.7344 4		
R1	Pond	0.3885	0.05798507	0.777	0.04080 9	0.00503 9	0.242812 5	0.579851/0.56	0.002775	0.303492	0.37	0.008231	0.6475 /0.324	0.57572 6	7.332	0.0023
R1	Stream	6.467	0.96522388	12.934	0.67930 7	0.08387 8	4.041875	9.652239/9.37	0.046192 86	5.051949	6.10	0.137013	10.7783 3 /5.389	9.58358	38.17	0.0119
R2	Stream	8.515	1.27089552	17.03	0.89443 3	0.11044 1	5.321875	12.70896	0.060821 43	6.651824		0.180403	14.1916 7	12.6185 5		
R3	Stream	9.111	1.35985075	18.222	0.95703 8	0.11817 1	5.694375	13.59851	0.065078 57	7.117413		0.19303	15.185	13.5017 8		
R4	Stream	6.479	0.96701493	12.958	0.68056 7	0.08403 4	4.049375	9.670149	0.046278 57	5.061323		0.137267	10.7983 3	9.60136 3		
VFS Mode - STEP 4 - 1800 g																
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %						
D2	Ditch	0.0851 2	0.01270448	0.17024	0.00894 1	0.00110 4	0.0532	0.127045	0.000608	0.066495		0.001803	0.14186 7	0.12614 1		
D2	Stream	0.0967 8	0.01444478	0.19356	0.01016 6	0.00125 5	0.060487 5	0.144448	0.000691 29	0.075603		0.00205	0.1613	0.14342		
D3	Ditch	0.0842 7	0.01257761	0.16854	0.00885 2	0.00109 3	0.052668 8	0.125776/0.12 2	0.000601 93	0.065831	0.08	0.001785	0.14045 /0.070	0.12488 1		
D4	Pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.010106 3	0.024134/0.02 3	0.000115 5	0.012632	0.02	0.000343	0.02695 /0.013	0.02396 3		
D4	Stream	0.0867 1	0.01294179	0.17342	0.00910 8	0.00112 5	0.054193 8	0.129418/0.12 6	0.000619 36	0.067737	0.08	0.001837	0.14451 7 /0.072	0.12849 7		
D6	Ditch	0.0964 1	0.01438955	0.19282	0.01012 7	0.00125	0.060256 3	0.143896	0.000688 64	0.075314		0.002043	0.16068 3	0.14287 2		
R1	Pond	0.0161 7	0.00241343	0.03234	0.00169 9	0.00021	0.010106 3	0.024134/0.02 3	0.000115 5	0.012632	0.02	0.000343	0.02695 /0.013	0.02396 3		
R1	Stream	0.3371	0.05031343	0.6742	0.03541	0.00437 2	0.210687 5	0.503134/0.48 9	0.002407 86	0.263339	0.32	0.007142	0.56183 3 /0.281	0.49955 5		
R2	Stream	0.0984 8	0.01469851	0.19696	0.01034 5	0.00127 7	0.06155	0.146985	0.000703 43	0.076931		0.002086	0.16413 3	0.14594		
R3	Stream	0.1054	0.01573134	0.2108	0.01107	0.00136	0.065875	0.157313	0.000752	0.082337		0.002233	0.17566	0.15619		

	m				1	7			86			7	4		
R4	Stream	0.07656	0.01142687	0.15312	0.008042	0.000993	0.04785	0.114269	0.00054686	0.059808		0.001622	0.1276	0.113456	
STEP 4 - 1500 g															
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %					
D2	Ditch	0.07096	0.01059104	0.14192	0.007454	0.00092	0.04435	0.10591	0.00050686	0.055433		0.001503	0.118267	0.105157	
D2	Stream	0.08059	0.01202836	0.16118	0.008465	0.001045	0.0503688	0.120284	0.00057564	0.062956		0.001707	0.134317	0.119428	
D3	Ditch	0.07027	0.01048806	0.14054	0.007381	0.000911	0.0439188	0.104881/0.102	0.00050193	0.054894	0.07	0.001489	0.117117/0.059	0.104135	
D4	Pond	0.0134	0.002	0.0268	0.001408	0.000174	0.008375	0.02/0.019	9.5714E-05	0.010468	0.01	0.000284	0.022333/0.011	0.019858	
D4	Stream	0.0722	0.01077612	0.1444	0.007584	0.000936	0.045125	0.107761/0.105	0.00051571	0.056402	0.07	0.00153	0.120333/0.060	0.106995	
D6	Ditch	0.07762	0.01158507	0.15524	0.008153	0.001007	0.0485125	0.115851	0.00055443	0.060636		0.001644	0.129367	0.115027	
R1	Pond	0.04383	0.00654179	0.08766	0.004604	0.000568	0.0273938	0.065418/0.063	0.00031307	0.03424	0.04	0.000929	0.073057/0.037	0.064953	
R1	Stream	0.4522	0.06749254	0.9044	0.0475	0.005865	0.282625	0.674925/0.655	0.00323	0.353254	0.43	0.009581	0.753667/0.377	0.670124	
R2	Stream	0.1382	0.02062687	0.2764	0.014517	0.001792	0.086375	0.206269	0.00098714	0.10796		0.002928	0.230333	0.204801	
R3	Stream	0.5131	0.07658209	1.0262	0.053897	0.006655	0.3206875	0.765821	0.003665	0.400828		0.010871	0.855167	0.760373	
R4	Stream	0.8598	0.12832836	1.7196	0.090315	0.011152	0.537375	1.283284	0.00614143	0.671666		0.018216	1.433	1.274155	
STEP 4 - 900 g															
Vegetative strip: 20 m				No spray buffer: 20 m						Nozzle reduction: 90 %					
D2	Ditch	0.04267	0.00636866	0.08534	0.004482	0.000553	0.0266688	0.063687	0.00030479	0.033333		0.000904	0.071117	0.063234	
D2	Stream	0.04821	0.00719552	0.09642	0.005064	0.000625	0.0301313	0.071955	0.00034436	0.037661		0.001021	0.080357	0.071443	
D3	Ditch	0.04227	0.00630896	0.08454	0.00444	0.000548	0.0264188	0.06309/0.0613	0.00030193	0.033021	0.04	0.000896	0.070457/0.035	0.062641	
D4	Pond	0.0080	0.00120597	0.01616	0.00084	0.00010	0.00505	0.01206/0.011	5.7714E-	0.006312	0.01	0.000171	0.01346	0.01197	

		8			9	5		7	05				7	4		
													/0.007			
D4	Stream	0.04319	0.00644627	0.08638	0.004537	0.00056	0.0269938	0.064463/0.0626	0.0003085	0.03374	0.04	0.000915	0.071983/0.036	0.064004		
D6	Ditch	0.04226	0.00630746	0.08452	0.004439	0.000548	0.0264125	0.063075	0.00030186	0.033013		0.000895	0.070433	0.062626		
R1	Pond	0.02538	0.00378806	0.05076	0.002666	0.000329	0.0158625	0.037881/0.0368	0.00018129	0.019827	0.02	0.000538	0.0423/0.021	0.037611		
R1	Stream	0.2607	0.03891045	0.5214	0.027384	0.003381	0.1629375	0.389104/0.378	0.00186214	0.203656	0.25	0.005523	0.4345/0.217	0.386337		
R2	Stream	0.07937	0.01184627	0.15874	0.008337	0.001029	0.0496063	0.118463	0.00056693	0.062003		0.001682	0.132283	0.11762		
R3	Stream	0.2952	0.0440597	0.5904	0.031008	0.003829	0.1845	0.440597	0.00210857	0.230607		0.006254	0.492	0.437463		
R4	Stream	0.4947	0.07383582	0.9894	0.051964	0.006416	0.3091875	0.738358	0.00353357	0.386454		0.010481	0.8245	0.733106		

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

zRMS comments:

According to Part B8 field beans is the surrogate crop for:
- soybeans for D3, D4 and R1 scenarios.

Use in pome fruits as the surrogate crop for:

- tobacco for D3, D4, D5, R1 and R4 scenarios,
- trees, ornamental shrubs for D3, D4, D5, R1, R3, R4 scenarios.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 (optionally Step 4) calculations for the use of AKO 600 SC in pome fruits (dose: 1.8 kg; 1.4 kg)

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae			Algae	Sed. dwell. prolonged	Higher plant		Sed. dwell. prolonged
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Desmodesmus subspicatus</i>	<i>Anabaena</i>	<i>Pseudokirchnerella</i>	HC5	<i>Chironomus riparius</i>	<i>Lemna gibba</i>		<i>Chironomus riparius</i>

Endpo- int			LC50 – EU level	NOEC – EU level	EC50 – EU level	ErC50 – study code	NOEC – EU level	ErC50/EyC50 – EU level	ErC50 – study code	ErC50 – study code	EC50	NOEC – EU level	EbC50 – EU level	ErC50 – study code		NOEC	
(µg/L)			670	5	952	7710	16	6.7/6.9	1400	12.801	5.3	472	6/12	6.748		32000	
AF			100	10	100	100	10	10	10	10	5	10	10	10		10	
RAC (µg/L)			6.7	0.5	9.52	77.1	1.6	0.67/0.69	140	1.2801	1.6	47.2	0.6/1.2	0.6748		3200	
FOCUS Scenario		PEC _{gl- max} (µg/L)													PEC _{sed max} (µg/kg) *		
Step 1																	
		235.19	35.1029851	470.38	24.7048 3	3.05045 4	146.99375	351.0299/340.9	1.6799285 7	183.7278	221.9	4.982839	391.983 3 /195.99	348.532 9	4050	1.266	
Step 2																	
N-Europe		175.18	26.1462687	350.36	18.4012 6	2.27211 4	109.4875	261.4627/253.9	1.2512857 1	136.8487	165.3	3.711441	291.966 7 /145.98	259.602 8	1910	0.597	
Step 3																	
D3	Ditch	138.9	20.7313433	277.8	14.5903 4	1.80155 6	86.8125	207.3134/201.3 0	0.9921428 6	108.5071	131.0	2.942797	231.5 /115.75	205.838 8	101.6	0.032	
D4	Pond	8.433	1.25865672	16.866	0.88581 9	0.10937 7	5.270625	12.58657/12.22	0.0602357 1	6.587767	7.96	0.178665	14.055 /7.03	12.4970 4	57.10	0.018	
D4	Strea m	131.4	19.6119403	262.8	13.8025 2	1.70428	82.125	196.1194/190.4 3	0.9385714 3	102.6482	104.3	2.783898	219 /109.5	194.724 4	4.950	0.002	
D5	Pond	8.434	1.25880597	16.868	0.88592 4	0.10939	5.27125	12.58806/12.22	0.0602428 6	6.588548	7.96	0.178686	14.0566 7 /7.028	12.4985 2	56.33	0.018	
D5	Strea m	137.8	20.5671642	275.6	14.4747 9	1.78728 9	86.125	205.6716/199.7 1	0.9842857 1	107.6478	130	2.919492	229.666 7 /114.83	204.208 7	4.247	0.001	
R1	Pond	8.433	1.25865672	16.866	0.88581 9	0.10937 7	5.270625	12.58657/12.22	0.0602357 1	6.587767	6.69	0.178665	14.055 /7.028	12.4970 4	52.58	0.016	
R1	Strea m	112.3	16.761194	224.6	11.7962 2	1.45655	70.1875	167.6119/162.7 5	0.8021428 6	87.72752	105.9	2.379237	187.166 7 /93.58	166.419 7	14.47	0.005	
R2	Strea m	148.8	22.2089552	297.6	15.6302 5	1.92996 1	93	222.0896	1.0628571 4	116.2409		3.152542	248	220.509 8			
R3	Strea m	159	23.7313433	318	16.7016 8	2.06225 7	99.375	237.3134	1.1357142 9	124.209		3.368644	265	235.625 4			
R4	Strea m	112.4	16.7761194	224.8	11.8067 2	1.45784 7	70.25	167.7612/162.9 0	0.8028571 4	87.80564	106.0	2.381356	187.333 3 /93.67	166.567 9	14.57	0.005	

VFS Mode- STEP 4 - 1800 g																
Vegetative strip: 20 m				No spray buffer: 90 m						Nozzle reduction: 0 %						
D3	Ditch	0.4208	0.06280597	0.8416	0.04420 2	0.00545 8	0.263	0.62806/0.61	0.0030057 1	0.328724	0.40	0.008915	0.70133 3 /0.351	0.62359 2		
D4	Pond	0.0915 3	0.01366119	0.18306	0.00961 4	0.00118 7	0.0572063	0.136612/0.133	0.0006537 9	0.071502	0.09	0.001939	0.15255 /0.076	0.13564		
D4	Stream	0.435	0.06492537	0.87	0.04569 3	0.00564 2	0.271875	0.649254/0.630	0.0031071 4	0.339817	0.41	0.009216	0.725 /0.363	0.64463 5		
D5	Pond	0.0915 7	0.01366716	0.18314	0.00961 9	0.00118 8	0.0572313	0.136672/0.133	0.0006540 7	0.071533	0.09	0.00194	0.15261 7 /0.076	0.13569 9		
D5	Stream	0.4564	0.0681194	0.9128	0.04794 1	0.00592	0.28525	0.681194/0.661	0.00326	0.356535	0.43	0.009669	0.76066 7 /0.380	0.67634 9		
R1	Pond	0.0915 3	0.01366119	0.18306	0.00961 4	0.00118 7	0.0572063	0.136612/0.133	0.0006537 9	0.071502	0.09	0.001939	0.15255 /0.076	0.13564		
R1	Stream	0.372	0.05552239	0.744	0.03907 6	0.00482 5	0.2325	0.555224/0.539	0.0026571 4	0.290602	0.35	0.007881	0.62 /0.31	0.55127 4		
R2	Stream	0.4929	0.07356716	0.9858	0.05177 5	0.00639 3	0.3080625	0.735672	0.0035207 1	0.385048		0.010443	0.8215	0.73043 9		
R3	Stream	0.5894	0.08797015	1.1788	0.06191 2	0.00764 5	0.368375	0.879701	0.00421	0.460433		0.012487	0.98233 3	0.87344 4		
R4	Stream	1.024	0.15283582	2.048	0.10756 3	0.01328 1	0.64	1.528358/1.484	0.0073142 9	0.799938	0.97	0.021695	1.70666 7 /0.85	1.51748 7		
STEP 4 - 1400 g																
Vegetative strip: 20 m				No spray buffer: 90 m						Nozzle reduction: 0 %						
D3	Ditch	0.3272	0.04883582	0.6544	0.03437	0.00424 4	0.2045	0.488358/0.474	0.0023371 4	0.255605	0.31	0.006932	0.54533 3 /0.273	0.48488 4		
D4	Pond	0.0711 7	0.01062239	0.14234	0.00747 6	0.00092 3	0.0444813	0.106224/0.103	0.0005083 6	0.055597	0.07	0.001508	0.11861 7 /0.059	0.10546 8		
D4	Stream	0.3383	0.05049254	0.6766	0.03553 6	0.00438 8	0.2114375	0.504925/0.490	0.0024164 3	0.264276	0.32	0.007167	0.56383 3 /0.282	0.50133 4		
D5	Pond	0.0712 1	0.01062836	0.14242	0.00748	0.00092 4	0.0445063	0.106284/0.103	0.0005086 4	0.055628	0.07	0.001509	0.11868 3 /0.059	0.10552 8		
D5	Stream	0.355	0.05298507	0.71	0.03729	0.00460 4	0.221875	0.529851/0.514	0.0025357 1	0.277322	0.33	0.007521	0.59166 7 /0.296	0.52608 2		
R1	Pond	0.0711 7	0.01062239	0.14234	0.00747 6	0.00092 3	0.0444813	0.106224/0.103	0.0005083 6	0.055597	0.07	0.001508	0.11861 7	0.10546 8		

													0.059			
R1	Stream	0.2893	0.0431791	0.5786	0.03038 9	0.00375 2	0.1808125	0.431791/0.419	0.0020664 3	0.225998	0.27	0.006129	0.48216 7 0.241	0.42872		
R2	Stream	0.3833	0.05720896	0.7666	0.04026 3	0.00497 1	0.2395625	0.57209	0.0027378 6	0.29943		0.008121	0.63883 3	0.56802		
R3	Stream	0.449	0.06701493	0.898	0.04716 4	0.00582 4	0.280625	0.670149	0.0032071 4	0.350754		0.009513	0.74833 3	0.66538 2		
R4	Stream	0.4868	0.07265672	0.9736	0.05113 4	0.00631 4	0.30425	0.726567/0.706	0.0034771 4	0.380283	0.46	0.010314	0.81133 3 0.406	0.72139 9		

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

zRMS comments:

According to Part B8 pome fruits is the surrogate crop for:

- tobacco for D3, D4, D5, R1 and R4 scenarios,
- trees, ornamental shrubs for D3, D4, D5, R1, R4 scenarios.

Use in pumpkin

According to Part B8 potatoes for D3, D4, R1, R3 scenarios and legumes for D5, R4 scenarios are the surrogate crops for pumpkin due to lack of scenarios.

The application rates for pumpkin is from 1.17 to 1.8 kg a.s./ha. PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower doses.

However the risk assessment for the following application rates was assessed by Applicant:

- potatoes as surrogate crop for scenarios D3, D4, R1, R3: 1.8 kg/ha, 1.5 kg/ha and 1.4 kg/ha.
- legumes as surrogate crop for scenario D5, R4: 1.8 kg/ha and 0.8 kg/ha.

Therefore the following risk mitigation measures should be considered:

Application rate 1.8 kg a.s./ha (surrogate crop potatoes, VFS mode – Step 4):

- D3 ditch, D4 stream, R1 stream, R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.5 kg a.s./ha (surrogate crop potatoes, Step 4):

- D3 ditch, D4 stream, R1 stream, – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;
- R3 stream - risk is unresolved

Application rate 1.4 kg a.s./ha (surrogate crop potatoes, Step 4):

- D3 ditch, D4 pond, D4 stream, R1 pond, R1 stream, R3 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction;

Application rate 1.8 kg a.s./ha (surrogate crop legumes, VFS mode – Step 4):

- D5 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction. For R4 stream scenario the risk is unacceptable.
- R4 stream – risk is unresolved.

Application rate 0.8 kg a.s./ha (surrogate crop legume, Step 4):

- D5 stream, R4 stream – 20 m vegetative filter strip, 20 m no spray buffer zone and 90% nozzle reduction.

Use in trees and ornamental shrubs

According to Part B8 pome fruits is the surrogate crop for trees and ornamental shrubs (D3, D4, D5, R1, R3, R4 scenarios) due to lack of scenarios.

The application rate for forest nurseries plants is from 1.17 to 1.8 kg a.s./ha.

The application rate for ornamental shrubs, basket willow, purple willow, forest tree, Christmas tree and energetic willow is from 1.17 to 1.50 kg a.s./ha.

The PEC/RAC ratios for D3, D4, D5, R1 and R4 scenarios for application rates at 1.8 kg/ha and 1.4 kg/ha in pome fruits are presented in Table 9.5-12 below.
PEC/RAC calculations at application rate 1.8 kg a.s./ha covers the lower dose rate.

The following risk mitigation measures should be considered:

Application rate 1.8 kg a.s./ha (surrogate crop - Pome fruits, Step 4):

- D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream, R3 stream – 20 m vegetative filter strip and 90 m no spray buffer zone.
- R4 stream – risk is unresolved

Application rate 1.4 kg a.s./ha (surrogate crop - Pome fruits, Step 4):

- D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, R1 pond, R1 stream, R3 stream, R4 stream – 20 m vegetative filter strip and 20 m no spray buffer zone.

9.5.2.1 Risk assessment for AKO 600 SC to aquatic organisms

Table 9.5-14 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in potatoes (3 L/ha)

Group				Inverteb. acute	Higher plant	Algae	Algae
Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Potatoes - Application rate: 3635 g of formulation/ha							
0		ditch	19.3088	0.124895	14.30281	7.72352	0.068715
		pond	0.77	0.005	0.57	0.308	0.002
		stream	15.04	0.097	11.14	6.016	0.053
30		ditch	1.1827	0.00765	0.876074	0.47308	0.004209
		pond	0.25	0.002	0.185	0.10	0.0009
		stream	1.18	0.008	0.874	0.472	0.004

zRMS comment:

For potatoes 30 m buffer zone is required.

For potatoes, based on the surrogate crop legumes, 30 m buffer zone is required.

Potatoes is also surrogate crop for pumpkin.

Table. 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in sunflower (3 L/ha)

Group				Inverteb. acute	Higher plant	Algae	Algae
Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Sunflower - Applicaiton rate: 3635 g of formulation/ha							
0	ditch	19.3088	0.124895	14.30281	7.72352	0.068715	
	pond	0.77	0.005	0.57	0.308	0.002	
	stream	15.04	0.097	11.14	6.016	0.053	
30	ditch	1.1827	0.00765	0.876074	0.47308	0.004209	
	pond	0.25	0.002	0,185	0.10	0.0009	
	stream	1.18	0.008	0.874	0.472	0.004	

zRMS comment:

For sunflower 30 m buffer zone is required.

For sunflowers, based on the surrogate crop maize, 30 m buffer zone is required.

Table. 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in maize (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
Test species			<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint			EC50	ERC50	ERC50	ERC50
(µg/L)			15460	13.5	25	2810
AF			100	10	10	10
RAC (µg/L)			154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)				
Maize - Applicaiton rate: 3635 g of formulation/ha						
0	ditch	19.3088	0.124895	14.30281	7.72352	0.068715
	pond	0.77	0.005	0.57	0.308	0.002
	stream	15.04	0.097	11.14	6.016	0.053
30	ditch	1.1827	0.00765	0.876074	0.47308	0.004209
	pond	0.25	0.002	0.185	0.10	0.0009
	stream	1.18	0.008	0.874	0.472	0.004

zRMS comment:

For maize as surrogate crop for sunflower 30 m buffer zone is required.

Table. 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in spring oilseeds (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
Test species			<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint			EC50	ERC50	ERC50	ERC50
(µg/L)			15460	13.5	25	2810
AF			100	10	10	10
RAC (µg/L)			154.6	1.35	2.5	281
Buffer zone [m]			PEC _{gl-max} (µg/L)			
Spring oilseeds - Applicaiton rate: 3635 g of formulation/ha						
0	ditch	23.3536	0.151058	17.29896	9.34144	0.083109
	pond	0.80	0.005	0.59	0.32	0.003
	stream	17.33	0.112	12.84	6.93	0.062
30	ditch	1.1827	0.00765	0.876074	0.47308	0.004209
	pond	0.25	0.002	0.185	0.10	0.0009
	stream	1.18	0.008	0.874	0.472	0.004

zRMS comment:

For spring oilseeds 30 m buffer zone is required.

For spring oilseeds, based on the surrogate crop winter oilseeds, 30 m buffer zone is required.

Spring oilseeds is also surrogate crop for flax, breadssd poppy, mustard and hemp.

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in winter oilseeds (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
Test species			<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint			EC50	ERC50	ERC50	ERC50
(µg/L)			15460	13.5	25	2810
AF			100	10	10	10
RAC (µg/L)			154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)				
Winter oilseeds - Applicaiton rate: 3635 g of formulation/ha						
0	ditch	23.3536	0.151058	17.29896	9.34144	0.083109
	pond	0.80	0.005	0.59	0.32	0.003
	stream	17.33	0.112	12.84	6.93	0.062
30	ditch	1.1827	0.00765	0.876074	0.47308	0.004209
	pond	0.25	0.002	0.185	0.10	0.0009
	stream	1.18	0.008	0.874	0.472	0.004

zRMS comment:

For winter oilseeds as surrogate crop for spring oilseeds, flax, breadssd poppy, mustard and hemp.30 m buffer zone is required.

Table. 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in legumes (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
Test species			<i>Daphnia magna</i>	<i>0Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint			EC50	ERC50	ERC50	ERC50
(µg/L)			15460	13.5	25	2810
AF			100	10	10	10
RAC (µg/L)			154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)				
Legumes - Applicaiton rate: 3635 g of formulation/ha						
0	ditch	19.3088	0.124895	14.30281	7.72352	0.068715
	pond	0.77	0.005	0.592	0.308	0.003
	stream	15.04	0.097	11.141	6.016	0.054
30	ditch	1.1827	0.00765	0.876074	0.47308	0.004209
	pond	0.25	0.002	0.185	0.10	0.0009
	strea	1.18	0.008	0.874	0.472	0.004

zRMS comment:

For legumes as surrogate crop for potatoes, spring oilseeds, soybeans, flax, breadseed poppy, mustard and hemp. 30 m buffer zone is required.

Table. 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in soybeans (3 L/ha)

Group				Inverteb. acute	Higher plant	Algae	Algae
Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Soybeans - Applicaiton rate: 3635 g of formulation/ha							
0	Ditch	19.3088	0.124895	14.30281	7.72352	0.068715	
	Pond	0.77	0.005	0.592	0.308	0.003	
	Stream	15.04	0.097	11.141	6.016	0.054	
30	Ditch	1.1827	0.00765	0.876074	0.47308	0.004209	
	Pond	0.25	0.002	0.185	0.10	0.0009	
	Stream	1.18	0.008	0.874	0.472	0.004	

zRMS comment:

For soybeans 30 m buffer zone is required.

Table. 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in tobacco (2.5 L/ha)

Group				Inverteb. acute	Higher plant	Algae	Algae
Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Tobacco - Applicaiton rate: 3029.5 g of formulation/ha							
0	Ditch	14.4443	0.09343	10.69948	5.77772	0.051403	
	Pond	0.63	0.004	0.467	0.252	0.002	
	Stream	11.53	0.075	8.541	4.612	0.041	
25	Ditch	1.1743	0.007596	0.869852	0.46972	0.004179	
	Pond	0.24	0.002	0.178	0.096	0.0009	
	stream	1.17	0.008	0.867	0.468	0.004	

zRMS comment:

For tobacco 25 m buffer zone is required.

For tobacco, based on the surrogate crop pome fruits, 75 m buffer zone is required.

Table. 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in leafy vegetables

Group	-	-	Inverteb. acute	Higher plant	Algae	Algae
Test species	-		<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint	-		EC50	ERC50	ERC50	ERC50
(µg/L)	-		15460	13.5	25	2810
AE	-		100	10	10	10
RAC (µg/L)	-		154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)	-			
Leafy vegetables – Applicaiton rate: 3635 g of formulation/ha						
0	Ditch	23.3536	0.151058	17.29896	9.34144	0.083109
	Pond	0.80				
	Stream	17.33				
30	Ditch	1.1827	0.00765	0.876074	0.47308	0.004209
	Pond	0.25				
	stream	1.18				

Table. 9.5-23 22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in field beans (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
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Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Field beans - Application rate: 3635 g of formulation/ha							
0		Ditch	19.3088	0.124895	14.30281	7.72352	0.068715
		Pond	0.77	0.005	0.570	0.308	0.0003
		Stream	15.04	0.097	11.141	6.016	0.054
30		Ditch	1.1827	0.00765	0.876074	0.47308	0.004209
		Pond	0.25	0.002	0.185	0.10	0.0009
		Stream	1.18	0.008	0.874	0.472	0.0042

zRMS comment:

For field beans as surrogate crop for soybeans 30 m buffer zone is required.

Table. 9.5-24 23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AKO 600 SC on each organism group based on Drift calculator SWASH Models ver. 5.3 for the use of AKO 600 SC in pome fruits (3 L/ha)

Group			Inverteb. acute	Higher plant	Algae	Algae
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Test species				<i>Daphnia magna</i>	<i>Lemma gibba</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i> <i>UTEX B 1444</i>
Endpoint				EC50	ERC50	ERC50	ERC50
(µg/L)				15460	13.5	25	2810
AF				100	10	10	10
RAC (µg/L)				154.6	1.35	2.5	281
Buffer zone [m]		PEC _{gl-max} (µg/L)					
Pome fruits - Applicaiton rate: 3635 g of formulation/ha							
0	Ditch	285.9371	1.849528	211.8053	114.3748	1.01757	
	Pond	17.19	0.111	12.734	6.876	0.819	
	Stream	261.51	1.692	193.711	104.604	0.931	
75	Ditch	1.3472	0.008714	0.997926	0.53888	0.004794	
	Pond	0.27	0.002	0.2	0.108	0.001	
	Stream	1.3472	0.009	0.998	0.539	0.005	

zRMS comment:

For pome fruits as surrogate crop for tobacco, trees and ornamental shrugs 75 m buffer zone is required.

zRMS comment:

For flax, breadseed poppy, mustard and hemp, based on the surrogate crops spring oilseed rape, winter oilseed rape and legumes 30 m buffer zone is required.

zRMS comment:

For pumpkin, based on the surrogate crops potatoes and legumes, 30 m buffer zone is required.

zRMS comment:

For trees and ornamental shrugs, based on the surrogate crops pome fruits, 75 m buffer zone is required.

Combined exposure of a mixture AKO 600 SC and BOA PRO 480 EC

Clomazone risk assessment on aquatic organisms is not the subject of this document. However, it's required for combined exposure assessment of a mixture: AKO 600 SC and BOA PRO 480 EC. Therefore, for details of ecotoxicological assessment of clomazone please refer to core dossier of BOA PRO 480 EC.

Decision scheme for mixture toxicity risk assessment for AKO 600 SC and BOA PRO 480 EC for potatoes

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

a) Only for the a.s. (ECxa.s.): → Go to 7

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

ANSWER: Measured toxicity data are provided for active substances only. Studies on a mixture product of AKO 600 SC and BOA PRO 480 EC have not been carried out → Go to STEP 7

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

ANSWER: No. → Go to STEP 8

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

Step 2 values for clomazone have been found in BOA PRO 480 EC core dossier.

Tab. 1 Calculations of aquatox exposure

	Exposure	(lower exposure tier)	(higher exposure tier)				
ACLONIFEN	Exposure tier (FOCUS step)	Step 2	Step 4 (D3 ditch)	Step 4 (D4 pond)	Step 4 (D4 stream)	Step 4 (R1 pond)	Step 4 (R1 stream)
	PECsw [mg a.s./L]	0.011040	0.000563	0.0000108	0.000597	0.00011	0.000499

	Relative proportions of the individual mixture components in the environment (p_{i-PEC})	0.693	0.103	0.022	0.109	0.022	0.092
CLOMAZONE	Exposure tier (FOCUS-step)	Step 2	Step 2	Step 2	Step 2	Step 2	Step 2
	PEC _{sw} [mg a.s./L]	0.004901	0.004901	0.004901	0.004901	0.004901	0.004901
	Relative proportions of the individual mixture components in the environment (p_{i-PEC})	0.307	0.897	0.978	0.891	0.978	0.908
	Total exposure concentration of the mixture (a.s.-based) (PEC _{mixture}) [mg/L]	0.015941	0.005464	0.005009	0.005498	0.005011	0.005400

Tab. 2 Calculations of a mixture toxicity

Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC _{mixture}) ($EC_{x-mix-CA} = \sum (p_{i-PEC}/EC_{x,i})$) [mg a.s./L]					
LC50 fish		0.949	4.723	10.496	4.553	10.438	5.090
EC50 daphnids		1.663	6.388	10.527	6.222	10.496	6.735
EbC50 algae		0.268	0.147	0.138	0.147	0.138	0.146
EC50 Higher plants		0.009	0.058	0.276	0.055	0.272	0.065

Tab. 3 Summary of mixture exposure

Endpoint/Test species		$ETR_{mix} = PEC_{mix} / EC_{x-PPP}$						Trigger values
LC50 fish		0.017	0.001	0.000	0.001	0.000	0.001	0.01
EC50 daphnids		0.010	0.001	0.000	0.001	0.000	0.001	0.01
EbC50 algae		0.060	0.037	0.036	0.037	0.036	0.037	0.1
EC50 Higher plants		1.840	0.094	0.018	0.100	0.018	0.083	0.1

The evaluation of the mixture toxicity risks for aquatic (and sediment-dwelling) organisms was performed in accordance with the “Guidance 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) and in EFSA Journal 2013;11(7):3290 (Aquatic Guidance Document, abbreviated as EFSA, 2013). The whole mixture assessment is based on the model of concentration addition (CA) and particularly the decision scheme as provided by EFSA (2013, section 10.3.11) was applied. The calculations were performed with the tool “AGD_AquaMix”. To keep the assessment concise the focus of this section of this registration report is on the parts of the assessment leading to the most critical results. It does not cover each calculation step for each organism group.

Combined exposure of a mixture AKO 600 SC and BOA PRO 480 EC

Table 9.5-24.2.1-1: Summary of assessment steps considered for the mixture risk assessment

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	Not relevant	Not relevant	Not relevant	Not relevant
Screening 2: Driver (optional Step)	Not relevant	Not relevant	Not relevant	Not relevant

Step 1: data available?	Endpoints only available for the a.s., go to Step 7.	Endpoints only available for the a.s., go to Step 7.	Endpoints only available for the a.s., go to Step 7.	Endpoints only available for the a.s., go to Step 7.
Step 2: apparent synergism or antagonism?	Not relevant	Not relevant	Not relevant	Not relevant
Step 3: mixture similar or not?	Not relevant	Not relevant	Not relevant	Not relevant
Step 4: ETRmix assessment (ECxPPP)	Not relevant	Not relevant	Not relevant	Not relevant
Step 5: driver available?	Not relevant	Not relevant	Not relevant	Not relevant
Step 6: driver assessment	Not relevant	Not relevant	Not relevant	Not relevant
Step 7: synergism assessment (few data)	Mixture toxicity calculation feasible: go to Step 8	Mixture toxicity calculation feasible: go to Step 8	Mixture toxicity calculation feasible: go to Step 8	Mixture toxicity calculation feasible: go to Step 8

Step 8a: ETRmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 8b: RQmix assessment	Not relevant	Not relevant	Not relevant	Not relevant
Step 9: antagonism assessment	Not relevant	Not relevant	Not relevant	Not relevant
Step 10: synergism assessment	Not relevant	Not relevant	Not relevant	Not relevant

Overview of the assessment steps: Table 9.5.2.1-1 gives a summary of the decisions made for each organism group during the mixture risk assessment with respect to the decision scheme as provided by EFSA (2013, section 10.3.11). A discussion of all details of every decision is not intended in this section of this registration report. Further in-depth justification and explanation for the decisions made are documented in the attached mixture risk calculation file. However, at least data for some important steps are documented in this section of this registration report, beginning with the check for (and potentially assessment of) synergistic or antagonistic effects (Step 2 and Steps 7, 9 and 10) as well as the ETR_{mix} or RQ_{mix} calculation results for the assessed FOCUS Scenarios (Steps 4 and 8a/b).

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

- a) Only for the a.s. (EC_xa.s.): → Go to 7
- b) For both formulation (EC_xPPP) and a.s. (EC_xa.s.): → Go to 2

ANSWER: Measured toxicity data are provided for active substances only. Studies on a mixture product of AKO 600 SC and BOA PRO 480 EC have not been carried out → Go to STEP 7

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

ANSWER: No evidence that synergistic interactions between mixture components might or might not occur. No studies on the mixture of AKO 600 SC and BOA PRO 480 EC have not been submitted → Go directly to STEP 8 for fish/invertebrates/algae/macrophytes

Step 8a: Mixture risk assessment based on measured and calculated mixture toxicity

Decisions taken when using the scheme as provided by EFSA (2013, section 10.3.11) lead to several assessment steps, which give a conclusion on the risk of a mixture or provide additional information important for the assessment. In the tables below the conclusions of different steps of the mixture risk assessment are summarized for the FOCUS Scenarios of concern. Information is reported on:

- a) Step 2 and associated steps, in which the question is answered if concentration addition can be assumed (i.e. a standard mixture toxicity risk assessment is appropriate) or if an antagonism/synergism is possible (more detailed information on Step 2 is given above). This is important to derive if further, specific considerations have to be made in the risk assessment. Associated steps are Step 7, Step 9 and Step 10.
- b) Step 5, in which the question is answered if there is a driver of risk and if so, which of the substance can be classified as driver (more detailed information on Step 5 is given the attached mixture risk calculation file. This is reported as additional information in case an assessment based on a driver would also be possible; for comparative reasons the table always lists the results from the mixture risk assessment according to Step 4 and/or Step 8. For an assessment based on a particular a.s. (the driver) please be referred to the individual a.s. risk assessment.
- c) Step 4 with the assessment results for cases where product data are usable (according to Step 3) and the risk assessment can be carried out based on the measured toxicity.
- d) **Step 8 (a/b) with the assessment results for cases where product data are not usable (according to Step 3) and the risk assessment has to be based on calculated mixture toxicity.**

The respective assessment conclusions are presented in Table 9.5.2.1-2 (FOCUS Step 1 – 3) or Table 9.5.2.1-6 (FOCUS Step 4). The assessment is based either on the exposure-toxicity ratio (ETR_{mix}) or the risk quotient (RQ_{mix}); the ETR_{mix} and RQ_{mix} are compared to respective trigger values: 1/10 or 1/100 for the ETR_{mix} (depending on the species assessed) and 1 for RQ_{mix} . Values below the trigger indicating a high risk are marked in bold. For the sake of a concise assessment the results below are limited to the organism groups most at risk. Further in-depth justification and explanation for other organism groups are documented in the attached mixture risk calculation file.

Table 9.5.2.1-2.1: Summary of the mixture toxicity risk assessment (related to Steps 8a) for fish for FOCUS Step 1 – 3 for application of the tank mix AKO 600 SC and BOA PRO 480 EC in potatoes

FOCUS Scenario	ETR_{mix-CA} (Step 8a)
----------------	-----------------------------

Step 1	0.08
Step 2	
N-Europe	0.02
S-Europe	
Step 3	
D3/ ditch	0.01
D4/ pond	0.00
D4/ stream	0.01
D6/ ditch	0.01
R1/ pond	0.00
R1/ stream	0.01
R2/ stream	0.01
R3/ stream	0.01

Table 9.5-26-2.1-3.2: Summary of the mixture toxicity risk assessment (related to Steps 8a) for invertebrate for FOCUS Step 1 – 3 for application of the tank mix AKO 600 SC and BOA PRO 480 EC in potatoes

FOCUS Scenario	ETR _{mix-CA} (Step 8a)
Step 1	0.06

Step 2	
N-Europe	0.01
S-Europe	
Step 3	
D3/ ditch	0.01
D4/ pond	0.00
D4/ stream	0.01
D6/ ditch	0.01
R1/ pond	0.00
R1/ stream	0.00
R2/ stream	0.00
R3/ stream	0.01

Table 9.5-27-2.1-4.3: Summary of the mixture toxicity risk assessment (related to Steps 8a) for algae for FOCUS Step 1 – 3 for application of the tank mix AKO 600 SC and BOA PRO 480 EC in potatoes

FOCUS Scenario	ETR _{mix-CA} (Step 8a)
Step 1	0.29
Step 2	

N-Europe	0.06
S-Europe	
Step 3	
D3/ ditch	0.02
D4/ pond	0.01
D4/ stream	0.02
D6/ ditch	0.02
R1/ pond	0.00
R1/ stream	0.02
R2/ stream	0.02
R3/ stream	0.03

Table 9.5-28.2.1-5.3: Summary of the mixture toxicity risk assessment (related to Steps 8a) for macrophytes for FOCUS Step 1 – 3 for application of the tank mix AKO 600 SC and BOA PRO 480 EC in potatoes

FOCUS Scenario	ETR _{mix-CA} (Step 8a)
Step 1	8.51
Step 2	
N-Europe	1.84

S-Europe	1
Step 3	1
D3/ ditch	1.04
D4/ pond	0.04
D4/ stream	0.86
D6/ ditch	1.03
R1/ pond	0.04
R1/ stream	0.72
R2/ stream	0.95
R3/ stream	1.02

Table 9.5-29 ~~2.1-6.1~~: Summary of the mixture toxicity risk assessment (related to Step 8a) for macrophytes, based on FOCUS Step 4 calculations for application of the tank mix AKO 600 SC and BOA PRO 480 EC in potatoes

[illegible]

90%	I	I	I	I	I	I	I	I	I	0.01
None	D4 Pond	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.00
None	D4 Stream	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.01
None	D6 Ditch	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.01
None	R1 pond	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.01
None	R1 stream	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.06
None	R2 stream	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.02
None	R3 stream	I	I	I	I	I	I	I	I	I
90%	I	I	I	I	I	I	I	I	I	0.07

Combined exposure conclusion:

ANSWER: ETR_{mix} values are below trigger values. Therefore, a mixture of AKO 600 SC and BOA PRO 480 EC poses no unacceptable risk to aquatic species. However, use of 20 meters vegetative strip and 20 meters no-spray buffer zone and 90% of nozzle reduction is required.

zRMS comments:

Combination toxicity is assessed for formulations containing more than one active substance, and for combinations of products (i.e. tank mixes) that are specified on the label.

For tank-mixes which are included on the label of use with a clear name and dose rate, the same approach should be followed as for formulations with multiple substances.

Tank-mix is proposed for use in potatoes at dose rate:

- AKO 600 SC: 0.48 – 1.2 kg aclonifen./ha (0.8 – 2.0 Lproduct/ha)
- BOA PRO 480 EC: 0.096 kg clomazone/ha (0.2 Lproduct/ha)

Combitox was repeated using AGD AquaMix tool.

Input Tox:

Product data	
Product name	
Density of product [g/cm ³]	1,18
LC ₅₀ fish [mg prod./L]	
LC ₅₀ fish a.s. based [mg sum of a.s./L]	
EC ₅₀ invertebrates [mg prod./L]	
EC ₅₀ invertebrates a.s. based [mg sum of a.s./L]	
EC ₅₀ algae [mg prod./L]	
EC ₅₀ algae a.s. based [mg sum of a.s./L]	
EC ₅₀ macrophytes [mg prod./L]	
EC ₅₀ macrophytes a.s. based [mg sum of a.s./L]	
Calculated mixture toxicity (Eq. 13) based on Tier 1 data only	[mg sum a.s./L]
EC _{50mixture} fish	1,165689698
EC _{50mixture} invertebrates	0,804705882
EC _{50mixture} algae	0,012060151
EC _{50mixture} macrophytes	0,021593903
Calculated mixture toxicity (Eq. 13) based also on additional data	[mg sum a.s./L]
EC _{50mixture} fish	1,165689698
EC _{50mixture} invertebrates	0,804705882
EC _{50mixture} algae	0,012060151
EC _{50mixture} macrophytes	0,021593903

Options

Show Species

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	Aclonifen	Clomazone		
Concentration in product [g a.s./L or g a.s./kg]	600	480		
p(X) (fraction in product)	0,56	0,44		
LC ₅₀ fish [mg a.s./L]	0,67	15,5		
LC ₅₀ invertebrates [mg a.s./L]	1,2	0,57		
EC ₅₀ algae [mg a.s./L]	0,0069	0,185		
EC ₅₀ macrophytes [mg a.s./L]	0,012	34		
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC ₅₀ fish [mg a.s./L]				
LC ₅₀ invertebrates [mg a.s./L]				
EC ₅₀ algae [mg a.s./L]				
EC ₅₀ macrophytes [mg a.s./L]				
AF for RAC				
Fish	100	100	100	100
Invertebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	10	10	10
RAC				
Fish	0,0067	0,155		
Invertebrates	0,012	0,0057		
Algae	0,00069	0,0185		
Macrophytes	0,0012	3,4		
Data used for calculation (after Step 3)				
Active substances	Aclonifen	Clomazone		
Concentration in Product [g a.s./L]	600	480		
p(X) (fraction in product)	0,56	0,44		
LC ₅₀ fish [mg a.s./L]	0,67	15,5		
LC ₅₀ invertebrates [mg a.s./L]	1,2	0,57		
EC ₅₀ algae [mg a.s./L]	0,0069	0,185		
EC ₅₀ macrophytes [mg a.s./L]	0,012	34		

Input PEC assessed and accepted in Part B8:

Used FOCUS Scenarios	Adonifen		Clomazone	
	PECs [µg/L]	Max PECsw	PECs [µg/L]	Max PECsw
Step 1	Step 1	51,4	Step 1	25,11
Step 2	Step 2		Step 2	
N-Europe	N-Europe	11,04	N-Europe	5,46
S-Europe	S-Europe		S-Europe	
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	6,247	D3 Ditch	0,5033
D4 Pond	D4 Pond	0,2516	D4 Pond	0,6784
D4 Stream	D4 Stream	5,156	D4 Stream	0,6741
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch	6,177	D6 Ditch	0,551
R1 Pond	R1 Pond	0,262	R1 Pond	0,076
R1 Stream	R1 Stream	4,315	R1 Stream	1,116
R2 Stream	R2 Stream	5,715	R2 Stream	0,899
R3 Stream	R3 Stream	6,095	R3 Stream	1,749
R4 Stream	R4 Stream		R4 Stream	

Active substance 1: Atraziflufen											
PEC _{calc} [µg/L]	Scenario	FOCUS Step 4									
Node	Vegetative state (m)	Name	Name	Name	Name	Name	5	10	15	20	
reduction	No spray buffer (m)	FOCUS default	5	10	15	20	5	10	15	20	
None	D1 Ditch										
50%											
75%											
90%											
None	D1 Stream										
50%											
75%											
90%											
None	D2 Ditch										
50%											
75%											
90%											
None	D2 Stream										
50%											
75%											
90%											
None	D3 Ditch										
50%											
75%											
90%											0.06
None	D4 Pond										
50%											
75%											
90%											0.06
None	D4 Stream										
50%											
75%											
90%											0.09
None	D5 Pond										
50%											
75%											
90%											
None	D5 Stream										
50%											
75%											
90%											
None	D6 Ditch										
50%											
75%											
90%											0.07
None	R1 Pond										
50%											
75%											
90%											0.01
None	R1 Stream										
50%											
75%											
90%											0.07
None	R2 Stream										
50%											
75%											
90%											0.11
None	R3 Stream										
50%											
75%											
90%											0.02
None	R4 Stream										
50%											
75%											
90%											

Active substance 2: Omisoproc											
PEC _{calc} [µg/L]	Scenario	FOCUS Step 4									
Node	Vegetative state (m)	Name	Name	Name	Name	Name	5	10	15	20	
reduction	No spray buffer (m)	FOCUS default	5	10	15	20	5	10	15	20	
None	D1 Ditch										
50%											
75%											
90%											
None	D1 Stream										
50%											
75%											
90%											
None	D2 Ditch										
50%											
75%											
90%											
None	D2 Stream										
50%											
75%											
90%											
None	D3 Ditch										
50%											
75%											
90%											0.00
None	D4 Pond										
50%											
75%											
90%											0.08
None	D4 Stream										
50%											
75%											
90%											0.07
None	D5 Pond										
50%											
75%											
90%											
None	D5 Stream										
50%											
75%											
90%											
None	D6 Ditch										
50%											
75%											
90%											0.05
None	R1 Pond										
50%											
75%											
90%											0.01
None	R1 Stream										
50%											
75%											
90%											0.04
None	R2 Stream										
50%											
75%											
90%											0.01
None	R3 Stream										
50%											
75%											
90%											0.03
None	R4 Stream										
50%											
75%											
90%											

Final CONCLUSION	low risk
	high risk

FOCUS Step 1, Step 2 and Step 3

Fish		Invertebrates		Algae		Macrophytes	
ETRmix-CA		ETRmix-CA		ETRmix-CA		ETRmix-CA	
Step 1	0,08	Step 1	0,09	Step 1	7,59	Step 1	4,28
Step 2		Step 2		Step 2		Step 2	
N-Europe	0,02	N-Europe	0,02	N-Europe	1,63	N-Europe	0,92
S-Europe		S-Europe		S-Europe		S-Europe	
Step 3		Step 3		Step 3		Step 3	
D1 Ditch		D1 Ditch		D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream		D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch		D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream		D2 Stream		D2 Stream	
D3 Ditch	0,01	D3 Ditch	0,01	D3 Ditch	0,91	D3 Ditch	0,52
D4 Pond	0,00	D4 Pond	0,00	D4 Pond	0,04	D4 Pond	0,02
D4 Stream	0,01	D4 Stream	0,01	D4 Stream	0,75	D4 Stream	0,43
D5 Pond		D5 Pond		D5 Pond		D5 Pond	
D5 Stream		D5 Stream		D5 Stream		D5 Stream	
D6 Ditch	0,01	D6 Ditch	0,01	D6 Ditch	0,90	D6 Ditch	0,51
R1 Pond	0,00	R1 Pond	0,00	R1 Pond	0,04	R1 Pond	0,02
R1 Stream	0,01	R1 Stream	0,01	R1 Stream	0,63	R1 Stream	0,36
R2 Stream	0,01	R2 Stream	0,01	R2 Stream	0,83	R2 Stream	0,48
R3 Stream	0,01	R3 Stream	0,01	R3 Stream	0,89	R3 Stream	0,51
R4 Stream		R4 Stream		R4 Stream		R4 Stream	

Algae											
FOCUS Step 4	Scenario Vegetative strip [m]	Efficacy CA									
Nozzle reduction	No spray buffer [m]	FOCUS default	5	10	15	20	5	10	15	20	
None	D1 Ditch										
50%											
75%											
90%	D1 Stream										
None											
50%											
75%	D2 Ditch										
50%											
75%											
90%	D2 Stream										
None											
50%											
75%	D3 Ditch										
50%											
75%											
90%	D4 Pond										
None											
50%											
75%	D4 Stream										
50%											
75%											
90%	D5 Pond										
None											
50%											
75%	D5 Stream										
50%											
75%											
90%	D6 Ditch										
None											
50%											
75%	D6 Stream										
50%											
75%											
90%	R1 Pond										
None											
50%											
75%	R1 Stream										
50%											
75%											
90%	R2 Stream										
None											
50%											
75%	R3 Stream										
50%											
75%											
90%	R4 Stream										
None											
50%											
75%											
90%											

Macrophytes											
FOCUS Step 4	Scenario Vegetative strip [m]	Efficacy CA									
Nozzle reduction	No spray buffer [m]	FOCUS default	5	10	15	20	5	10	15	20	
None	D1 Ditch										
50%											
75%											
90%	D1 Stream										
None											
50%											
75%	D2 Ditch										
50%											
75%											
90%	D2 Stream										
None											
50%											
75%	D3 Ditch										
50%											
75%											
90%	D4 Pond										
None											
50%											
75%	D4 Stream										
50%											
75%											
90%	D5 Pond										
None											
50%											
75%	D5 Stream										
50%											
75%											
90%	D6 Ditch										
None											
50%											
75%	R1 Pond										
50%											
75%											
90%	R1 Stream										
None											
50%											
75%	R2 Stream										
50%											
75%											
90%	R3 Stream										
None											
50%											
75%	R4 Stream										
50%											
75%											
90%											

Conclusion:

The risk for fish and aquatic invertebrates is acceptable using FOCUS Step 3 PECsw values.
 For algae and aquatic macrophyte 20 meters vegetated buffer zone and 90% of nozzle reduction is required.

9.5.3 Overall conclusions

For the intended uses, calculated PEC/RAC ratios did indicate an unacceptable risk for most sensitive groups of aquatic organisms in FOCUS 1-3 scenarios relevant for RMS and other Central Zone members. Therefore, the further, risk assessment was based on FOCUS Step 4 PEC_{sw} calculations (SWAN ver. 4 and 5) considering reduced exposure of surface water bodies. Calculations and parameters were made and selected for highest acceptable doses according to PART B8 results. According to the GAP, both Aclonifen and its formulation achieve the acceptability criterium $PEC/RAC < 1$ for run-off exposure, however risk mitigation is required. The mixture of AKO 600 SC and BOA PRO 480 EC poses no unacceptable risk to aquatic species. However, use of 20 meters vegetative strip, 20 meters no-spray buffer zone and 90% of nozzle reduction is required

Required risk mitigation and maximum doses of aclonifen that are safe for aquatic organisms are summarized below:

Potatoes:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.5 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
1.4 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Sunflower:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.5 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
0.9 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Maize:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.5 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
0.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Spring oilseeds:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.7 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Winter oilseeds:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
1.3 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Soybeans:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	-
1.2 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 50%	Scenarios relevant for EU Central Zone

Tobacco:

1.5 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	-
1.4 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone

Leafy vegetables:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.6 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
1 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Field beans:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland (VFSmode)
1.5 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
0.9 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

Pome fruits:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 90 m	Nozzle reduction: 0%	Scenarios relevant for Poland
1.4 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 90 m	Nozzle reduction: 0%	Scenarios relevant for EU Central Zone (including Poland)

Legumes:

1.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for Poland
0.8 kg of aclonifen	Vegetative strip: 20 m	No spray buffer: 20 m	Nozzle reduction: 90%	Scenarios relevant for EU Central Zone (including Poland)

According to available data and calculations AKO 600 SC will not pose a risk to aquatic organisms (PEC_{sw}/RAC <1) if:

- 30m buffer zone is used on potatoes, sunflower, flax, spring oilseeds, breadseed poppy, mustard, soyabeans, pumpkin and hemp
- 25m buffer zone is used on tobacco
- 75m buffer zone is used on forest nurseries plants (pine tree, spruce, fir tree, larch), ornamental shrubs, basket willow, purple willow, forest tree, christmas tree, energetic willow in Poland

zRMS conclusion:

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290).

For the risk assessment in general the UE agreed endpoints for the active substance were used.

For AKO 600 SC tests on *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Lemna gibba* and *Anabaena flos-aquae* were provided by Applicant, but no test is reported for fish.

According to the Commission Regulation (EU) No 284/2013, point 10.2.1 Acute toxicity:

“Test shall be carried out on one species from each of the three/four groups of aquatic organisms, that is to say fish, aquatic invertebrates, algae...”

and

“Testing shall be performed where:

(a) the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance;...”

Species	Aclonifen	AKO 600 SC
	Endpoint	Endpoint
<i>Oncorhynchus mykiss</i>	0.670 mg a.s./L	-
<i>Daphnia magna</i>	1.2 mg a.s./L	EC ₅₀ = 15.46mg test item/L _{nom} EC ₅₀ = 7.71 mg aclonifen/L _{nom}
<i>Pseudokirchneriella subcapitata</i>	13 mg a.s./L	E _r C ₅₀ = 0.025 mg test item/L _{nom} E _r C ₅₀ = 12.801 µg aclonifen/L
<i>Desmodesmus subspicatus</i>	0.0069 mg/L	-
<i>Lemna gibba</i>	0.012mg a.s./L	E _r C ₅₀ frond number = 0.0135 mg test item/L _{nom} E _r C ₅₀ frond number = 0.006748 mg/L _{nom}
<i>Anabaena flos-aquae</i>		E _r C ₅₀ = 2.81 mg test item/L _{nom} E _r C ₅₀ = 1.40 mg aclonifen/L _{nom}

Based on the acute aquatic data for active substance is not expected that the formulation to be more sensitive to fish than to algae and macrophytes. It can be assumed that acute toxicity to fish for formulation can be predicted on the basis of data for the active.

Based on the PEC/RAC calculations for the active substance aclonifen, at the highest doses, unacceptable risk for aquatic organisms is indicated for some scenarios.

Therefore doses reduction was proposed by Applicant. However, in the case of surrogate crops, reduced doses is not always comparable to the proposed lower dose for main crop.

Risk mitigation measures and dose reduction should therefore be considered at national level.

Based on the PEC/RAC calculations for the formulation AKO 600 SC, at the highest doses, no unacceptable risk for aquatic organisms is indicated with the appropriate risk mitigation measures.

Additionally tank-mix is proposed for use in potatoes at dose rate:

- AKO 600 SC: 0.48 – 1.2 kg aclonifen/ha (0.8 – 2.0 Lproduct/ha)

- BOA PRO 480 EC: 0.096 kg clomazone/ha (0.2 L_{product}/ha)
 Calculations of PEC_{sw} values at proposed dose rates for aclonifen (1.2 kg a.s./ha) and clomazone (0.096 kg a.s./ha) were assessed and accepted in Part B8 and used in Mixture Toxicity.
 Based on the combitox results the risk is acceptable when 20 m vegetative buffer zone and 90% of nozzle reduction is applied

Conclusion:

Overview on appropriate risk mitigation measures to receive an acceptable acute and chronic risk for the aquatic organisms through the use of AKO 600 SC in various crops is presented below.

ACLONIFEN				
Potatoes				
1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L _{product} /ha) (PL, DE)				
0.48 – 1.2 kg a.s./ha (0.8 – 2.0 L _{product} /ha) (PL)				
1.8 kg a.s./ha				
	D3 ditch		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D4 pond		Step 3	acceptable
	D4 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D6 ditch		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R1 pond		Step 3	acceptable
	R1 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R2 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R3 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D5 pond	surrogate crop Legumes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D5 stream	surrogate crop Legumes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R4 stream	surrogate crop Legumes	Step 4 VFS mode	unresolved
	1.5 kg/ha			
	D3 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	D4 pond		Step 3	acceptable
	D4 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	D6 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R1 pond		Step 3	acceptable
	R1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R2 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R3 stream		Step 4	unresolved
	D5 pond	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
	D5 stream	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
	R4 stream	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
1.4 kg/ha				
	D3 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	D4 pond		Step 3	acceptable

	D4 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	D6 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R1 pond		Step 3	acceptable
	R1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R2 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
	R3 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	D5 pond	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
	D5 stream	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
	R4 stream	surrogate crop Legumes	Step 4	Lack of PEC/RAC calculation
0.8 kg/ha				
	D5 pond	surrogate crop Legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	D5 stream	surrogate crop Legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	R4 stream	surrogate crop Legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction;
Sunflower				
1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (HU, RO, SK, PL)				
1.8 kg/ha				
	D5 pond		Step 3	acceptable
	D5 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
	R1 pond		Step 3	acceptable
	R1 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R3 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	R4 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D3 ditch	surrogate crop Maize	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
	D4 pond	surrogate crop Maize	Step 3	acceptable
	D4 stream	surrogate crop Maize	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
1.5 kg/ha				
	D5 pond		Step 3	acceptable
	D5 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	R1 pond		Step 3	acceptable
	R1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	R3 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	R4 stream		Step 4	unresolved
	D3 ditch	surrogate crop Maize	Step 4	Not assessed in Part B8
	D4 pond	surrogate crop Maize	Step 3	Not assessed in Part B8
	D4 stream	surrogate crop Maize	Step 4	Not assessed in Part B8;
0.9 kg/ha				
	D5 pond		Step 3	acceptable
	D5 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
	R1 pond		Step 3	acceptable
	R1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;

R3 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
R4 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction;
D3 ditch	surrogate crop Maize	Step 4	Lack of PEC/RAC calculation
D4 pond	surrogate crop Maize	Step 4	Lack of PEC/RAC calculation
D4 stream	surrogate crop Maize	Step 4	Lack of PEC/RAC calculation
0.8 kg/ha			
D3 ditch	surrogate crop Maize	Step 4	Not assessed in Part B8
D4 pond	surrogate crop Maize	Step 3	Not assessed in Part B8
D4 stream	surrogate crop Maize	Step 4	Not assessed in Part B8
Spring Oilseed rape 1.17 – 1.8 kg a.s./ha (1.95 – 3.0 Lproduct/ha) (PL, DE, SK)			
1.8 kg/ha			
D1 ditch		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
D1 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
D3 ditch		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
D4 pond		Step 3	acceptable
D4 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
D5 pond		Step 3	acceptable
D5 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
R1 pond		Step 3	acceptable
R1 stream		Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction;
R3 stream	surrogate crop winter OSR	Step 4 VFS mode	unresolved
R4 stream	surrogate crop legumes	Step 4 VFS mode	unresolved
1.7 kg/ha			
D1 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D3 ditch		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D4 pond		Step 3	acceptable
D4 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D5 pond		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D5 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
R1 pond		Step 3	acceptable
R1 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
R3 stream			Lack of PEC/RAC calculation
R4 stream			Lack of PEC/RAC calculation
1.3 kg/ha			
R3 stream	surrogate crop winter OSR	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R4 stream	surrogate crop legumes		Lack of PEC/RAC calculation
0.8 kg/ha			
R4 stream	surrogate crop legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction

Flax (surrogate crops: spring oilseed rape, winter oilseed rape and legumes)

1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL, HU, RO, SK)

- see table above

Breadseed poppy (surrogate crops: spring oilseed rape, winter oilseed rape and legumes)

1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL, DE, HU, RO, SK)

- see table above

Mustard (surrogate crops: spring oilseed rape, winter oilseed rape and legumes)

1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL, RO, SK)

- see table above

Hemp (surrogate crops: spring oilseed rape, winter oilseed rape and legumes)

1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL, DE, HU, RO, SK)

- see table above

Soybeans

1.17 – 1.8 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL)

1.8 kg/ha

R3 stream		Step 4	unresolved
R4 stream		Step 4	unresolved
D3 ditch	surrogate crop - Field beans	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop - Field beans	Step 3	acceptable
D4 stream	surrogate crop - Field beans	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop - Field beans	Step 3	acceptable
R1 stream	surrogate crop - Field beans	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction

1.5 kg/ha

R3 stream			Lack of PEC/RAC calculation
R4 stream			Lack of PEC/RAC calculation
D3 ditch	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop - Field beans	Step 3	acceptable
D4 stream	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop - Field beans	Step 3	acceptable
R1 stream	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes		Lack of PEC/RAC calculation

1.2 kg/ha

R3 stream		Step 4	20 m vegetative filter strip and 50% nozzle reduction
R4 stream		Step 4	20 m vegetative filter strip and 50% nozzle reduction
D3 ditch	surrogate crop - Field beans		Lack of PEC/RAC calculation

D4 pond	surrogate crop - Field beans	Step 3	acceptable
D4 stream	surrogate crop - Field beans		Lack of PEC/RAC calculation
R1 pond	surrogate crop - Field beans	Step 3	acceptable
R1 stream	surrogate crop - Field beans		Lack of PEC/RAC calculation
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes		Lack of PEC/RAC calculation
0.9 kg/ha			
R3 stream			Lack of PEC/RAC calculation
R4 stream			Lack of PEC/RAC calculation
D3 ditch	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop - Field beans	Step 3	acceptable
D4 stream	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop - Field beans	Step 3	acceptable
R1 stream	surrogate crop - Field beans	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes		Lack of PEC/RAC calculation
0.8 kg/ha			
R3 stream			Lack of PEC/RAC calculation
R4 stream			Lack of PEC/RAC calculation
D3 ditch			Lack of PEC/RAC calculation
D4 pond			Lack of PEC/RAC calculation
D4 stream			Lack of PEC/RAC calculation
R1 pond			Lack of PEC/RAC calculation
R1 stream			Lack of PEC/RAC calculation
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
Tobacco 1.08 – 1.5 kg a.s./ha (1.95 – 3.0 L_{product}/ha) (PL, HU, RO, SK)			
1.8 kg/ha			
R3 stream			Lack of PEC/RAC calculation
D3 ditch	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R4 stream	surrogate crop pome	Step 4	unresolved

	fruits		
1.5 kg/ha			
R3 stream		Step 4	unresolved
D3 ditch			Lack of PEC/RAC calculation
D4 pond			Lack of PEC/RAC calculation
D4 stream			Lack of PEC/RAC calculation
D5 pond			Lack of PEC/RAC calculation
D5 stream			Lack of PEC/RAC calculation
R1 pond			Lack of PEC/RAC calculation
R1 stream			Lack of PEC/RAC calculation
R4 stream			Lack of PEC/RAC calculation
1.4 kg/ha			
R3 stream		Step 4	20 m vegetative filter strip and 90% nozzle reduction
D3 ditch	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
Pumpkin			
1.17 – 1.8 kg a.s./ha (1.95 – 3.0 Lproduct/ha) (PL, DE, HU, RO, SK)			
1.8 kg/ha			
D3 ditch	surrogate crop potatoes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop potatoes	Step3	acceptable
D4 stream	surrogate crop potatoes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop potatoes	Step3	acceptable
R1 stream	surrogate crop potatoes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
R3 stream	surrogate crop potatoes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
D5 pond	surrogate crop legumes	Step3	acceptable
D5 stream	surrogate crop legumes	Step 4 VFS mode	20 m vegetative filter strip and 90% nozzle reduction
R4 stream	surrogate crop legumes	Step 4 VFS mode	unresolved
1.5 kg/ha			
D3 ditch	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop potatoes	Step 3	acceptable
D4 stream	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop potatoes	Step 3	acceptable
R1 stream	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R3 stream	surrogate crop potatoes	Step 4	unresolved

D5 pond	surrogate crop legumes	Step3	acceptable
D5 stream	surrogate crop legumes		Lack of PEC/RAC calculation
R4 stream	surrogate crop legumes		Lack of PEC/RAC calculation
1.4 kg/ha			
D3 ditch	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D4 pond	surrogate crop potatoes	Step 3	acceptable
D4 stream	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R1 pond	surrogate crop potatoes	Step 3	acceptable
R1 stream	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R3 stream	surrogate crop potatoes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes		Lack of PEC/RAC calculation
R4 stream	surrogate crop legumes		Lack of PEC/RAC calculation
0.8 kg/ha			
D3 ditch	surrogate crop potatoes		Lack of PEC/RAC calculation
D4 pond	surrogate crop potatoes		Lack of PEC/RAC calculation
D4 stream	surrogate crop potatoes		Lack of PEC/RAC calculation
R1 pond	surrogate crop potatoes		Lack of PEC/RAC calculation
R1 stream	surrogate crop potatoes		Lack of PEC/RAC calculation
R3 stream	surrogate crop potatoes		Lack of PEC/RAC calculation
D5 pond	surrogate crop legumes	Step 3	acceptable
D5 stream	surrogate crop legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
R4 stream	surrogate crop legumes	Step 4	20 m vegetative filter strip and 90% nozzle reduction
Trees and ornamental shrugs: Forest nurseries plants 1.17 – 1.8 kg a.s./ha (1.95 – 3.0 Lproduct/ha) (PL, HU, RO, SK) Ornamental shrubs, Basket willow, Purple willow, Forest tree, Christmas tree, Energetic willow 1.17 – 1.50 kg a.s./ha (1.95 – 2.5 Lproduct/ha) (PL, HU, RO, SK)			
1.8 kg/ha			
D3 ditch	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R3 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R4 stream	surrogate crop pome fruits	Step 4	unresolved
1.4 kg/ha			
D3 ditch	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D4 pond	surrogate crop pome	Step 4	90 m buffer zone with 20 m vegetative filter strip

	fruits		
D4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
D5 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 pond	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R1 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R3 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip
R4 stream	surrogate crop pome fruits	Step 4	90 m buffer zone with 20 m vegetative filter strip

AKO 600 SC				
Potatoes	3 L/ha		30 m buffer zone	30 m buffer zone
		based on the surrogate crop legumes	30 m buffer zone	
Sunflower	3 L/ha		30 m buffer zone	30 m buffer zone
		based on the surrogate crop maize	30 m buffer zone	
Spring oilseeds	3 L/ha		30 m buffer zone	30 m buffer zone
		based on the surrogate crop winter oilseeds	30 m buffer zone	
Soybeans	3 L/ha		30 m buffer zone	30 m buffer zone
		based on the surrogate crop field beans	30 m buffer zone	
Tobacco	2.5 L/ha		25 m buffer zone	75 m buffer zone
		based on the surrogate crop pome fruits	75 m buffer zone	
Flax, breadseed poppy, mustard and hemp	3 L/ha	based on the surrogate crops spring oilseed rape, winter oilseed rape and legumes	30 m buffer zone	30 m buffer zone
Pumpkin	3 L/ha	based on the surrogate crops potatoes and legumes	30 m buffer zone	30 m buffer zone
Trees and ornamental shrubs	3L/ha	based on the surrogate crops pome fruits	75 m buffer zone	75 m buffer zone

Tank-mix:

AKO 600 SC: 0.48 – 1.2 kg aclonifen/ha (0.8 – 2.0 Lproduct/ha) with BOA PRO 480 EC: 0.096 kg clomazone/ha (0.2 Lproduct/ha) - 20 m vegetative buffer zone and 90% of nozzle reduction.

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

Effects on bees of AKO 600 SC were not evaluated as part of the EU assessment of Aclonifen. .

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

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Aclonifen	Oral	LD ₅₀ > 107 µg a.s./bee	EFSA Scientific Report (2008) 149, 1-80
<i>Apis mellifera</i>	Aclonifen	Contact	LD ₅₀ > 100 µg a.s./bee	EFSA Scientific Report (2008) 149, 1-80
<i>Apis mellifera</i>	AKO 600 SC	Oral	LD ₅₀ > 200 µg/bee LD ₅₀ > 99.8 µg a.s./bee	Wojciech A. Study code: B-80-23 Date: 10.2023
<i>Apis mellifera</i>	AKO 600 SC	Contact	LD ₅₀ > 200 µg/bee LD ₅₀ > 99.8 µg a.s./bee	Wojciech A. Study code: B-81-23 Date: 10.2023
<i>Apis mellifera</i>	AKO 600 SC	Larval	LOED > 33.33 µg of the test item/larva (> 16.63 µg a.s./larva) NOED > 11.11 µg of the test item/larva (> 5.54 µg a.s./larva)	Woźniak A. Study code: 0038/0162E Date: 01.2024
<i>Apis mellifera</i>	AKO 600 SC	Chronic	LDD50 > 93.58 µg of the test item/bee/day (> 46.7 µg a.s./bee/day) NOEDD > 93.58 µg of the test item/bee/day (> 46.7 µg a.s./bee/day) LOEDD > 93.58 µg of the test item/bee/day (> 46.7 µg a.s./bee/day)	Zawrońska K. Study code: 0038/0164/E Date: 11.2023
<i>Bombus spp.</i>	AKO 600 SC	Oral	LD ₅₀ > 200 µg/bumblebee LD ₅₀ > 99.8 µg a.s./bumblebee	Wojciech A. Study code: B-74-23 Date: 01.2024
<i>Bombus spp.</i>	AKO 600 SC	Contact	LD ₅₀ > 200 µg/bumblebee LD ₅₀ > 99.8 µg a.s./bumblebee	Wojciech A. Study code: B-75-23 Date: 01.2024

Species	Substance	Exposure System	Results	Reference
Higher-tier studies (tunnel test, field studies)				
n/a				

9.6.1.1 Justification for new endpoints

No new endpoints were established

9.6.2 Risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for bees from all other intended uses in groups described in GAP (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of AKO 600 SC in group no. 1

Intended use		Group no. 1	
Active substance		Aclonifen	
Application rate (g/ha)		1 × 1800 g/ha	
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	107	1800	16.82
Contact toxicity	100		18
Product		AKO 600 SC	
Application rate (g/ha)		1 × 3635.4 g/ha	
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	200	3635.4	18.18
Contact toxicity	200		18.18

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

The risk assessment for bees which are presented below contains only assessment for honey bees larvae and assessment of the chronic risk for bees after use of the product AKO 600 SC. Acute contact and oral risk has already been assessed and presented in table 9.6-2.

The assessment was done by using Bee Tool v.3

a) Screening step

Table 9.6-3 Screening step assessment of the chronic risk for bees and assessment for honey bees

larvae due to the use of AKO 600 SC

Intended uses	Potato, sunflower, rapeseed, flax, poppy, mustard, soyabean, pumpkin, hemp, tobacco, forest nurseries plants, ornamental shrubs, absket willow, purple willow, forest tree, christmas tree, energetic willow.			
Product	AKO 600 SC			
Application rate (g product/ha)	1x 3635.4			
Test design	Endpoints (µg/bee/d or µg/larva)	Ef x SV	ETR	Trigger
Chronic oral toxicity	LDD50= 93.58 µg/bee/day	7.6	0.297	0.03
Larvae toxicity	NOED= 11.11 µg ai/larva	4.4	1.45	0.2
Oral toxicity	200	7.6	0.14	0.2
Contact toxicity	200		18.2	42

The calculated ETR values of honey bees larvae is higher than the trigger values of 0.2, therefore Tier 1 assessment is required and provided below

The calculated ETR values of chronic oral toxicity for adult bees is higher than the trigger values of 0.03, therefore Tier 1 assessment is required and provided below

b) First tier assessment

Table. 9.6-4 First tier assessment of the chronic oral risk for bees due to the use of AKO 600 SC

Intended use	Potato, sunflower, rapeseed, flax, poppy, mustard, soyabean, pumpkin, hemp, tobacco, forest nurseries plants, ornamental shrubs, absket willow, purple willow, forest tree, christmas tree, energetic willow.					
Product	AKO 600 SC					
Application rate (g product/ha)	1 x 3635.4					
Crop category	Application type	Category	Scenario	BBCH	Honeybee	
					ETR	Trigger
Bare soil - crop attractive for pollen and nectar	spray DW	chronic	treated crop	< 10	0.02	0.03
Bare soil - crop attractive for pollen and nectar	spray DW	chronic	weeds	< 10	0.01	0.03
Bare soil - crop attractive for pollen and nectar	spray DW	chronic	field margin	< 10	0.00	0.03
Bare soil - crop attractive for pollen and nectar	spray DW	chronic	adjacent crop	< 10	0.00	0.03

Bare soil - crop attractive for pollen and nectar	spray DW	chronic	next crop	< 10	0.02	0.03
Bare soil - crop attractive for pollen, only	spray DW	chronic	treated crop	< 10	0.00	0.03
Bare soil - crop attractive for pollen, only	spray DW	chronic	weeds	< 10	0.01	0.03
Bare soil - crop attractive for pollen, only	spray DW	chronic	field margin	< 10	0.00	0.03
Bare soil - crop attractive for pollen, only	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Bare soil - crop attractive for pollen, only	spray DW	chronic	next crop	< 10	0.02	0.03
Bare soil - crop attractive for nectar, only	spray DW	chronic	treated crop	< 10	0.02	0.03
Bare soil - crop attractive for nectar, only	spray DW	chronic	weeds	< 10	0.01	0.03
Bare soil - crop attractive for nectar, only	spray DW	chronic	field margin	< 10	0.00	0.03
Bare soil - crop attractive for nectar, only	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Bare soil - crop attractive for nectar, only	spray DW	chronic	next crop	< 10	0.02	0.03
Potatoes	spray DW	chronic	treated crop	< 10	0.00	0.03
Potatoes	spray DW	chronic	weeds	< 10	0.08	0.03
Potatoes	spray DW	chronic	field margin	< 10	0.00	0.03
Potatoes	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Potatoes	spray DW	chronic	next crop	< 10	0.02	0.03
Oilseed rape (rapeseed)	spray DW	chronic	treated crop	< 10	0.02	0.03
Oilseed rape (rapeseed)	spray DW	chronic	weeds	< 10	0.08	0.03
Oilseed rape (rapeseed)	spray DW	chronic	field margin	< 10	0.00	0.03
Oilseed rape (rapeseed)	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Oilseed rape (rapeseed)	spray DW	chronic	next crop	< 10	0.02	0.03
Sunflower	spray DW	chronic	treated crop	< 10	0.02	0.03
Sunflower	spray DW	chronic	weeds	< 10	0.08	0.03
Sunflower	spray DW	chronic	field margin	< 10	0.00	0.03
Sunflower	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Sunflower	spray DW	chronic	next crop	< 10	0.02	0.03

Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	treated crop	< 10	0.02	0.03
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	weeds	< 10	0.08	0.03
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	field margin	< 10	0.00	0.03
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	next crop	< 10	0.02	0.03
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	chronic	treated crop	< 10	0.02	0.03
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	chronic	weeds	< 10	0.08	0.03
Fruiting vegetables 1: Chillies, Peppers, Cu-	spray DW	chronic	field margin	< 10	0.00	0.03

cumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Water-melons						
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Water-melons	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Water-melons	spray DW	chronic	next crop	< 10	0.02	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	treated crop	< 10	0.02	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	weeds	< 10	0.08	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	field margin	< 10	0.00	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	next crop	< 10	0.02	0.03
Poppy seed	spray DW	chronic	treated crop	< 10	0.00	0.03
Poppy seed	spray DW	chronic	weeds	< 10	0.08	0.03
Poppy seed	spray DW	chronic	field margin	< 10	0.00	0.03
Poppy seed	spray DW	chronic	adjacent crop	< 10	0.00	0.03
Poppy seed	spray DW	chronic	next crop	< 10	0.02	0.03

The calculated ETR values of chronic oral toxicity for field margin, adjacent crop, next crops and treated crops scenarios are lower than the trigger value of 0.03. AKO 600 SC poses no unacceptable risk to bees at the proposed label rate.

The calculated ETR value of chronic oral toxicity for weeds scenario exceeds the trigger value of 0.03 which means AKO 600 SC poses a risk to bees at the proposed label rate.

Table. 9.6-5 First tier assessment of risk for larval bees due to the use of AKO 600 SC

Intended use	Potato, sunflower, rapeseed, flax, poppy, mustard, soyabean, pumpkin, hemp, tobacco, forest nurseries plants, ornamental shrubs, absket willow, purple willow, forest tree, christmas tree, energetic willow.
Product	AKO 600 SC
Application rate (g	1 x 3635.4

product/ha)						
Crop category	Application type	Category	Scenario	BBCH	Larval bees	
					ETR	Trigger value
Bare soil - crop attractive for ollen and nectar	spray DW	larva	treated crop	< 10	0.11	0.2
Bare soil - crop attractive for pollen and nectar	spray DW	larva	weeds	< 10	0.06	0.2
Bare soil - crop attractive for pollen and nectar	spray DW	larva	field margin	< 10	0.01	0.2
Bare soil - crop attractive for pollen and nectar	spray DW	larva	adjacent crop	< 10	0.00	0.2
Bare soil - crop attractive for pollen and nectar	spray DW	larva	next crop	< 10	0.11	0.2
Bare soil - crop attractive for pollen, only	spray DW	larva	treated crop	< 10	0.00	0.2
Bare soil - crop attractive for pollen, only	spray DW	larva	weeds	< 10	0.06	0.2
Bare soil - crop attractive for pollen, only	spray DW	larva	field margin	< 10	0.01	0.2
Bare soil - crop attractive for pollen, only	spray DW	larva	adjacent crop	< 10	0.00	0.2
Bare soil - crop attractive for pollen, only	spray DW	larva	next crop	< 10	0.11	0.2
Bare soil - crop attractive for nectar, only	spray DW	larva	treated crop	< 10	0.11	0.2
Bare soil - crop attractive for nectar, only	spray DW	larva	weeds	< 10	0.06	0.2

Bare soil - crop attractive for nectar, only	spray DW	larva	field margin	< 10	0.01	0.2
Bare soil - crop attractive for nectar, only	spray DW	larva	adjacent crop	< 10	0.00	0.2
Bare soil - crop attractive for nectar, only	spray DW	larva	next crop	< 10	0.11	0.2
Potatoes	spray DW	larva	treated crop	< 10	0.00	0.2
Potatoes	spray DW	larva	weeds	< 10	0.62	0.2
Potatoes	spray DW	larva	field margin	< 10	0.01	0.2
Potatoes	spray DW	larva	adjacent crop	< 10	0.00	0.2
Potatoes	spray DW	larva	next crop	< 10	0.11	0.2
Oilseed rape (rapeseed)	spray DW	larva	treated crop	< 10	0.11	0.2
Oilseed rape (rapeseed)	spray DW	larva	weeds	< 10	0.62	0.2
Oilseed rape (rapeseed)	spray DW	larva	field margin	< 10	0.01	0.2
Oilseed rape (rapeseed)	spray DW	larva	adjacent crop	< 10	0.00	0.2
Oilseed rape (rapeseed)	spray DW	larva	next crop	< 10	0.11	0.2
Sunflower	spray DW	larva	treated crop	< 10	0.11	0.2
Sunflower	spray DW	larva	weeds	< 10	0.62	0.2
Sunflower	spray DW	larva	field margin	< 10	0.01	0.2
Sunflower	spray DW	larva	adjacent crop	< 10	0.00	0.2
Sunflower	spray DW	larva	next crop	< 10	0.11	0.2
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegeta-	spray DW	larva	treated crop	< 10	0.11	0.2

bles, Lentis, Lupins, Peas, Soybeans, Vetches						
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Le- guminous for silage, Leguminous vegeta- bles, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	larva	weeds	< 10	0.62	0.2
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Le- guminous for silage, Leguminous vegeta- bles, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	larva	field margin	< 10	0.01	0.2
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Le- guminous for silage, Leguminous vegeta- bles, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	larva	adjacent crop	< 10	0.00	0.2
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Le- guminous for silage, Leguminous vegeta- bles, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	larva	next crop	< 10	0.11	0.2
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gher- kins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	larva	treated crop	< 10	0.11	0.2
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gher- kins, Pumpkins,	spray DW	larva	weeds	< 10	0.62	0.2

Squash, Gourds, Melon, Watermelons						
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	larva	field margin	< 10	0.01	0.2
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	larva	adjacent crop	< 10	0.00	0.2
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	larva	next crop	< 10	0.11	0.2
Flax: Linseed, Mustard seed	spray DW	larva	treated crop	< 10	0.11	0.2
Flax: Linseed, Mustard seed	spray DW	larva	weeds	< 10	0.62	0.2
Flax: Linseed, Mustard seed	spray DW	larva	field margin	< 10	0.01	0.2
Flax: Linseed, Mustard seed	spray DW	larva	adjacent crop	< 10	0.00	0.2
Flax: Linseed, Mustard seed	spray DW	larva	next crop	< 10	0.11	0.2
Poppy seed	spray DW	larva	treated crop	< 10	0.00	0.2
Poppy seed	spray DW	larva	weeds	< 10	0.62	0.2
Poppy seed	spray DW	larva	field margin	< 10	0.01	0.2
Poppy seed	spray DW	larva	adjacent crop	< 10	0.00	0.2
Poppy seed	spray DW	larva	next crop	< 10	0.11	0.2

The calculated ETR values of larval toxicity for field margin, adjacent crop, next crops and treated crops scenarios are lower than the trigger value of 0.032. AKO 600 SC poses no unacceptable risk to larvae bees at the proposed label rate.

The calculated ETR value of larval toxicity for weeds scenario exceeds the trigger value of 0.032 which means AKO 600 SC poses a risk to larvae bees at the proposed label rate.

c) Risk refinements

Table 9.6-6 Intended uses not passing the 1st oral tier risk assessment of AKO 600 SC

Scenario	Application method	Category	Scenario	BBCH	ETR	Trigger value
Potatoes	spray DW	chronic	weeds	< 10	0.08	0.03
Oilseed rape (rapeseed)	spray DW	chronic	weeds	< 10	0.08	0.03
Sunflower	spray DW	chronic	weeds	< 10	0.08	0.03
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	chronic	weeds	< 10	0.08	0.03
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	chronic	weeds	< 10	0.08	0.03
Flax: Linseed, Mustard seed	spray DW	chronic	weeds	< 10	0.08	0.03
Poppy seed	spray DW	chronic	weeds	< 10	0.08	0.03
Potatoes	spray DW	larva	weeds	< 10	0.62	0.2
Oilseed rape (rapeseed)	spray DW	larva	weeds	< 10	0.62	0.2
Sunflower	spray DW	larva	weeds	< 10	0.62	0.2
Pulses: Beans, Broad beans, Horse beans, Buckwheat, Chick peas, Cow peas, Leguminous for silage, Leguminous vegetables, Lentis, Lupins, Peas, Soybeans, Vetches	spray DW	larva	weeds	< 10	0.62	0.2
Fruiting vegetables 1: Chillies, Peppers, Cucumbers, Gherkins, Pumpkins, Squash, Gourds, Melon, Watermelons	spray DW	larva	weeds	< 10	0.62	0.2
Flax: Linseed, Mustard seed	spray DW	larva	weeds	< 10	0.62	0.2
Poppy seed	spray DW	larva	weeds	< 10	0.62	0.2

Risk refinements for the crops which are described in table above are necessary for improving assessment of the chronic oral risk for bees and bee larvae.

According to Guidance Document on risk assessment on bees – (EFSA Journal 2013;11(7):3295) to improve the chronic risk assessment for weeds:

- It is recommended to remove weeds before flowering period

Due to AKO 600 SC being herbicide used for selective or non-selective weed control in crops, its uses at early plant growth stages (BBCH < 10 for all intended uses) causes the lowerence or fully removing presence of weeds on field before their flowering period. Due to that, weeds scenarios should be considered not relevant. Therefore AKO 600 SC pose no unacceptable chronic risk to bees and its larvae.

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

Not relevant.

9.6.3 Effects on bumble bees

Table 9.6-3: First-tier assessment of the risk for bumblebees due to the use of AKO 600 SC in group no. 1

Product		AKO 600 SC	
Application rate (g/ha)		1 × 3635.4 g/ha	
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	200	3635.4	18.18
Contact toxicity	200		18.18

9.6.4 Effects on solitary bees

Not relevant

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that AKO 600 SC applied at the maximum use rate in potatoes (worst case scenario application rate) poses low risk to bees

zRMS Comments:

The submitted risk assessment is based on the recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2) and new EU guidance (2013).

The EU agreed endpoints for active substance were used in risk assessment.

In addition to that, the Applicant submitted studies on chronic toxicity of formulation AKO 600 SC to adult bees and larvae. New studies were accepted. Therefore, the requirements set out in Regulation 284/2013 are considered fulfilled.

The acute risk assessment performed in accordance with the SANCO guidance presented by the Applicant was accepted.

There is currently no EU agreed chronic risk assessment scheme for bees. However, as agreed in the Central Zone a risk assessment based on the EFSA bee GD is presented below for illustrative purposes.

The ETR values are less than the Tier 1 trigger values for downward sprays indicating that the chronic risk to honeybee larvae and bees is acceptable following use of AKO 600 SC according to the proposed

use pattern excluding weeds scenario.

In the Tier 1 assessment, an unacceptable chronic risk was indicated for adult bees foraging on weeds in the treated fields. As no further studies were submitted to solve the chronic risk indicated for adult bees, the risk assessment could not be finalized. For bee larvae an unacceptable risk was indicated for weeds scenario. As no further studies were submitted to solve the chronic risk indicated for adult bees and larvae the risk assessment could not be finalized..

Final decision of this issue and its consequence on the registration process will have to be dealt with at the product authorization by the CMS that consider indications of EFSA (2013) at the national level, since at the zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level.

An acceptable risk to bees of the formulation AKO 600 SC can be concluded, based on the risk assessment scheme of the Guidance Document on Terrestrial Eco-toxicology (SANCO/10329/2002 rev 2).

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

Effects on non-target arthropods of AKO 600 SC were not evaluated as part of the EU assessment Aclonifen. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Coccinella septempunctata</i>	AKO 600 SC	Extended laboratory test	ER ₅₀ > 3L/ha which is equivalent to 3654 g/ha	Wojciech A. Study code: B-76-23 Date: 02.2024
<i>Typhlodromus pyri</i> (Sch.)	AKO 600 SC	Extended laboratory test	ER ₅₀ > 1.871L/ha which is equivalent to 2279 g/ha	Wojciech A. Study code: B-77-23 Date: 03.2024
<i>Aphidius rhopalosiphi</i>	AKO 600 SC	Extended laboratory test	ER ₅₀ > 4.119L/ha which is equivalent to 5017 g/ha	Wojciech A. Study code: B-78-23 Date: 01.2024
<i>Chrysoperla carnea</i>	AKO 600 SC	Extended laboratory test	ER ₅₀ > 3L/ha which is equivalent to 3654 g/ha	Wojciech A. Study code: B-79-23 Date: 01.2024
<i>Typhlodromus pyri</i>	AKO 600 SC	Aged-residue extended laboratory test	> 6090 g/ha (at 28 and 42 DAT)	Zaworska K. Study code: 0038/0197/E Date:10.2024

9.7.1.1 Justification for new endpoints

Not required

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the group no. 1 also covers the risk for non-target arthropods from all other intended uses described in the GAP (see 9.1.2).

Table 9.7-2: Higher tier assessment of the in-field risk for non-target arthropods due to the use of AKO 600 SC in group no. 1

Intended use	Group no. 1		
Active substance/product	AKO 600 SC		
Application rate (g/ha)	1 x 3654 g/ha		
MAF	1		
Test species Higher tier	ER50 (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 1
<i>Coccinella septempunctata</i>	3654	3654	1
<i>Typhlodromus pyri</i> (Sch.)	2279		1.6
<i>Aphidius rhopalosiphi</i>	5017		0.73
<i>Chrysoperla carnea</i>	3654		1

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

* If an LR50 or ER50 from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Intended use	Bare soil		
Active substance/product	Aclonifen		
Application rate (g/ha)	1 x 3654 g/ha		
MAF	1		
Test species Higher-tier	Rate with ≤ 50 % effect (g/ha)	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Coccinella septempunctata</i>	3654	3654	Yes
<i>Typhlodromus pyri</i> (Sch.)	2279		No
<i>Aphidius rhopalosiphi</i>	5017		Yes

<i>Chrysoperla carnea</i>	3654		Yes
Aged residues			
<i>Typhlodromus pyri</i>	> 6090 (at 28 and 42 DAT)	3654	Yes

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

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the potato also covers the risk for non-target arthropods from all other intended uses described in the GAP (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of AKO 600 SC in group no . 1

Intended use		Group no. 1			
Active substance/product		AKO 600 SC			
Application rate (g/ha)		1 x 3654 g/ha			
MAF		1			
vdf		1 (higher tier)			
Test species	ER₅₀ (lab.) (g/ha)	Drift rate [%/100]	PER_{off-field} (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 1
<i>Coccinella septempunctata</i>	3654	0.0277	101.22	5	0.14
<i>Typhlodromus pyri</i> (Sch.)	2279		101.22		0.22
<i>Aphidius rhopalosiphi</i>	5017		101.22		0.10
<i>Chrysoperla carnea</i>	3654		101.22		0.14

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

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Combined exposure

Combined exposure risk assessment on NTAs is not a main subject of this document. Therefore risk assessment has not been made.

9.7.2.3 Additional higher-tier risk assessment

HQ value for *Typhlodromus pyri* is > 1, therefore additional higher tier risk assessment is necessary.

Due to the in-field HQ values for exposure to maximum residues on leaves for *Typhlodromus pyri* (Sch.) falls above the trigger value, further assessment has been done. Such assessment was made for AKO 600 SC with aged residues study.

Results proves that 14 days after treatment its possible to regrowth the arthropods colony

Detailed results are presented below:

Table 1. Final results

Treatment	DAT	Mortality assessment		Reproduction assessment		
		Mortality [%]	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Reproduction reduction [%]	Significance*
Control	0	4.0		not tested		
	14	3.0		5.2	not applicable	not applicable
	28	3.0		5.3		
	42	4.0		5.3		
3 L of the test item/200 L of water/ha	0	54.0	52.1	not tested		
	14	37.0	35.1	2.5	52.2	+
	28	28.0	25.8	4.4	16.3	+
	42	23.0	19.8	4.7	12.4	+
Reference item (6 g of dimethoate/ 200 L of water/ha)	0	95.0	94.8	not tested		
	14	96.0	95.9			
	28	94.0	93.8			
	42	96.0	95.8			

* value calculated using ToxRat using test with significance level $p=0.05$

Conclusion

The impact of the freshly-dried and field-aged residues of the CHR/H/AKO 600 S.C. on the *Typhlodromus pyri* was evaluated under laboratory test conditions in the following bioassays: 0 DAT, 14 DAT, 28 DAT, and 42 DAT. Reproduction assessment on 0 DAT was not performed due to mortality $\geq 50\%$ of introduced organisms. The test item caused both $<50\%$ mortality and $<50\%$ reduction in reproductive performance (relative to the control) in two subsequent bioassays at 28 DAT and 42 DAT.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in-field HQ value for *Typhlodromus pyri*. exceeds the trigger value, therefore additional assessment was necessary. Such assessment was made for AKO 600 SC.

AKO 600 SC applied at the maximum use rates poses no risk to non-target arthropods.

No risk mitigation are needed.

zRMS Comments:

The submitted risk assessment based on the “Guidance Document on Terrestrial Ecotoxicology” (2002) was accepted.

Acceptable risk may be concluded for in-field and off-field populations of non-target arthropods from the intended uses of AKO 600 SC.

Conclusion

The risk to arthropods other than bees is acceptable if the AKO 600 SC is applied in accordance with proposed use pattern.

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 aclonifen. 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 AKO 600 SC were not evaluated as part of the EU assessment of aclonifen. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Aclonifen	acute, 14 days	LC _{50corr} = 150 mg as/kg dw soil	EFSA Scientific Report (2008) 149, 1-80
<i>Eisenia fetida</i>	Aclonifen (tested as BANDUR SC 600 g/L)	28 d, chronic	Reproduction: NOEC _{corr} = 45 mg a.s./kg dw soil	EFSA Scientific Report (2008) 149, 1-80
<i>Eisenia andre</i>	AKO 600 SC	Reproduction Test Test duration: 8 weeks 10% sphagnum peat, 20% kaolin clay, 69.775% air-dried quartz sand, 0.225% calcium carbonate;	NOEC _(rep) = 560 mg as/kg dw soil NOEC _{(rep)(corr)} = 280 mg as/kg dw soil* NOEC _(surv) = 1000 mg as/kg dw soil NOEC _{(surv)(corr)} = 500 mg as/kg dw soil* EC _{10(repro)} = 889.96 mg as/kg dw soil EC _{10(corr)} = 444.98 mg as/kg dw soil	Gierbuszewska A.. Study code: G-35-23 Date: 03.2024
<i>Folsomia candida</i>	AKO 600 SC	Reproduction Test Test duration: 28 d 5% sphagnum peat, 20% kaolin clay, 74.88% air-dried	NOEC = 100 mg as/kg dw soil NOEC _{corr} = 50 mg as/kg dw soil*	Górska M. Study code: G-36-23 Date: 03.2024

Species	Substance	Exposure System	Results	Reference
		industrial sand and 0.12% calcium carbonate	EC ₁₀ = 103.42 mg as/kg dw soil EC _{10(corr)} = 51.71 mg as/kg dw soil	
<i>Hypoaspis aculeifer</i>	Aclonifen (tested as BANDUR SC 600 g/L)	Chronic 28 days	NOEC Mortality 13.3 mg as/kg dw soil (10 kg as/ha)	EFSA Scientific Report (2008) 149, 1-80
<i>Hypoaspis (Geolaelaps) aculeifer</i>	AKO 600 SC	Reproduction Test Terst duration: 14 d 5% sphagnum peat, 20% kaolin clay, and 74.88% air-dried industrial sand, 0.12% calcium carbonate.	NOEC = 180mg/kg dw NOEC _{corr} = 90 mg as/kg dw* EC ₁₀ = 172.46 mg as/kg dw soil EC _{10(corr)} = 86.23 mg as/kg dw soil	Górska M. Study code: G-37-23 Date: 03.2024
Field studies				
n/a				
Litter bag test				
n/a				

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

9.8.1.1 Justification for new endpoints

Not required

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the group no. 1 also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in groups described in the GAP (see 9.1.2).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of AKO 600 SC in group no. 1

Intended use			
Acute effects on earthworms			
Product/active substance	LC₅₀ (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_a (criterion TER ≥ 10)
Aclonifen	Not relevant	Not relevant	Not relevant
AKO 600 SC	Not relevant	Not relevant	Not relevant
Chronic effects on earthworms - <i>Eisenia andre</i>			
Product/active substance	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
Aclonifen (tested as BANDUR SC 600 g/L)	45	2.6256	17.14
AKO 600 SC	280	4.8472	57.77
Chronic effects on other soil macro- and mesofauna - <i>Folsomia candida</i>			
Product/active substance	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (NOEC / Pecsoil) (criterion TER ≥ 5)
AKO 600 SC	50	4.8472	10.32
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis (Geolaelaps) aculeifer</i>			
Product/active substance	EC₁₀ (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (NOEC / Pecsoil) (criterion TER ≥ 5)
Aclonifen (tested as BANDUR SC 600 g/L)	13.3	2.6256	5.1
AKO 600 SC	86.23	4.8472	17.79

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

AKO 600 SC TER values were above required criterion (TER ≥ 5) therefore the acute and long term risk assessment for earth-worms and other non-target soil organisms (meso- and macrofauna) is considered acceptable and not harm for those organisms

Intended uses of the product are considered safe and acceptable.

Combined exposure

Combined exposure risk assessment on in-soil organisms is not a main subject of this document. Therefore risk assessment has not been made.

zRMS comments:

PEC_{soil} values were calculated considering GAP of AKO 600 SC. The highest predicted environmental concentrations (PEC_{soil}) of the active substance and formulation were taken into account for the risk assessment.

The lower of NOEC and EC₁₀ value was used in the risk assessment.

Earthworms

Aclonifen - according to the EFSA Scientific Report (2008) 149 the chronic risk for earthworms was based on the result of study with BANDUR SC 600 g/L (reproduction NOECcorr at 45 mg as/kg dw soil). Therefore the TER calculation with this endpoint was added by RMS above.

AKO 600 SC - for the assessment of long-term risk to earthworms the NOEC reproduction value from study with the formulation was used.

All TER_{LT} values are above trigger value of 5.

Other soil macro-organisms

Aclonifen - according to the EFSA Scientific Report (2008) 149 for, the chronic risk for *Hypoaspis aculeifer* was based on the result of study with BANDUR SC 600 g/L (mortality NOECcorr at 13.3 mg as/kg dw soil). Therefore the TER calculation with this endpoint was added by RMS above. Additionally, according to EFSA Scientific Report (2008) for aclonifen: "...the long-term risk to soil dwelling mites was considered to be low since reproduction was not affected and the mortality was only 13% at the higher tested concentration of 42.1 mg a.s./kg soil."

AKO 600 SC - for the assessment of long-term risk to other soil macro-organisms the NOEC reproduction value from study with the formulation was used.

All TER_{LT} values are above trigger value of 5.

Conclusion:

According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substance following use of AKO 600 SC in compliance with proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

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

Effects on soil microorganisms of AKO 600 SC were not evaluated as part of the EU assessment of Aclonifen. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Aclonifen	28 d	20 mg as/kg dw soil (15 kg as/ha): 22 % inhibition on N _{tot} at day 28	EFSA Scientific Report (2008) 149, 1-80
N-mineralisation	AKO 600 SC	28 d	nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 4.87 mg test item/kg dry weight of soil and 5 x PEC: 24.36 mg test item/kg dry weight of soil did not exceed 25% on 28 day of analysis.	Pieczka P. Study code: G-38-23 Date: 03.2024

9.9.1.1 Justification for new endpoints

Not relevant

9.9.2 Risk assessment

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the potatoes also covers the risk for the soil microorganisms from all other intended uses described in the GAP (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of AKO 600 SC in potatoes

Intended use			
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Aclonifen	20 mg as/kg dw soil (15 kg as/ha): 22 % inhibition on N _{tot} at day 28	2.6171 2.6256*	Yes

AKO 600 SC	Nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 4.87 mg test item/kg dry weight of soil and 5 x PEC: 24.36 mg test item/kg dry weight of soil did not exceed 25% on 28 day of analysis.	4.872 4.8472*	Yes
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*Corrected according to Part B8

9.9.3 Overall conclusions

On the basis of the results, it was concluded that AKO 600 SC at the concentrations corresponding to the PEC: 4.87 mg test item/kg dry weight of soil and 5 x PEC: 24.36 mg test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Intended use of AKO 600 SC poses an acceptable risk to soil microorganisms.

zRMS comments:

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

The worst-case predicted environmental concentrations in soil (PEC_{soil}) of the active substance and formulation were taken into account for the risk assessment.

For the assessment of risk for formulation the endpoints from study, presented in Appendix 2, were used. For active substance the EU agreed endpoint was used.

Conclusion:

Since no effects (> 25%) were seen at application rates far higher than the values of PEC_{soil} for active substance and formulation it can be concluded that application of AKO 600 SC, according to the GAP, will not cause any detrimental effect to soil micro-organisms.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of AKO 600 SC were not evaluated as part of the EU assessment of aclonifen. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results [mL]	Reference
<i>Helianthus annuus</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Pieczka P. Study code: G-40-23 Date: 04.2024
<i>Brassica oleracea</i> <i>var. capitata</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 1010.14 ER ₅₀ (Shoot dry weight) > 94.98 ER ₅₀ (Plant damage) > 116.15	Pieczka P. Study code: G-40-23 Date: 04.2024
<i>Pisum sativum</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Pieczka P. Study code: G-40-23 Date: 04.2024
<i>Daucus carota</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) >	Pieczka P. Study code: G-40-23 Date: 04.2024

Species	Substance	Exposure System	Results [mL]	Reference
			3000.0 ER ₅₀ (Plant damage) > 3000.0	
<i>Allium cepa</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Pieczka P. Study code: G-40-23 Date: 04.2024
<i>Avena sativa</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 2358.90 ER ₅₀ (Shoot length) > 484.52 ER ₅₀ (Shoot dry weight) > 569.21 ER ₅₀ (Plant damage) > 850.97	Pieczka P. Study code: G-40-23 Date: 04.2024
<i>Beta vulgaris</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 187.88 ER ₅₀ (Shoot length) > 327.87 ER ₅₀ (Shoot dry weight) > 114.10 ER ₅₀ (Plant damage) > 158.73	Pieczka P. Study code: G-09-24 Date: 04.2024
<i>Brassica napus</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 623.15	Pieczka P. Study code: G-09-24 Date: 04.2024

Species	Substance	Exposure System	Results [mL]	Reference
			ER ₅₀ (Shoot dry weight) > 51.63 ER ₅₀ (Plant damage) > 110.82	
<i>Zea mays</i>	AKO 600 SC	Seedling Emergence and Seedling Growth	ER ₅₀ (Emergence of plants) > 3000.0 ER ₅₀ (Survival of plants) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Pieczka P. Study code: G-09-24 Date: 04.2024
<i>Helianthus annuus</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Gierbuszewska A. Study code: G-39-23 Date: 04.2024
<i>Brassica oleracea</i> <i>var. capitata</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 794.31 ER ₅₀ (Shoot dry weight) > 100.92 ER ₅₀ (Plant damage) > 130.15	Gierbuszewska A. Study code: G-39-23 Date: 04.2024
<i>Pisum sativum</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0	Gierbuszewska A. Study code: G-39-23 Date: 04.2024

Species	Substance	Exposure System	Results [mL]	Reference
			ER ₅₀ (Plant damage) > 3000.0	
<i>Daucus carota</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 3000.0 ER ₅₀ (Plant damage) > 3000.0	Gierbuszewska A. Study code: G-39-23 Date: 04.2024
<i>Allium cepa</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 1820.74 ER ₅₀ (Plant damage) > 2347.81	Gierbuszewska A. Study code: G-39-23 Date: 04.2024
<i>Avena sativa</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 1595.78 ER ₅₀ (Shoot dry weight) > 403.61 ER ₅₀ (Plant damage) > 1084.76	Gierbuszewska A. Study code: G-39-23 Date: 04.2024
<i>Beta vulgaris</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 192.43 ER ₅₀ (Shoot length) > 194.19 ER ₅₀ (Shoot dry weight) > 14.39 ER ₅₀ (Plant damage) > 39.43	Gierbuszewska A. Study code: G-10-24 Date: 04.2024
<i>Brassica napus</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0	Gierbuszewska A. Study code: G-10-24 Date: 04.2024

Species	Substance	Exposure System	Results [mL]	Reference
			ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 27.67 ER ₅₀ (Plant damage) > 106.88	
<i>Zea mays</i>	AKO 600 SC	Vegetative Vigour	ER ₅₀ (Plant number at the end of the experiment) > 3000.0 ER ₅₀ (Shoot length) > 3000.0 ER ₅₀ (Shoot dry weight) > 2957.90 ER ₅₀ (Plant damage) > 3000.0	Gierbuszewska A. Study code: G-10-24 Date: 04.2024

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Not required

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”, (SAN-CO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the potatoes also covers the risk for non-target terrestrial plants from all other intended uses described in the GAP (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of AKO 600 SC in potato

Intended use	Group no. 1	
Active substance/product	AKO 600 SC	
Application rate (g/ha)	1 x 3654 g/ha	

MAF		1			
Test species	ER ₅₀ (g of the product /ha)	Drift rate	PER _{off-field} (g/ha)	TER criterion: TER ≥ 5	
<i>Helianthus annuus</i>	3654	0.0277	101.22	36.1	21 days Seedling Emergence and Seedling Growth
<i>Brassica oleracea var. capitata</i>	115.6856	0.0277	101.22	1.14	21 days Seedling Emergence and Seedling Growth
<i>Pisum sativum</i>	3654	0.0277	101.22	36.1	21 days Seedling Emergence and Seedling Growth
<i>Daucus carota</i>	3654	0.0277	101.22	36.1	21 days Seedling Emergence and Seedling Growth
<i>Allium cepa</i>	3654	0.0277	101.22	36.1	21 days Seedling Emergence and Seedling Growth
<i>Avena sativa</i>	590.1454	0.0277	101.22	5.83	21 days Seedling Emergence and Seedling Growth
<i>Beta vulgaris</i>	138.9738	0.0277	101.22	1.37	21 days Seedling Emergence and Seedling Growth
<i>Brassica napus</i>	62.88534	0.0277	101.22	0.62	21 days Seedling Emergence and Seedling Growth
<i>Zea mays</i>	3654	0.0277	101.22	36.1	21 days Vegetative Vigour 21 days Seedling Emergence and Seedling Growth
<i>Helianthus annuus</i>	3654	0.0277	101.22	36.1	21 days Vegetative Vigour
<i>Brassica oleracea var. capitata</i>	122.9206	0.0277	101.22	1.21	21 days Vegetative Vigour
<i>Pisum sativum</i>	3654	0.0277	101.22	36.1	21 days Vegetative Vigour
<i>Daucus carota</i>	3654	0.0277	101.22	36.1	21 days Vegetative Vigour
<i>Allium cepa</i>	2217.661	0.0277	101.22	21.91	21 days Vegetative Vigour
<i>Avena sativa</i>	491.597	0.0277	101.22	4.86	21 days Vegetative Vigour
<i>Beta vulgaris</i>	17.52702	0.0277	101.22	0.17	21 days Vegetative Vigour
<i>Brassica napus</i>	33.70206	0.0277	101.22	0.33	21 days Vegetative Vigour

<i>Zea mays</i>	3602.722	0.0277	101.22	35.59	21 days Vegetative Vigour
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MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

zRMS comments: ER₅₀ values are expressed as g of the product/ha taking into account the formulation density d = 1.218 g/mL (20°C)

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of AKO 600 SC in group no. 1 considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Group no. 1			
Active substance/product		AKO 600 SC			
Application rate (g/ha)		1 x 3654			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50 % drift red. (g/ha)	PER_{off-field} 75 % drift red. (g/ha)	PER_{off-field} 90 % drift red. (g/ha)
1	0.0277	101.22	50.61	25.305	10.122
5	0.0057	20.83	10.415	5.2075	2.083
10	0.0029	10.6	5.3	2.65	1.06
15	0.002	7.308	7.31 3.654	3.655 1.827	1.8275 0.731
20	0.0015	5.481	5.48 2.741	2.74 1.370	1.37 0.548
30	0.001	3.654	3.65 1.827	1.825 0.914	0.9125 0.365
40	0.0007	2.5578	2.56 1.279	1.28 0.639	0.64 0.256
50	0.0006	2.1924	2.19 1.10	1.095 0.548	0.5475 0.219
Toxicity value		TER = 0.17			
Vegetative Vigour					
ER ₅₀ = 17.53 g/ha		criterion: TER ≥ 5			
1	0.0277	0.17	0.35	0.69	1.73

					1.71
5	0.0057	0.84	1.68	3.37	8.42
10	0.0029	1.65	3.31	6.62	16.54
15	0.002	2.4	4.8	9.59	23.98
20	0.0015	3.2	6.4	12.8	31.99
30	0.001	4.8	9.61	19.2118	48.03
40	0.0007	6.85	13.7	27.3943	68.48
50	0.0006	8	16.01 15.94	32.02 31.99	80.05
Toxicity value					
Seedling Emergence and Seedling Growth					
ER50 = 62.89 g/ha					
1		0.62	1.24	2.49	6.21
5		3.02	6.04	12.08	-
10		5.93	-	-	-

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

During the comment period, HU and SI requested to provide more realistic risk mitigation measures for the approved lower application rate of 2 L/ha.

The risk assessment for application rate of 2.0 L/ha (1.2 kg a.s./ha) was presented below.

Intended use		Group no. 1			
Active substance/product		AKO 600 SC			
Application rate (g/ha)		1 x 2436			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50 % drift red. (g/ha)	PER_{off-field} 75 % drift red. (g/ha)	PER_{off-field} 90 % drift red. (g/ha)
1	0.0277	67.48	33.74	16.87	6.75
5	0.0057	13.89	6.95	3.47	1.39
10	0.0029	7.06	3.53	1.77	0.71
15	0.002	4.87	2.44	1.22	0.49
20	0.0015	3.65	1.83	0.91	0.365
30	0.001	2.44	1.22	0.61	0.24
Toxicity value		TER = 0.17			
Vegetative Vigour					
ER ₅₀ = 17.53 g/ha		criterion: TER ≥ 5			
1	0.0277	0.26	0.52	1.04	2.60
5	0.0057	1.26	2.52	5.05	12.61
10	0.0029	2.48	4.97	9.90	24.69

15	0.002	3.60	7.18	14.37	35.78
20	0.0015	4.80	9.58	19.26	48.03
30	0.001	7.18	14.37	28.74	73.04
Toxicity value					
Seedling Emergence and Seedling Growth					
ER50 = 62.89 g/ha					
1		0.93	1.86	3.73	9.39
5		4.53	9.05	18.12	45.24
10		8.91	17.82	35.53	88.58

9.10.3 Overall conclusions

In order to achieve an acceptable the off-field exposure ($TER \geq 5$) and minimize of a risk for non-target plants, one of the following risk mitigation measures has to be implemented

- 5 m buffer zone with 90% nozzle reduction
- 10 m buffer zone with 75% nozzle reduction
- 20 m buffer zone with 50% nozzle reduction
- 40 m buffer zone with 0% nozzle reduction

Combined exposure

Combined exposure risk assessment on NTPs is not a main subject of this document. Therefore risk assessment has not been made.

zRMS Comments:

The risk assessment was based on the results of studies with formulation presented in Appendix 2 (vegetative vigour and on seedling emergence) and considering the use in potatoes as a worst-case. *Beta vulgaris* was the most sensitive species in Vegetative Vigour tests and *Brassica napus* was the most sensitive species in Seedling Emergence tests.

All effects and endpoints were reported in studies summary, including phytotoxicity effects, and the lowest endpoints were used in the risk assessment.

For application rate of 3 L/ha the TER value are above the trigger value of 5 when following risk mitigation measures are applied:

- 5 m buffer zone with 90% nozzle reduction or
- 10 m buffer zone with 75% nozzle reduction or
- 20 m buffer zone with 50% nozzle reduction or
- 40 m buffer zone

For accepted max. application rate of 2 L/ha (1.2 kg as/ha) TER value are above the trigger value of 5 when following risk mitigation measures are applied:

- 5 m buffer zone with 75% nozzle reduction or
- 15 m buffer zone with 50% nozzle reduction or
- 30 m buffer zone

Conclusion:

The risk for terrestrial plants following use of AKO 600 SC / Tanger 600 SC, Libretto 600 SC could be considered as low when the appropriate risk mitigation measures were applied.:

Member States should consider risk mitigation measures at national level.

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

Not relevant

9.12 Monitoring data (KCP 10.8)

Not relevant

9.13 Classification and Labelling

zRMS comment:

For details see Part C

Based on the *Lemna gibba* ErC₅₀ equal to 0.0135 mg test item/L and in accordance to CLP Regulation the following classification for formulation is proposed:

Aquatic acute toxicity Category 1, H 400 Very toxic to aquatic life

Based on the *Pseudokirchneriella subcapitata* NOEC = 0.004 mg test item/L and in accordance to CLP Regulation the following classification for formulation is proposed:

Aquatic chronic toxicity Category 1, H 410 Very toxic to aquatic life with long lasting effects

Pictogram: GHS09

Signal word: Warning

P-statements

P391 Collect spillage.

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	Czarnecka M.	2024	Daphnia magna, Acute Immobilisation Test Study code: W-19-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.2	Czarnecka M.	2024	Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test Study code: W-20-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.2	Czarnecka M.	2024	Lemna gibba CPCC 310, Growth inhibition test Study code: W-21-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.2	Czarnecka M.	2024	Anabaena flos-aquae UTEX B 1444, Growth inhibition test Study code: W-22-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.3.1	Wojciech A.	2023	Honeybees (Apis mellifera L.), Acute Oral Toxicity Test Study code: B-80-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.

KCP 10.3.1	Wojciech A.	2023	Honeybees (<i>Apis mellifera</i> L.), Acute Contact Toxicity Test Study code: B-81-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 10.3.1	Wojciech A.	2024	Bumblebees (<i>Bombus</i> spp.), Acute Oral Toxicity Test Study code: B-74-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 10.3.1	Wojciech A.	2024	Bumblebees (<i>Bombus</i> spp.), Acute Contact Toxicity Test Study code: B-75-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 10.3.1	Zaworska K.	2023	Honey bee, chronic oral toxicity test of test item CHR/H/AKO 600 SC according to OECD 245 Guideline Study code: 0038/0164/E SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 10.3.1	Woźniak A.	2024	Honey bee larval toxicity test following repeated exposure of the test item CHR/H/AKO 600 SC according to OECD GD 239 ENV/JM/MONO(2016)34 Study code: 0038/0162/E SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.
KCP 10.3.2	Wojciech A.	2024	An extended laboratory test for evaluating effects of CHR/H/AKO 600 SC on the ladybird beetle, <i>Coccinella septempunctata</i> (L.) Study code: B-76-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemirol” Sp. z o. o.

KCP 10.3.2	Wojciech A.	2024	An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the predatory mite, <i>Typhlodromus pyri</i> (Sch.) Study code: B-77-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.3.2	Wojciech A.	2024	An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez) Study code: B-78-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.3.2	Wojciech A.	2024	An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the green lacewing, <i>Chrysoperla carnea</i> Study code: B-79-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.3.2	Zaworska K.	2024	Aged-residue extended laboratory test to determine the effects of the test item CHR/H/AKO 600 SC on the <i>Typhlodromus pyri</i> Scheuten according to the IOBC, BART and EPPO Joint Initiative, Blümel S. et al. (2000) and ESCORT 2, Candolfi M.P. et al. (2001) Study code: 0038/0197/E SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.4	Gierbuszewska A.	2024	Earthworm reproduction test (<i>Eisenia andrei</i>) Study code: G-35-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.4	Górska M.	2024	Collembolan (<i>Folsomia candida</i>) Reproduction Test Study code: G-36-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.

KCP 10.4	Górska M.	2024	Predatory mite (<i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>) reproduction test in soil Study code: G-37-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.5	Pieczka P.	2024	Soil Microorganisms: Nitrogen Transformation Test Study code: G-38-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.6	Pieczka P.	2024	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study code: G-09-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.6	Pieczka P.	2024	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study code: G-40-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.6	Gierbuszewska A.	2024	Terrestrial Plant Test: Vegetative Vigour Test Study code: G-10-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” Sp. z o. o.
KCP 10.6	Gierbuszewska A.	2024	Terrestrial Plant Test: Vegetative Vigour Test Study code: G-39-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP: Yes Unpublished	N	PUH „Chemiroł” 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
KCP 10.3.2	Heimann, D., Römcke, J. and Vickus, P	1992	A study of the acute toxicity for Pardosa sp. (spiders) of SAG 127 01 H. ' ES01AA01 ! R007269 GLP, unpublished ANA2000-765	N	BAY4
KCP 10.3.2	Jacobs G.	2002	AE F068300 00 SC50 A203 = EXP04209E (Bandur): Laboratory dose-reponse test to evaluate the effect on survival and reproduction of the predaceous mite Hypoaspis aculeifer Canestrini (Acari. Laelapidae) in standard soil (LUFA 2.1). C029557 GLP, unpublished ANA2005-30	N	BAY4
KCP 10.3.2	Lühns U.	1999	Effects of EXP04209E on the Predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory. 5642063 ! R006155 GLP, unpublished ANA2000-678	N	BAY4
KCP 10.3.2	Moll M.	1999	Effects of EXP04209E on the parasitoid Aphidius rhopalosiphii (Hymenoptera, Aphidiidae) in the laboratory. 5641001 ! R006177 GLP, unpublished ANA2000-679	N	BAY4
KCP 10.3.2	Römcke, J. and Vickus, P	1992	A study of the acute toxicity for Poecilus cupreus (Carabidae) of SAG 127 01 H. BE-E-148-92-01-CAK-1 ! R007267 GLP, unpublished ANA2000-764	N	BAY4
KCP 10.3.2	Römcke, J. and Vickus, P.	1992	A study of the acute toxicity for Aleochara bilineata (Staphylinidae) of SAG127 01 H. BE-E-148-92-01 STA1 ! R007268 GLP, unpublished ANA2000-1111	N	BAY4
KCP 10.3.2	Schmitzer S.	2000	Effects of EXP04209E on the Wolf spider Pardosa spec. (Araneae, Lycosidae) in the laboratory - extended laboratory study -. 7761066 ! B002997 GLP, unpublished ANA2000-1111	N	BAY4
KCP 3.2.10.	van Stratum P.	2000	EXP04209E: An Extended Laboratory Study to Evaluate the Effects on the Predaceous Mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae). B002976 GLP, unpublished ANA2005-28	N	BAY4
KCP 10.3.2	Waltersdorfer A.	2003	Toxicity to the Predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory acetonifen water miscible suspension concentrate 600 g/L code: AE F068300 00 SC50 A203.	N	BAY4

			C032823 ! CW02/082 GLP, unpublished ANA2005-27		
KCP 10.6	Spatz B.	2001	Effects of EXP 04209E on Terrestrial (Non-Target) Plants: Vegetative Vigour Test. 9523087 ! C016889 GLP, unpublished PFL2001-116	N	BAY4
KCP 10.6	Spatz B.	2001	ffects of EXP 04209E on Terrestrial (Non-Target) Plants: Seedling Emergence and Seed ling Growth Test. Proj. 9522086 ! C016892 GLP, unpublished PFL2001-115	N	BAY4
KCP 10.6	Spatz B.	2002	Effects of EXP 04209E on terrestrial (non-target) plants: Vegetative vigour test. Proj-No. 9527087 ! C023845 GLP, unpublished PFL2002-78	N	BAY4
KCP 10.6	Spatz B.	2002	Effects of EXP 04209E on terrestrial (non-target) plants: Seedling emergence and seedling growth test. Proj-No. 9526086 ! C023847 GLP, unpublished PFL2002-77	N	BAY4
KCP 10.6	Spatz, B. and Meinerling, M.	2003	Effects of AE F068300 00 SC50 A2 (EXP04209E) on Terrestrial (Non-Target) Plants - Higher Tier Test with Lactuca sativa. C040615 ! 17192094 GLP, unpublished PFL2004-83	N	BAY4
KCP 10.6	Spatz, B. and Meinerling, M.	2004	Effects of AE F068300 00 SC50 A2 (EXP04209E) on Terrestrial (Non-Target) Plants - Higher Tier Test with Brassica napus and Lolium perenne. C040617 ! 17191094 GLP, unpublished PFL2004-82	N	BAY4
KCP 10.1.2		1989	Acute oral toxicity study (and limit test) of EXP4209 in rats. GLP, unpublished	Y	BAY4
KCP 10.3.1	Kling, A.	2003	Assessment of side effects of AE F068300 00 SC50 A203 (BANDUR) to the Honey bee, Apis mellifera L. in the laboratory. C030375 GLP, unpublished BIE2005-17	N	BAY4
KCP 10.3.1	Tornier, I.	1991	Determination of side effects of SAG 127 01H in the Honey bee, Apis mellifera in laboratory. R007412 GLP, unpublished BIE2005-19	N	BAY4
KCP 10.3.1	Tornier, I.	1991	Laboratory study to determine the side effects of SAG 12701H in the Honey bee, Apis mellifera. C025164 ! 234/01 An GLP, unpublished ARW2005-19	N	BAY4
KCP 10.3.2	Handley, J.W. and Wetton, P.M.	1992	The acute toxicity of EXP04209 to earthworms (Eisenia foetida). R007910 GLP, unpublished ARW2005-19	N	BAY4

KCP 10.3.2	Lührs, U.	2001	Effects of EXP04209E on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil. Proj.9524022 ! C015297 ! C040616 GLP, unpublished ARW2001-131	N	BAY4
KCP 10.3.2	Meister A and Schwiening S.	2002	Effects of EXP04209E on the decomposition of organic material enclosed in litter bags in the field (amendment). C020883 GLP, unpublished ARW2005-22	N	BAY4
KCP 10.3.2	Meister, A. and Schwiening, S	2002	Effects of EXP04209E on the decomposition of organic material enclosed in litter bags in the field. C020616 GLP, unpublished ARW2005-21	N	BAY4
KCP 10.5	Aldred, D.	1993	A laboratory assessment of the effects of EXP4209 on soil microflora respiration and nitrogen turnover. R007278 ! ELL/726/3 GLP, unpublished BMF2005-13	N	BAY4
KCP 10.1.1	██████	1999	Aclonifen technical: Acute oral toxicity (LD50) to Bobwhite quail. ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.1.1	██████	2003	5-day-dietary LC50 for Bobwhite quail (<i>Colinus virginianus</i>), aclonifen (tech. as). ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.1.1	██████	1995	Aclonifen - Avian subchronic toxicity test - Oral toxicity in japanese quail (including effects on reproduction following a 6-week administration in the diet). ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.2	██████	1991	The acute toxicity of aclonifen to Common carp (<i>Cyprinus carpio</i>). ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.1.2	██████	1985	Two-generation reproduction toxicity study with CME - 127 in the rat. ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.2	██████	1991	The prolonged toxicity of aclonifen to Rainbow trout (<i>Oncorhynchus mykiss</i>). ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.2	██████	1993	Aclonifen: 21-day Rainbow trout toxicity study under flow-through conditions. ██████████ GLP, unpublished ██████████	Y	BAY4
KCP 10.2	██████	1997	Aclonifen - Early life stage toxicity test to Fathead minnow (<i>Pimephales promelas</i>). ██████████	Y	BAY4
KCP 10.2	██████	1995	Aclonifen: Bioconcentration of [14C]-residues in Rainbow trout. ██████████ GLP, unpublished	Y	BAY4

KCP 10.2		1995	Bioconcentration of [14C]-aclonifen in <i>Oncorhynchus mykiss</i> under flow-through conditions. 198/80-1018 ! C034500 GLP, unpublished WAT95-00601	Y	BAY4
KCP 10.2	Douglas, M.T., Halls, R.W.S. and MacDonald, I.A.	1991	The acute toxicity of aclonifen to <i>Daphnia magna</i> . RNP GLP, unpublished	N	BAY4
KCP 10.2	Handley, J.W., Sewell, I.G. and Bartlett, A.J.	1990	The algistatic activity of aclonifen CME 127. 282/53 ! R007145 GLP, unpublished WAT95-00157	N	BAY4
KCP 10.2	Hobert J.R	1998	Aclonifen - Toxicity to the freshwater diatom, <i>Navicula pelliculosa</i> . 98-7-7387 ! R005692 GLP, unpublished WAT1999-113	N	BAY4
KCP 10.2	MC Elligott A.	2001	Aclonifen freshwater algal growth inhibition study in a sediment water system (<i>Scenedesmus subspicatus</i>). 605325 ! C015751 GLP, unpublished WAT2001-585	N	BAY4
KCP 10.2	Sewell, I.G. and McKenzie, J.	2004	Sediment-water chironomid toxicity test using spiked sediment aclonifen (AE F068300). C039873 GLP, unpublished WAT2005-36	N	BAY4
KCP 10.2	Suteau, P.	1996	clonifen Toxicology to the Sediment Dwelling Chironomid Larvae (<i>Chironomus riparius</i>). SA 96243 ! R007434 GLP, unpublished WAT97-00168	N	BAY4
KCP 10.2	Hoberg J.R	1998	Aclonifen – Toxicity to the duckweed, <i>Lemna gibba</i> 98-7-7411 ! R005693 GLP, unpublished WAT1999-114	N	BAY4
KCP 10.3.1.	Schmitzer S.	1999	Final report - Laboratory Testing for Toxicity (Acute Contact and Oral LD50) of ACLONIFEN on Honey Bees (<i>Apis mellifera</i> L.) (Hymenoptera, Apidae). R007442 ! Proj.No. 5400036 GLP, unpublished BIE2005-13	N	BAY4

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

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 *Daphnia magna*

Comments of zRMS:	The study was performed according to OECD TG 202 and principles of GLP. The validity criteria are met: - the percentage of immobilisation of <i>Daphnia magna</i> in the control was 0% (criterion: not more than 10%), - the dissolved oxygen concentrations in the test vessels were within the range of 8.2 – 9.5 mg/L (criterion: not less than 3 mg/L).. All results refer to nominal concentrations since the measured concentrations were within 80 to 120% of nominal. The study is considered acceptable and suitable for the risk assessment. 48h-EC₅₀ = 15.46 mg test item/L 48h-EC₅₀ = 7.71 mg aclonifen/L
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Report	CHR/H/AKO 600 SC Daphnia magna, Acute Immobilisation Test; Study code: W-19-23 Study Director: M. Czarnecka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	OECD Guideline No. 202 (2004)/ EU method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item	CHR/H/AKO 600 SC; batch no. CHR/02/2023; the content of active substance (aclonifen): 49.9% (w/w), 607.8 g/L; manufacturing date: March 23, 2023; expiry date: not provided by the Sponsor.
Test system	Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.
Test design	Static test (48 h of exposure); 4 replicates per each test item concentration and the control; 5 Daphnia magna in each replicate.
Nominal test item concentration	40, 20, 10, 5.0, 2.5, 1.25, and 0.63 mg/L plus the control.
Nominal concentrations of aclonifen	19.96, 9.98, 4.99, 2.50, 1.25, 0.62 and 0.31 mg/L plus the control.
Test conditions	Temperature: 19.6 – 21.5°C; pH of the control: 7.71 – 7.89; dissolved oxygen concentration in the control: 8.8 – 8.9 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.
Statistics	Weibull analysis using linear max. likelihood regression and analyses by: Tarone's Test Procedure, Step-down Cochran Armitage Test Procedure.
Endpoints value	EC50/48 h, NOEC/48 h, and LOEC/48 h.

Results and discussions

Immobilisation of *Daphnia magna* exposed to the test item, CHR/H/AKO 600 SC was investigated during a 48-hour static test. The test item concentrations used in the definitive test were determined on the basis of the preliminary test results. The definitive test was performed with the test item concentrations of 40, 20, 10, 5.0, 2.5, 1.25 and 0.63 mg/L plus the control. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentrations or the control per replicate. Four replicates were used for the test item concentrations and the control, each with five *Daphnia magna*. The *Daphnia magna* were observed for immobilisation and any abnormal behavior or appearance after 24 and 48 h of exposure. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel. In the control and the test item concentration of 0.63 mg/L, no immobilisation of *Daphnia magna* was observed during exposure. At exposure termination in

the test item concentrations of 40, 20, 10, 5.0, 2.5 and 1.25 mg/L, the immobilisation of *Daphnia magna* was 90, 40, 35, 30, 15 and 10%, respectively. No abnormal behaviour of *Daphnia magna* was observed during exposure. At exposure termination, in the test item concentrations of 40, 20, 10 and 5.0 mg/L, the biofilm of the test item was observed on the surface of suspension test item in all test vessels. Moreover, particles of the test item in the form of sediment were observed at the bottom of all test vessels in the test item concentrations of 40 and 20 mg/L. In the test item concentrations in the range of 5.0 – 40 mg/L, daphnids were covered with particles of the test item. The concentrations of aclonifen were chemically determined using the validated high performance liquid chromatography (HPLC) with Diode Array Detection. Samples for chemical determination were collected from all test item concentrations and the control at exposure initiation and at exposure termination. In the samples collected at exposure initiation, the determined concentrations of aclonifen were in the range of 103.5 – 107.9% of nominal concentration. Therefore, the test item concentrations were prepared correctly. In the samples collected at exposure termination, the determined concentrations of aclonifen were in the range of 101.9 – 105.1% of nominal concentration. Therefore, the concentrations of aclonifen were stable during 48 h under test conditions. The endpoints value was determined based on the nominal test item concentrations as well as on the nominal concentrations of aclonifen.

Table 4. Immobilisation of *Daphnia magna*, first preliminary range-finding test (non-GLP)

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		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
0.01	20	0	0	0	0	0	0	0	0	0	0
0.1	20	0	0	0	0	0	0	0	0	0	0
1.0	20	0	0	0	0	0	0	0	0	0	0
10	20	0	0	0	0	1	1	2	1	0	25

Time of exposure: 04.10.2023 – 06.10.2023

Table 5. Immobilisation of *Daphnia magna*, second preliminary range-finding test (non-GLP)

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		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
1.6	20	0	0	0	0	1	2	1	2	0	30
8.0	20	1	0	2	3	2	2	3	5	30	60
40	20	2	3	3	2	5	5	5	5	50	100

Time of exposure: 12.10.2023 – 14.10.2023

Table 8. Immobilisation of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		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
0.63	20	0	0	0	0	0	0	0	0	0	0
1.25	20	0	0	0	0	0	2	0	0	0	10
2.5	20	0	0	0	0	0	1	1	1	0	15
5.0	20	2	1	0	0	2	2	1	1	15	30
10	20	2	2	1	0	2	2	2	1	25	35
20	20	1	1	1	2	2	1	2	3	25	40
40	20	3	2	1	2	5	5	4	4	40	90

Time of exposure: 28.11.2023 – 30.11.2023

Table 9. Endpoint values based on the nominal test item concentrations, definitive test

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC₅₀	47.24 (28.0 – 146.05)	15.46 (10.89 – 22.37)
EC₂₀	13.73 (7.24 – 22.31)	4.46 (2.23 – 6.77)
EC₁₀	6.06 (1.84 – 10.37)	1.96 (0.69 – 3.47)
LOEC	5.0	1.25
NOEC	2.5	0.63

Calculations were made according to [8], [SOP/W/68]
(-) - 95% confidence interval

Table 10. Endpoint values based on the nominal concentrations of aclonifen, definitive test

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC₅₀	23.57 (13.97 – 72.95)	7.71 (5.43 – 11.17)
EC₂₀	6.85 (3.61 – 11.13)	2.23 (1.11 – 3.38)
EC₁₀	3.02 (0.91 – 5.17)	0.98 (0.34 – 1.73)
LOEC	2.50	0.62
NOEC	1.25	0.31

Calculations were made according to [8], [SOP/W/68]
(-) - 95% confidence interval

The endpoints value based on the nominal test item concentrations:

The EC₅₀/48 h value is 15.46 mg/L (95% confidence interval 10.89 – 22.37)

The LOEC is 1.25 mg/L

The NOEC is 0.63 mg/L

The endpoints value based on the nominal concentrations of aclonifen:

The EC₅₀/48 h value is 7.71 mg/L (95% confidence interval 5.43 – 11.17)

The LOEC is 0.62 mg/L

The NOEC is 0.31 mg/L

TEST VALIDITY CRITERIA

In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004):

- the percentage of immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.2 – 9.5 mg/L (criterion: not less than 3 mg/L).

A 2.2.1.2 *Raphidocelis subcapitata*

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. - the biomass in the control increased by a factor of 168.1 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.5% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 7.4% (criterion: it must not exceed 35%). All results refer to nominal concentrations since the measured concentrations were
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	within 80 to 120% of nominal. The study is considered acceptable and suitable for the risk assessment. 72h-E_rC₅₀ = 0.025 mg test item/L 72h-E_rC₅₀ = 12.801 µg aclonifen/L
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Reference: 10.2.1

Report CHR/H/AKO 600 SC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test; Study code: W-20-23
Study Director: M. Czarnecka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): OECD Guideline No. 201 (2006)/EU method C.3.

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item	CHR/H/AKO 600 SC; batch no. CHR/02/2023; the content of active substance (aclonifen): 49.9% (w/w), 607.8 g/L; manufacturing date: March 23, 2023; expiry date: not provided by the Sponsor.
Test system	The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata (Korshikov) Hindák, Selenastrum capricornutum Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.
Test design	72 hours of exposure; three replicates per each test item concentration; six replicates per control; a background for each treatment; initial algal cell density: 1×10^4 cells/mL.
Nominal test item concentration	0.1, 0.033, 0.011, 0.004 and 0.001 mg/L plus the control.
Nominal concentrations of aclonifen	49.9, 16.467, 5.489, 1.996 and 0.499 µg/L plus the control.
Test conditions	Temperature: 21.9 – 22.8°C; pH of the control: 7.49 – 7.62; mean light intensity: 7386 – 7536 lux; constant illumination and shaking; medium: AAP.
Statistics	Non-linear 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.
Endpoints value	ErC ₅₀ /72 h, EyC ₅₀ /72 h, NOEC/72 h, LOEC/72 h.

Results and discussions

The growth of the green algae *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) exposed to the test item, CHR/H/AKO 600 SC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the algae was 1×10^4 cells/mL. . The range of the test item concentrations used in the definitive test was determined on the basis of the preliminary tests results. The definitive test was performed with the following test item concentrations: 0.1, 0.033, 0.011, 0.004, and 0.001 mg/L plus the control. The number of algal cells was determined with an indirect method, which involves a spectrophotometric measurement of the absorbance of algal suspension at 670 nm and converting its value into the number of cells using a standard curve. The absorbance for each replicate of each test item concentration and the control were measured after 24, 48, and 72 h of exposure. Morphology observations of the algae cells were performed at exposure termination. No growth rate and yield inhibitions were observed in the test item concentration of 0.001 mg/L. Calculated inhibition of growth rate for the test item concentrations ranging from 0.004 to 0.1 mg/L after 72 h of exposure was in the range of 2.1 – 79.2% when compared to the control. Inhibition of yield for the test item concentrations ranging 0.004 to 0.1 mg/L after 72 h of exposure was in the range of 10.1 – 98.8% when compared to the control. Morphology observations of the algae cells were performed at exposure termination. In the test item concentrations of 0.001, 0.004 and 0.011 mg/L, no differences in shape, size and colour of algal cells were reported as compared to the algal cells in the control. In the test item concentration of 0.033 mg/L, 50% of deformed algal cells were reported as compared to the algae cells in the control. No differences in shape, size and colour of 50% of algal cells were reported as compared to the algal cells in the control. In the test item concentration of 0.1 mg/L, 80% of deformed algal cells were reported as compared to the algae cells in the control. No differences in shape, size and colour of 20% of algal cells were reported as compared to the algal cells in the control. The concentrations of aclonifen were chemically analyzed using the validated high performance liquid chromatographic method with MS/MS detection (LC-MS/MS). Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.

At exposure initiation, the determined concentrations of aclonifen were in the range of 96.5 – 114.8% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of aclonifen were in the range of 85.1 – 93.4% of the nominal concentration. The results confirm, that the concentrations of aclonifen were stable under test conditions. The endpoints value was determined based on the nominal test item concentrations as well as on the nominal concentrations of aclonifen.



Table 4. Inhibition of growth rate and yield, first preliminary non-GLP test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.01	14.5	51.7
0.1	83.9	99.1
1.0	89.5	99.5
10	>100*	>100*

*Inhibition is lower than 0%, which means that the algal cell density at exposure termination was higher than the algal cell density in the control.

Time of exposure: 23.10.2023 – 26.10.2023

Table 5. Inhibition of growth rate and yield, second preliminary non-GLP test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.0001	3.7	16.9
0.001	3.7	16.6
0.01	10.6	40.1

Time of exposure: 31.10.2023 – 03.11.2023

Table 7. Observations of algal cell morphology at exposure termination, definitive test

Nominal test item concentration [mg/L]	Observations
Control	Normal colour, shape and size of the algal cells
0.001	No changes
0.004	No changes
0.011	No changes
0.033	50% of deformed algal cells
0.1	80% of deformed algal cells

Table 10. Growth rate and yield, definitive test

Nominal test item concentration [mg/L]	Growth rate* [10 ⁶ cells/mL]				Yield** [10 ⁶ cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.569	1.843	1.675	1.695	1.607
	1.569	1.809	1.726	1.701	1.636
	1.569	1.869	1.690	1.709	1.676
	1.629	1.833	1.687	1.716	1.713
	1.629	1.748	1.753	1.710	1.681
mean	1.589	1.834	1.701	1.708	1.671
standard deviation	0.031	0.053	0.031	0.008	0.042
0.001	1.629	1.849	1.709	1.729	1.780
	1.629	1.765	1.742	1.712	1.692
	1.569	1.836	1.686	1.697	1.615
mean	1.609	1.817	1.713	1.713	1.696
standard deviation	0.035	0.045	0.028	0.016	0.083
0.004	1.459	1.881	1.682	1.674	1.506
	1.459	1.881	1.711	1.683	1.551
	1.569	1.724	1.691	1.661	1.450
mean	1.495	1.828	1.695	1.673	1.502
standard deviation	0.064	0.091	0.0151	0.011	0.051
0.011	1.629	0.830	1.247	1.235	0.397
	1.504	1.084	1.392	1.327	0.525
	1.629	1.092	1.507	1.409	0.676
mean	1.588	1.002	1.382	1.324	0.533
standard deviation	0.072	0.149	0.131	0.087	0.140
0.033	1.459	0.171	0.601	0.743	0.083
	1.459	0.045	n.d.	0.486	0.033
	1.459	0.171	0.258	0.629	0.056
mean	1.459	0.129	n.d.	0.620	0.057
standard deviation	0.000	0.072	n.d.	0.129	0.025
0.1	1.253	0.000	n.d.	0.292	0.014
	1.308	0.196	n.d.	0.418	0.025
	1.386	n.d.	n.d.	0.355	0.019
mean	1.316	n.d.	n.d.	0.355	0.019
standard deviation	0.067	n.d.	n.d.	0.063	0.006

n.d. – not determined due mathematical reasons

* - Growth rate [10⁶ cells/mL] was calculated according to the following formula:

Table 11. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
0.001	-0.3*	-1.5*
0.004	2.1	10.1
0.011	22.5	68.1
0.033	63.7	96.6
0.1	79.2	98.8

*Inhibition is lower than 0%, which means that the algal cell density at exposure termination w higher than the algal cell density in the control.

Table 12. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC₅₀	n.d.	0.036 (0.012 – 0.104)	0.025 (0.016 – 0.041)
E_rC₂₀	0.130 (0.056 – 0.288)	0.006 (0.003 – 0.015)	0.009 (0.006 – 0.013)
E_rC₁₀	0.048 (0.026 – 0.088)	0.003 (0.001 – 0.006)	0.005 (0.003 – 0.007)
LOEC	0.033	0.011	0.011
NOEC	0.011	0.004	0.004

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d. – not determined

Table 13. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC₅₀	0.297 (0.042 – 2.019)	0.010 (0.007 – 0.015)	0.008 (0.007 – 0.010)
E_yC₂₀	0.054 (0.018 – 0.173)	0.005 (0.003 – 0.007)	0.005 (0.004 – 0.006)
E_yC₁₀	0.022 (0.008 – 0.064)	0.003 (0.002 – 0.004)	0.004 (0.003 – 0.005)
LOEC	0.033	0.004	0.004
NOEC	0.011	0.001	0.001

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

Table 14. Growth rate endpoint values based on the nominal concentrations of aclonifen, definitive test

Endpoint value [µg/L]	Time of exposure:		
	24 h	48 h	72 h
ErC₅₀	n.d.	19.742 (9.612 – 39.568)	12.801 (8.306 – 19.545)
ErC₂₀	65.100 (29.838 – 136.232)	4.052 (2.310 – 7.151)	4.426 (3.116 – 6.295)
ErC₁₀	23.834 (13.576 – 41.842)	1.771 (0.983 – 3.190)	2.541 (1.751 – 3.687)
LOEC	16.467	5.489	5.489
NOEC	5.489	1.996	1.996

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d. – not determined

Table 15. Yield endpoint values based on the nominal concentrations of aclonifen, definitive test

Endpoint value [µg/L]	Time of exposure:		
	24 h	48 h	72 h
EyC₅₀	149.722 (24.026 – 855.242)	4.995 (3.427 – 7.214)	4.154 (3.401 – 5.061)
EyC₂₀	26.975 (9.733 – 78.103)	2.371 (1.751 – 3.218)	2.511 (2.130 – 2.959)
EyC₁₀	11.012 (4.178 – 29.024)	1.606 (1.164 – 2.215)	1.930 (1.621 – 2.298)
LOEC	16.467	1.996	1.996
NOEC	5.489	0.499	0.499

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

The endpoints value based on the nominal test item concentrations:

The ErC₅₀/72 h value is 0.025 mg/L (95% confidence interval: 0.016 – 0.041).
The LOEC/72 h value for growth rate is 0.011 mg/L.
The NOEC/72 h value for growth rate is 0.004 mg/L.
The EyC₅₀/72 h value is 0.008 mg/L (95% confidence interval: 0.007 – 0.010).
The LOEC/72 h value for yield is 0.004 mg/L.
The NOEC/72 h value for yield is 0.001 mg/L.

The endpoints value based on the nominal concentrations of aclonifen:

The ErC₅₀/72 h value is 12.801 µg/L (95% confidence interval: 8.306 – 19.545).
The LOEC/72 h value for growth rate is 5.489 µg/L.
The NOEC/72 h value for growth rate is 1.996 µg/L.
The EyC₅₀/72 h value is 4.154 µg/L (95% confidence interval: 3.401 – 5.061).
The LOEC/72 h value for yield is 1.996 µg/L.
The NOEC/72 h value for yield is 0.499 µg/L.

TEST VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:

- the biomass in the control increased by a factor of 168.1 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.5% (criterion: it must not exceed 7%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 7.4% (criterion: it must not exceed 35%).

A 2.2.1.3 *Lemna gibba*

Comments of zRMS:	The study was performed according to OECD TG 221 and principles of GLP. The validity criteria are met: - the doubling time of frond number in the control was 1.9 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 12.7), - the average specific growth rate in the control between day 0 and day 7 was 0.362 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹). All results refer to nominal concentrations since the measured concentrations were within 80 to 120% of nominal. The study is considered acceptable and suitable for the risk assessment. 7d E_rC₅₀ frond number = 13.523 µg test item/L, 7d E_rC₅₀ dry weight = 17.020 µg test item/L, 7d E_rC₅₀ frond number = 6.748 µg aclonifen/L, 7d E_rC₅₀ dry weight = 8.493 µg aclonifen/L
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Reference:	10.2.1
Report	CHR/H/AKO 600 SC Lemna gibba CPCC 310, Growth inhibition test; Study code: W-21-23 Study Director: M. Czarnecka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	OECD Guideline No. 201 (2006)/EU method C.3 221 (2006)/ EU Method C.26.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item	CHR/H/AKO 600 SC; batch no. CHR/02/2023; the content of active substance (aclonifen): 49.9% (w/w), 607.8 g/L; manufacturing date: March 23, 2023; expiry date: not provided by the Sponsor.
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Test system	Freshwater aquatic plant <i>Lemna gibba</i> L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.
Test design	Semi-static system with two renewals (7 days of exposure); three replicates for each test item concentration and six replicates for the control.
Nominal test item concentration	200, 40, 8.0, 1.6, 0.32, and 0.064 µg/L plus the control.
Nominal concentrations of aclonifen	99.8, 19.96, 3.992, 0.798, 0.160 and 0.0319 µg/L plus the control.
Test conditions	Temperature: 22.6 – 22.8°C; pH of the control: 7.41 – 8.83; light intensity: 7840 – 8148 lux; constant illumination; test vessels: glass crystallizers containing 150 mL of each treatment; initial frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP.
Statistics	Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Step-down Jonckheere-Terpstra Test Procedure, Wilimas Multiple Sequential t-test Procedure.
Endpoints value	ErC50, ErC20, ErC10, EyC50, EyC20, EyC10, LOEC and NOEC, based on frond number and dry weight

Results and discussions

The growth of *Lemna gibba* exposed to the test item, CHR/H/AKO 600 SC, was investigated in a 7 day semi-static test with two 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: 200, 40, 8.0, 1.6, 0.32, and 0.064 µg/L plus the control. The total number of fronds in each test vessel was counted twice during exposure (day 3 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time. At exposure termination, in the test item concentrations of 0.064, 0.32, and 1.6 µg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of 8.0 µg/L, separated roots were observed. In the test item concentration of 40 µg/L, colony break-up was observed. In the test item concentration of 200 µg/L, smaller new fronds, colony break-up and necrosis were observed. The concentrations of aclonifen were chemically analyzed using a validated high performance liquid chromatographic method with MS/MS detection (LC-MS/MS). Samples of all fresh test item concentrations and the control collected at exposure initiation and all spent test item concentrations and the control collected at the first renewal were chemically determined. Moreover, fresh samples of the highest (200 µg/L) and the lowest test item concentration (0.064 µg/L) plus the control at both renewals as well as spent samples of the highest and the lowest test item concentration plus the control during the second renewal and at exposure termination were chemically analyzed. In fresh samples at exposure initiation and at renewals, the determined concentrations of aclonifen were in the range of 89.0 – 110% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. In spent samples at renewals and at exposure termination, the determined concentrations of aclonifen were in the range of 90.8 – 101% of the nominal concentration. Therefore, the concentrations of aclonifen were stable under test conditions. The endpoints value was determined

based on the nominal test item concentrations as well as on the nominal concentrations of aclon-
ifen.

Table 8. Frond number and dry weight – definitive test

Nominal test item concentration [µg/L]	Frond number			Dry weight [mg]
	day 3	day 5	day 7	day 7
Control	35	65	105	22.3
	33	71	116	22.6
	31	65	102	21.7
	30	64	103	22.5
	39	89	145	29.5
	32	66	115	19.2
	mean	33.3	70.0	114.3
standard deviation	3.27	9.63	16.19	3.44
0.064	33	69	107	25.4
	34	71	122	24.3
	34	72	116	22.2
	mean	33.7	70.7	115.0
standard deviation	0.58	1.53	7.55	1.63
0.32	30	67	109	20.9
	30	66	106	19.3
	32	68	115	20.1
	mean	30.7	67.0	110.0
standard deviation	1.15	1.00	4.58	0.80
1.6	31	65	97	17.4
	31	60	94	15.9
	29	61	88	16.7
	mean	30.3	62.0	93.0
standard deviation	1.15	2.65	4.58	0.75
8.0	23	29	31	5.4
	26	29	30	6.0
	23	30	30	5.5
	mean	24.0	29.3	30.3
standard deviation	1.73	0.58	0.58	0.32
40	17	18	18	3.0
	21	21	22	3.8
	20	20	20	2.6
	mean	19.3	19.7	20.0
standard deviation	2.08	1.53	2.00	0.61
200	15	16	16	2.7
	13	15	15	2.6
	14	14	14	2.9
	mean	14.0	15.0	15.0
standard deviation	1.00	1.00	1.00	0.15
Inoculum	day 0	9	1.5	
		9	1.2	
9		1.0		
mean		9	1.2	
standard deviation		0.0	0.3	

Dates of exposure: 02.02.2024 – 09.02.2024

Table 9. Section-by-section growth rate, growth rate and yield based on frond number – definitive test

Nominal test item concentration [µg/L]	Section-by-section growth rate *			Mean growth rate	Yield**		
	0 – 3 d	3 – 5 d	5 – 7 d	0 – 7 d	3 d	5 d	7 d
Control	0.453	0.310	0.240	0.351	26	56	96
	0.433	0.383	0.245	0.365	24	62	107
	0.412	0.370	0.225	0.347	22	56	93
	0.401	0.379	0.238	0.348	21	55	94
	0.489	0.413	0.244	0.397	30	80	136
	0.423	0.362	0.278	0.364	23	57	106
mean	0.435	0.369	0.245	0.362	24.3	61.0	105.3
standard deviation	0.0316	0.0340	0.0175	0.0189	3.27	9.63	16.19
n	6						
CV	7.3	9.2	7.1	5.2	13.4	15.8	15.4
0.064	0.433	0.369	0.219	0.354	24	60	98
	0.443	0.368	0.271	0.372	25	62	113
	0.443	0.375	0.238	0.365	25	63	107
	0.440	0.371	0.243	0.364	24.7	61.7	106
mean	0.440	0.371	0.243	0.364	24.7	61.7	106
standard deviation	0.0057	0.0039	0.0259	0.0095	0.58	1.53	7.55
N	3						
CV	1.3	1.0	10.7	2.6	2.3	2.5	7.1
0.32	0.401	0.402	0.243	0.356	21	58	100
	0.401	0.394	0.237	0.352	21	57	97
	0.423	0.377	0.263	0.364	23	59	106
	0.408	0.391	0.248	0.358	21.7	58.0	101
mean	0.408	0.391	0.248	0.358	21.7	58.0	101
standard deviation	0.0124	0.0128	0.0134	0.0059	1.15	1.00	4.58
n	3						
CV	3.0	3.3	5.4	1.7	5.3	1.7	4.5
1.6	0.412	0.370	0.200	0.340	22	56	88
	0.412	0.330	0.224	0.335	22	51	85
	0.390	0.372	0.183	0.326	20	52	79
	0.405	0.357	0.203	0.334	21.3	53.0	84.0
mean	0.405	0.357	0.203	0.334	21.3	53.0	84.0
standard deviation	0.0128	0.0236	0.0207	0.0071	1.15	2.65	4.58
N	3						
CV	3.2	6.6	10.2	2.1	5.4	5.0	5.5
8.0	0.313	0.116	0.033	0.177	14	20	22
	0.354	0.055	0.017	0.172	17	20	21
	0.313	0.133	0.000	0.172	14	21	21
	0.326	0.101	0.017	0.174	15.0	20.3	21.3
mean	0.326	0.101	0.017	0.174	15.0	20.3	21.3
standard deviation	0.0236	0.0412	0.0167	0.0027	1.73	0.58	0.58
n	3						
CV	7.2	40.7	99.5	1.6	11.5	2.8	2.7
40	0.212	0.029	0.000	0.099	8	9	9
	0.282	0.000	0.023	0.128	12	12	13
	0.266	0.000	0.000	0.114	11	11	11
	0.254	0.010	0.008	0.114	10.3	10.7	11.0
mean	0.254	0.010	0.008	0.114	10.3	10.7	11.0
standard deviation	0.0369	0.0165	0.0134	0.0143	2.08	1.53	2.00
n	3						
CV	14.5	173.2	173.2	12.6	20.1	14.3	18.2

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Table 9. Continued. Section-by-section growth rate, growth rate and yield based on frond number – definitive test

Nominal test item concentration [µg/L]	Section-by-section growth rate *			Mean growth rate	Yield**		
	0 – 3 d	3 – 5 d	5 – 7 d	0 – 7 d	3 d	5 d	7 d
200	0.170	0.032	0.000	0.082	6	7	7
	0.123	0.072	0.000	0.073	4	6	6
	0.147	0.000	0.000	0.063	5	5	5
mean	0.147	0.035	0.000	0.073	5.0	6.0	6.0
standard deviation	0.0239	0.0358	0.0000	0.0095	1.00	1.00	1.00
n	3						
CV	16.3	103.5	102.1	13.1	20.0	16.7	16.7

Example calculations: *growth rate = (ln (frond number on day 3) - ln (frond number on day 0))/3 days,
** yield = (frond number on day 7) - (frond number on day 0)
n: number of replicates
CV: coefficient of variation

Table 12. Results inhibition of growth rate and yield – definitive test

Nominal test item concentration [µg/L]	Based on frond number		Based on dry weight	
	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield
Control	0.0	0.0	0.0	0.0
0.064	-0.5*	-0.6*	-1.7*	-4.6*
0.32	1.2	4.1	4.3	13.2
1.6	7.9	20.3	10.6	28.9
8.0	52.1	79.7	47.5	79.6
40	68.6	89.6	67.8	91.1
200	79.9	94.3	72.1	93

Dates of exposure: 02.02– 09.02.2024

*inhibition is lower than 0% what means that growth rate and yield based on frond number and dry weight in the test item concentration at exposure termination was higher than in the control

Table 13. Growth rate endpoint values based on nominal test item concentrations [µg/L] – definitive test

Endpoint value [µg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E ₁ C ₁₀	1.708 (0.882 – 2.793)	0.938 (0.435 – 2.625)	0.735 (0.298 – 1.346)	0.543 (0.199 – 1.072)
E ₁ C ₂₀	5.940 (3.789 – 8.427)	2.776 (1.599 – 4.189)	1.997 (1.038 – 3.169)	1.772 (0.863 – 2.954)
E ₁ C ₃₀	64.469 (49.555 – 86.696)	22.099 (16.382 – 30.140)	13.523 (9.544 – 19.260)	17.020 (12.648 – 25.285)
LOEC	8.0	8.0	1.6	1.6
NOEC	1.6	1.6	0.32	0.32

Calculations according to [8], [SOP/W/68]
(-) - 95% confidence interval

Table 14. Yield endpoint values based on nominal test item concentrations [µg/L] – definitive test

Endpoint value [µg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E ₁ C ₁₀	0.648 (0.311 – 1.116)	0.794 (0.400 – 1.243)	0.947 (0.627 – 1.260)	0.400 (0.233 – 0.586)
E ₁ C ₂₀	2.168 (1.283 – 3.246)	1.575 (0.954 – 2.226)	1.500 (1.102 – 1.878)	0.794 (0.534 – 1.064)
E ₁ C ₃₀	21.868 (16.378 – 29.596)	5.845 (4.473 – 7.638)	3.614 (3.016 – 4.329)	2.942 (2.363 – 3.664)
LOEC	1.6	8.0	1.6	1.6
NOEC	0.32	1.6	0.32	0.32

Calculations according to [8], [SOP/W/68]
(-) - 95% confidence interval

Table 15. Growth rate endpoint values based on the nominal concentrations of aclonifen [µg/L] – definitive test

Endpoint value [µg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E ₁ C ₁₀	0.852 (0.440 – 1.393)	0.468 (0.217 – 0.811)	0.367 (0.149 – 0.672)	0.271 (0.099 – 0.535)
E ₁ C ₂₀	2.964 (1.891 – 4.204)	1.385 (0.798 – 2.090)	0.996 (0.518 – 1.581)	0.884 (0.432 – 1.474)
E ₁ C ₃₀	32.169 (24.733 – 43.258)	11.027 (8.175 – 15.036)	6.748 (4.762 – 9.610)	8.493 (5.813 – 12.617)
LOEC	3.992	3.992	0.798	0.798
NOEC	0.798	0.798	0.160	0.160

Calculations according to [8], [SOP/W/68]
(-) - 95% confidence interval

Table 16. Yield endpoint values based on the nominal concentrations of aclonifen [µg/L] – definitive test

Endpoint value [µg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E ₁ C ₁₀	0.323 (0.155 – 0.557)	0.396 (0.209 – 0.620)	0.472 (0.313 – 0.628)	0.200 (0.117 – 0.292)
E ₁ C ₂₀	1.082 (0.642 – 1.619)	0.786 (0.478 – 1.110)	0.748 (0.550 – 0.937)	0.396 (0.267 – 0.531)
E ₁ C ₃₀	10.912 (8.173 – 14.786)	2.916 (2.232 – 3.811)	1.803 (1.505 – 2.160)	1.468 (1.179 – 1.828)
LOEC	0.798	3.992	0.798	0.798
NOEC	0.160	0.798	0.160	0.160

Calculations according to [8], [SOP/W/68]

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:

The ErC50/7 d value is 13.523 µg/L (95% confidence interval 9.544 – 19.260).
The ErC20/7 d value is 1.997 µg/L (95% confidence interval 1.038 – 3.169).
The ErC10/7 d value is 0.735 µg/L (95% confidence interval 0.298 – 1.346).
The EyC50/7 d value is 3.614 µg/L (95% confidence interval 3.016 – 4.329).

The EyC20/7 d value is 1.500 µg/L (95% confidence interval 1.102 – 1.878).

The EyC10/7 d value is 0.947 µg/L (95% confidence interval 0.627 – 1.260).

For growth rate and yield, the NOEC/7 d values are 0.32 µg/L, whereas the LOEC/7 d values are 1.6 µg/L.

Endpoints based on the dry weight:

The ErC50/7 d value is 17.020 µg/L (95% confidence interval 11.648 – 25.285).

The ErC20/7 d value is 1.772 µg/L (95% confidence interval 0.863 – 2.954).

The ErC10/7 d value is 0.543 µg/L (95% confidence interval 0.199 – 1.072).

The EyC50/7 d value is 2.942 µg/L (95% confidence interval 2.363 – 3.664).

The EyC20/7 d value is 0.794 µg/L (95% confidence interval 0.534 – 1.064).

The EyC10/7 d value is 0.400 µg/L (95% confidence interval 0.233 – 0.586).

For growth rate and yield, the NOEC/7 d values are 0.32 µg/L, whereas the LOEC/7 d values are 1.6 µg/L.

The endpoint values based on the nominal concentrations of aclonifen:

Endpoints based on the frond number:

The ErC50/7 d value is 6.748 µg/L (95% confidence interval 4.762 – 9.610).

The ErC20/7 d value is 0.996 µg/L (95% confidence interval 0.518 – 1.581).

The ErC10/7 d value is 0.367 µg/L (95% confidence interval 0.149 – 0.672).

The EyC50/7 d value is 1.803 µg/L (95% confidence interval 1.505 – 2.160).

The EyC20/7 d value is 0.748 µg/L (95% confidence interval 0.550 – 0.937).

The EyC10/7 d value is 0.472 µg/L (95% confidence interval 0.313 – 0.628).

For growth rate and yield, the NOEC/7 d values are 0.160 µg/L, whereas LOEC/7 d values are 0.798 µg/L.

Endpoints based on the dry weight:

The ErC50/7 d value is 8.493 µg/L (95% confidence interval 5.813 – 12.617).

The ErC20/7 d value is 0.884 µg/L (95% confidence interval 0.431 – 1.474).

The ErC10/7 d value is 0.271 µg/L (95% confidence interval 0.099 – 0.535).

The EyC50/7 d value is 1.468 µg/L (95% confidence interval 1.179 – 1.828).

The EyC20/7 d value is 0.396 µg/L (95% confidence interval 0.267 – 0.531).

The EyC10/7 d value is 0.200 µg/L (95% confidence interval 0.117 – 0.292).

For growth rate and yield, the NOEC/7 d values are 0.160 µg/L, whereas LOEC/7 d values are 0.798 µg/L.

TEST VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 221 and the EU Method C.26. were met:

- the doubling time of frond number in the control was 1.9 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 12.7),
- the average specific growth rate in the control between day 0 and day 7 was 0.362 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹).

A 2.2.1.4 *Anabaena flos-aquae* UTEX B 1444

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. - the biomass in the control increased by a factor of 43.5 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 4.3% (criterion: it must not exceed 10%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 34.3% (criterion: it must not exceed 35%). All results refer to nominal concentrations since the measured concentrations were within 80 to 120% of nominal. The study is considered acceptable and suitable for the risk assessment. 72h-ErC50 = 2.81 mg test item/L 72h-ErC50 = 1.40 mg aclonifen/L
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Reference: 10.2.1

Report CHR/H/AKO 600 SC *Anabaena flos-aquae* UTEX B 1444, Growth inhibition test; Study code: W-22-23 Study Director: M. Czarnecka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): OECD Guideline No. 201 (2006)/EU method C.3.

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item CHR/H/AKO 600 SC; batch no. CHR/02/2023; the content of active substance (aclonifen): 49.9% (w/w), 607.8 g/L; manufacturing date: March 23, 2023; expiry date: not provided by the Sponsor.

Test system The freshwater cyanobacteria, *Anabaena flos-aquae* (Lyng.) Bréb UTEX B 1444 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.

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

Nominal test item concentration 10, 3.3, 1.1, 0.37, 0.12 and 0.04 mg/L plus the control.
4.99, 1.65, 0.55, 0.19, 0.06 and 0.02 mg/L plus the control.

Nominal concentrations of aclonifen

Test conditions Temperature: 22.5 – 23.0°C; pH of the control: 7.54 – 7.88; mean light intensity: 3326 – 3380 lux; constant illumination and shaking; medium: AAP.

Statistics Non-linear 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.

Endpoints value ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results and discussions

The growth of the cyanobacteria, *Anabaena flos-aquae* UTEX B 1444 exposed to the test item, CHR/H/AKO 600 SC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the cyanobacteria was 1×10^4 cells/mL. The definitive test was performed with the following test item concentrations: 10, 3.3, 1.1, 0.37, 0.12 and 0.04 mg/L plus the control. The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope. In case of each replicate, the number of cells was determined after 24, 48, and 72 h of exposure. Morphology observations of the cyanobacteria cells were performed at exposure termination. No growth rate and yield inhibitions were observed in the test item concentrations of 0.12 and 0.04 mg/L. Calculated inhibition of growth rate for the test item concentrations ranging from 0.37 to 10 mg/L after 72 h of exposure was in the range of 11.5 – 68.7% when compared to the control. Inhibition of yield for the test item concentrations ranging 0.37 to 10 mg/L after 72 h of exposure was in the range of 33.2 – 94.3% when compared to the control. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control. The concentrations of aclonifen were chemically analyzed using the validated high performance liquid chromatography (HPLC) with Diode Array Detection. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined. At exposure initiation, the determined concentrations of aclonifen were in the range of 96.7 – 101% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of aclonifen were in the range of 88.5 – 94.3% of the nominal concentration. The results confirm, that the concentrations of aclonifen were stable under test conditions. The endpoints value were determined based on the nominal test item concentrations as well as on the nominal concentrations of aclonifen.

Table 4. Inhibition of growth rate and yield, first preliminary non-GLP test

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.0001	-1.7*	-9.4*
0.001	4.2	15.3
0.01	5.9	22.1
0.1	7.8	25.7

*Inhibition is lower than 0%, which means that the cyanobacterial cell density at exposure termination was higher than the cyanobacterial cell density in the control.

Time of exposure: 27.10.2023 – 30.10.2023

Table 5. Inhibition of growth rate and yield, second preliminary non-GLP test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.1	7.5	28.0
1.0	53.0	86.9
10	67.5	93.4
100	69.8	94.1

Time of exposure: 06.11.2023 – 09.11.2023

Table 8. Cyanobacterial cell density – definitive test

Nominal test item concentration [mg/L]	Cyanobacterial cell density [$\times 10^6$ cells/mL]		
	24 h	48 h	72 h
Control	0.050	0.219	0.475
	0.060	0.241	0.544
	0.047	0.203	0.431
	0.053	0.178	0.400
	0.050	0.166	0.425
	0.041	0.172	0.335
mean	0.050	0.197	0.435
standard deviation	0.006	0.030	0.070
0.04	0.069	0.266	0.541
	0.060	0.206	0.581
	0.044	0.169	0.560
mean	0.058	0.214	0.561
standard deviation	0.013	0.049	0.020
0.12	0.085	0.150	0.550
	0.141	0.185	0.410
	0.081	0.238	0.466
mean	0.102	0.191	0.475
standard deviation	0.034	0.044	0.070
0.37	0.088	0.110	0.175
	0.119	0.197	0.335
	0.066	0.135	0.372
mean	0.091	0.147	0.294
standard deviation	0.027	0.045	0.105
1.1	0.147	0.081	0.122
	0.081	0.141	0.160
	0.038	0.100	0.103
mean	0.089	0.107	0.128
standard deviation	0.055	0.031	0.029
3.3	0.050	0.081	0.060
	0.066	0.131	0.066
	0.106	0.078	0.044
mean	0.074	0.097	0.057
standard deviation	0.029	0.030	0.011
10	0.088	0.091	0.022
	0.022	0.053	0.031
	0.050	0.044	0.050
mean	0.053	0.063	0.034
standard deviation	0.033	0.025	0.014

Time of exposure: 30.03.2024 – 01.03.2024

Table 9. Growth rate and yield, definitive test

Table 3. Growth rate and yield, derivative test					
Nominal test item concentration [mg/L]	Growth rate* [10 ⁴ cells/mL·day ⁻¹]				Yield** [10 ⁴ cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.609	1.477	0.774	1.287	0.465
	1.792	1.390	0.814	1.332	0.534
	1.548	1.463	0.753	1.255	0.421
	1.668	1.211	0.810	1.230	0.390
	1.609	1.200	0.940	1.250	0.415
	1.411	1.434	0.667	1.171	0.325
mean	1.606	1.363	0.793	1.254	0.425
standard deviation	0.126	0.125	0.090	0.054	0.070
0.04	1.932	1.349	0.710	1.330	0.531
	1.792	1.234	1.037	1.354	0.571
	1.482	1.346	1.198	1.342	0.550
	mean	1.735	1.310	0.982	1.342
standard deviation	0.230	0.066	0.249	0.012	0.020
0.12	2.140	0.568	1.299	1.336	0.540
	2.646	0.272	0.796	1.238	0.400
	2.092	1.078	0.672	1.281	0.456
	mean	2.293	0.639	0.922	1.285
standard deviation	0.307	0.408	0.332	0.049	0.070
0.37	2.175	0.223	0.464	0.954	0.165
	2.477	0.504	0.531	1.171	0.325
	1.887	0.716	1.014	1.205	0.362
	mean	2.179	0.481	0.670	1.110
standard deviation	0.295	0.247	0.300	0.136	0.105
1.1	2.688	n.d.	0.410	0.834	0.112
	2.092	0.554	0.126	0.924	0.150
	1.335	0.968	0.030	0.777	0.093
	mean	2.038	n.d.	0.189	0.845
standard deviation	0.678	n.d.	0.197	0.074	0.029
3.3	1.609	0.482	n.d.	0.597	0.050
	1.887	0.686	n.d.	0.629	0.056
	2.361	n.d.	n.d.	0.494	0.034
	mean	1.952	n.d.	n.d.	0.573
standard deviation	0.380	n.d.	n.d.	0.071	0.011
10	2.175	0.034	n.d.	0.263	0.012
	0.788	0.879	n.d.	0.377	0.021
	1.609	n.d.	0.128	0.536	0.040
	mean	1.524	n.d.	n.d.	0.392
standard deviation	0.697	n.d.	n.d.	0.137	0.014

n.d. - not determined due mathematical reasons

* Growth rate (day⁻¹) was calculated according to the following formula:

Table 11. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E ₁ C ₅₀	n.d.	>10	2.81 (1.20 - 6.39)
E ₁ C ₂₀	n.d.	1.40 (0.30 - 6.75)	0.53 (0.27 - 1.05)
E ₁ C ₁₀	n.d.	0.34 (0.08 - 1.54)	0.22 (0.11 - 0.45)
LOEC	>10	1.10	0.37
NOEC	≥10	0.37	0.12

(-) - 95% confidence interval

Calculations were made according to [9], [SOP/W/68]

n.d. - not determined

Table 12. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E ₁ C ₅₀	n.d.	1.85 (0.16 - 20.24)	0.55 (0.19 - 1.55)
E ₁ C ₂₀	n.d.	0.25 (0.04 - 1.86)	0.23 (0.10 - 0.56)
E ₁ C ₁₀	n.d.	0.09 (0.01 - 0.72)	0.15 (0.06 - 0.37)
LOEC	>10	0.37	0.37
NOEC	≥10	0.12	0.12

(-) - 95% confidence interval

Calculations were made according to [9], [SOP/W/68]

n.d. - not determined

Table 13. Growth rate endpoint values based on the nominal concentrations of aclonifen, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
ErC ₅₀	n.d.	>4.99	1.40 (0.60 – 3.20)
EyC ₅₀	n.d.	0.69 (0.14 – 3.52)	0.27 (0.14 – 0.53)
ErC ₁₀	n.d.	0.17 (0.03 – 0.79)	0.11 (0.05 – 0.23)
LOEC	>4.99	0.55	0.19
NOEC	≥4.99	0.19	0.06

(-) = 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d. = not determined

Table 14. Yield endpoint values based on the nominal concentrations of aclonifen, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	n.d.	0.92 (0.08 – 10.68)	0.27 (0.10 – 0.79)
E _y C ₅₀	n.d.	0.13 (0.02 – 0.98)	0.12 (0.05 – 0.28)
E _r C ₁₀	n.d.	0.04 (0.01 – 0.37)	0.07 (0.03 – 0.18)
LOEC	>4.99	0.19	0.19
NOEC	≥4.99	0.06	0.06

(-) = 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d. = not determined

The endpoint values based on the nominal test item concentrations:

The ErC₅₀/72 h value is 2.81 mg/L (95% confidence interval: 1.20 – 6.39).

The EyC₅₀/72 h value is 0.55 mg/L (95% confidence interval: 0.19 – 1.55).

The LOEC/72 h value for growth rate and yield is 0.37 mg/L.

The NOEC/72 h value for growth rate and yield is 0.12 mg/L.

The endpoint values based on the nominal concentrations of aclonifen:

The ErC₅₀/72 h value is 1.40 mg/L (95% confidence interval: 0.60 – 3.20).

The EyC₅₀/72 h value is 0.27 mg/L (95% confidence interval: 0.10 – 0.79).

The LOEC/72 h value for growth rate and yield is 0.19 mg/L.

The NOEC/72 h value for growth rate and yield is 0.06 mg/L.

TEST VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:

- the biomass in the control increased by a factor of 43.5 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 4.3% (criterion: it must not exceed 10%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 34.3% (criterion: it must not exceed 35%).

A 2.2.2

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.a *Coccinella septempunctata* (L.)

Comments of zRMS:	The test was conducted in compliance with Good Laboratory Practice (GLP) principles and holds an ISO 9001:2015 certification. The methodology followed established ecotoxicological guidelines, including ESCORT 1 and 2, and guidelines from the IOBC, BART, and EPPO Joint Initiative. Deviations: The use of leaf discs instead of plastic discs was noted as a deviation from the guideline but was pre-defined in the Study Plan and internal SOPs. This is generally considered a minor deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ was determined to be > 3.0 L/ha Study accepted.
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Reference: 10.3

Report An extended laboratory test for evaluating effects of CHR/H/AKO 600 SC on the ladybird beetle, *Coccinella septempunctata* (L.); Study code: B-76-23
Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

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

Deviations: The experimental part of the study was conducted according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Schmuck V. et al., 2000), SOP/B/63 and other procedures related with the study and the Study Plan. In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/63. The deviation is due to the type of study ordered by the Sponsor. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses will not be carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1

GLP: Yes

Acceptability:

Duplication No

(if vertebrate study)

Materials and methods

Test item:	CHR/H/AKO 600 SC Content: 607.8 g/L of acetonifin Batch number: CHR/02/2023 Manufacturing date: 23 March 2023 Expiry date: not determined by the Sponsor 1
Biological test system:	the ladybird beetle, <i>C. septempunctata</i> L. (Arthropoda: Coccinellidae) – age: 5-day-old larvae – source: Commercial supplier (Katz Biotech AG, Germany)
Experimental design:	5 study groups: – a control group (0.0 L/ha) – CHR/H/AKO 600 SC at the rates of: – the test item at the rate of 0.75 L/ha – the test item at the rate of 1.5 L/ha – the test item at the rate of 3.0 L/ha – pyrazophos at the rate of 3.0 g/ha – number of replicates: 40 replicates/group – number of larvae: 1 larva of <i>Coccinella septempunctata</i> /replicate
Test conditions:	– temperature: 23.0 – 27.0°C – relative air humidity: 60.0 – 80.1% – photoperiod: 16 hours light : 8 hours dark – light intensity 2078 lx
Statistical analysis:	probit analysis using linear max. likelihood regression, Chi2 2x2

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/AKO 600 SC on mortality and reproductive capacity of the ladybird beetle, *Coccinella septempunctata*. In a definitive test, three test item application rates of 0.75, 1.5 and 3.0 L/ha were used. To assess mortality of the ladybird beetles, *Coccinella septempunctata* L., 5-day-old larvae were exposed to the test item applied to leaf discs. There were 40 replicates of each treated group. Each replicate contained 1 larva of *C. septempunctata* L. The larvae were fed with the fresh aphids, *Acyrtosiphon pisum* until pupation. During the exposure phase, survival, condition and development of the ladybird beetles were regularly assessed until the end of pupation. After emergence of the adults, pre-imaginal mortality was calculated on the basis of the numbers of dead larvae, pupae, and adults which died during emergence. After completion of mortality assessment, healthy hatched beetles from the control group and from group treated with the test item at the rates of 0.75, 1.5 and 3.0 L/ha were subjected to evaluate the reproductive performance. To allow egg-laying, adult ladybirds were transferred to separate reproduction units. The beetles had continuous access to food in the form of a honey-water solution (2:1), pine pollen (*Pinus* sp.) and the broad bean plants infested with the aphid, *A. pisum*. Reproductive performance observations, concerning the numbers of eggs laid and their fertility were made between 27 and 47 day after treatment. To check the relative susceptibility of the test system and the sensitivity of the test method, pyrazophos was used as a reference item. The rate of the reference item was 3.0 g/ha. Control beetles had contact with leaf discs sprayed with distilled water.

The effects of the test item, CHR/H/AKO 600 SC on mortality and reproductive capacity of the ladybird beetle, *Coccinella septempunctata* L. in the laboratory test are summarized below.

Study group	Parameters (endpoints)						
	Mortality				Reproduction		
Test item [L/ha]	[no.]	[%]	Corr. [%] ^a	LR ₅₀ [L/ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]
Test item: CHR/H/AKO 600 SC							
Control (0.0)	12	30.0	-	> 3.0	17.9	13.8	-
0.75	10	25.0	(-7.1) ^b		13.1	10.5	23.9
1.5	6	15.0	(-21.4) ^b		10.4	8.3	40.0
3.0	15	38.0	10.7		17.0	13.6	1.7
NOER _{mortality}	≥ 3.0 [L test item/ha]						
Reference item: pyrazophos							
[g/ha]	Mortality [%]				Reproduction		
3.0	100.0				not assessed		

^a: Mortality corrected according to the Abbott formula [10]

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

Conclusions

The validity criterion concerning mortality was met, because mortality of the ladybird beetle, *Coccinella septempunctata* L. in the control group was equal to 30.0% ($\leq 30.0\%$). The mortality of the ladybird beetles exposed to the test item at the rates of 0.75, 1.5 and 3.0 L/ha, after Abbott correction, were (-7.1), (-21.4) and 10.7%, respectively. The negative value indicates that mortality in the group treated with the test item was lower than in the control group.

At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.75, 1.5 and 3.0 L/ha of CHR/H/AKO 600 SC and the control group.

The LR₅₀ value is higher than 3.0 L/ha of CHR/H/AKO 600 SC. The NOER_{mortality} is higher than or equal to 3.0 L/ha of CHR/H/AKO 600 SC.

The mortality of the ladybird beetles exposed to the reference item at the rate of 3.0 g of pyrazophos/ha, was equal to 100.0%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to pyrazophos.

The mean number of fertile eggs/female/day in the control group was 13.8 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha were equal to 10.5, 8.3 and 13.6, it refers to 23.9, 40.0 and 1.7% of reproduction reduction.

It can be concluded that CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha had no adverse effect on mortality of the ladybird beetle.

Based on the results, it can be stated that CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha has no adverse effect on the reproduction capacity of the ladybird beetle.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- pre-imaginal mortality of the control group was 30.0% (criterion: a maximum of 30.0%),
- mean corrected mortality of the reference item group was 100.0% (criterion: a minimum of 40%),
- fertility (the mean number of fertile eggs/female/day) in the control group was 13.8 (criterion: ≥ 2 fertile eggs/female).

A 2.3.b *Typhlodromus pyri* (Sch.)

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles and according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000). The study successfully met all three of its mandatory validity criteria : • Mortality in the control group was 0.0% (criterion: $\leq 20\%$) . • Mortality in the reference item group was 96.67%, confirming the sensitivity of the test population (criterion: 50% to 100%) . • The cumulative mean number of eggs per female in the control group was 8.1, demonstrating a healthy and reproductive population (criterion: ≥ 4) . Deviations: The use of an additional food source (<i>T. urticae</i> eggs) was noted as a deviation from the guideline but was justified as preventing mites from escaping. This is generally considered a minor deviation. The LR_{50} = 1.5256 L/ha. The NOER = 0.33 L/ha. The NOER <0.0371 L/ha The ER_{50} = 1.8710 L/ha Study is accepted.
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Reference: 10.3

Report An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the predatory mite, *Typhlodromus pyri* (Sch.); Study code: B-77-23
Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

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

Deviations: The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/36: 'An extended laboratory test for evaluating the effects of plant

protection products on the predatory mite, *Typhlodromus pyri* (Sch.)', other references given in section 9 and the SOP's listed in section 10 of the report and the Study Plan. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses were not carried out. The waiver of these analyses constitutes a deviation from the OECD Principles of Good Laboratory Practice Number 1. According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen should be used. However, in the experiment additional food in the form of the two-spotted spider mite (*T. urticae*) eggs, was used. Another food source prevents the mites from escaping from discs. These deviations have no impact on the quality, integrity and final results of the study

GLP: Yes

Acceptability:

Duplication
(if vertebrate study) No

Materials and methods

Test item:	CHR/H/AKO 600 SC Active substance: 607.8 g/L of aclonifen Batch number: CHR/02/2023 Production date: 23.03.2023 Expiry date: unspecified
Biological test system:	the predatory mite, <i>Typhlodromus pyri</i> (Sch.) (Acari: Phytoseiidae) – age: 24-hour-old protonymphs – source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented from a commercial breeder
Experimental design:	7 study groups: – a control group (0.0 L/ha) – CHR/H/AKO 600 SC at the rate of 0.0371 L/ha – CHR/H/AKO 600 SC at the rate of 0.11 L/ha – CHR/H/AKO 600 SC at the rate of 0.33 L/ha – CHR/H/AKO 600 SC at the rate of 1.0 L/ha – CHR/H/AKO 600 SC at the rate of 3.0 L/ha – reference item: dimethoate at the rate of 4.0 g/ha number of replicates: 3/group number of mites in each replicate: 20
Test conditions:	– temperature: 23.5 – 26.0 °C – relative air humidity: 68 – 70 % – photoperiod: 16 h light : 8 h dark – light intensity: 718 lx
Statistical analysis:	Probit analysis using linear max. likelihood regression Step-down Cochran-Armitage test procedure Shapiro Wilk's Test on Normal Distribution

Levene's Test on Variance Homogeneity (with Residuals)
Williams Multiple Sequential t-test Procedure

Endpoints:

- mite mortality after 7 days of the treatment
- LR50 and NOERmortality
- reproduction reduction (Pr) after 14 days of the treatment
- ER50 and NOERreproduction

Results and discussions

The aim of the extended laboratory test was to evaluate the effects of the test item, CHR/H/AKO 600 SC on mortality and reproduction of the predatory mite, *T. pyri* (Sch.). On the basis of the preliminary test results, it was decided to use five rates of the test item in the definitive test. These were 0.0371, 0.11, 0.33, 1.0 and 3.0 L/ha (i.e. 22.55, 66.86, 200.57, 607.8 and 1823.4 g a.s. /ha, respectively). The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (*Pinus* sp.) and *T. urticae* eggs. Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and groups treated with the test item at rates 0.0371, 0.11, 0.33 and 1.0 L/ha were made after 8, 11, and 14 days of the treatment. Mortality of *T. pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints. To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 4.0 g/ha. The control group was treated with distilled water.

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to CHR/H/AKO 600 SC at rates of 0.0371, 0.11, 0.33, 1.0 and 3.0 L/ha, the percentages of mortality, were 0.0, 0.0, 1.67, 16.67 and 91.67%, respectively. There were no statistically significant differences in mortality in the group treated with the test item at the rates of 0.0371, 0.11, 0.33 L/ha in comparison to the control group. There were statistically significant differences in mortality between groups treated with the test item at the rates of 1.0 and 3.0 L/ha in comparison to the control group (Step-down Cochran-Armitage test procedure, $p(\text{trend}) < \alpha 0.05$). The LR50 value is 1.5256 L/ha. NOERmortality is 0.33 L/ha.

After 7 days of exposure to dimethoate at the rate of 4.0 g/ha, mortality was 96.67%. Therefore, the validity criterion specified in the method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Reproduction of the surviving mites from the control group and groups treated with test item at the rates of 0.0371, 0.11, 0.33 and 1.0 L/ha were assessed since mortality of these groups was $\leq 50.0\%$.

The mean reproduction rate (Rr) in the control group was 8.1 eggs/female. The mean Rr after 14 days of exposure to test item at the rates of 0.0371, 0.11, 0.33 and 1.0 L/ha were 6.7, 6.6, 5.1 and 4.6 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.0371, 0.11, 0.33 and 1.0 L/ha were 17.5, 18.7, 36.3 and 42.5%, respectively.

There were statistically significant differences in reproduction between the groups treated with the test item at the rates of 0.0371, 0.11, 0.33 and 1.0 L/ha and the control group (Williams Multiple Sequential t-test Procedure, $|t| > |t^*|$). The calculated ER50 value is 1.8710 L/ha. NOERreproduction is lower than 0.0371 L/ha.

Parameter (endpoint)				
Mortality (dead + escaped mites)		Reproduction		
Test item rate [L/ha]	Total [%]	Test item rate [L/ha]	Mean number of eggs/ female (Rr) [no.]	Reproduction reduction Pr [%]
control	0.0	control	8.1	–
0.0371	0.0	0.0371 ⁺	6.7	17.5
0.11	0.0	0.11 ⁺	6.6	18.7
0.33	1.67	0.33 ⁺	5.1	36.3
1.0 ⁺	16.67	1.0 ⁺	4.6	42.5
3.0 ⁺	91.67	3.0	not assessed	
LR ₅₀	1.5256 L/ha (1.0040 – 2.3485)*	ER ₅₀	1.8710 L/ha (0.7375 – 56.4056)*	
NOER _{mortality}	0.33 L/ha	NOER _{reproduction}	< 0.0371 L/ha	
Reference item: dimethoate				
Rate [g/ha]	Total [%]	Reproduction		
4.0	96.67	not assessed		

⁺: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [11], [SOP/B/67]

*: 95%-confidence limits

Conclusions

Based on the results it can be stated that CHR/H/AKO 600 SC at the rates 1.0 and 3.0 L/ha has an adverse effect on mortality and at the rates 0.0371, 0.11, 0.33 and 1.0 L/ha has an adverse effect on reproduction of the mites.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
- mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 96.67% on day 7 of exposure (criterion: from 50 to 100%),
- the cumulative mean number of eggs per female in the control group was 8.1 (required: ≥ 4 eggs per female).

A 2.3.c *Aphidius rhopalosiphi*

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles. The methodology followed established international guidelines, including ESCORT 1, ESCORT 2, and specific guidelines for <i>A. rhopalosiphi</i> from the IOBC, BART, and EPPO Joint Initiative ((Mead-Briggs M.A. et al., 2010). The study successfully met all five of its mandatory validity criteria : • Mortality in the control group was 0.0% (criterion: $\leq 10.0\%$) . • Mortality in the reference item group was 86.7%, confirming the sensitivity of the test population (criterion: $\geq 50\%$) . • The mean number of mummies per female in the control group was 35.8, demonstrating a healthy and reproductive population (criterion: ≥ 5.0) . • All control wasps survived oviposition and produced offspring, meeting the final two criteria. The $LR_{50} > 3.0$ L/ha The $ER_{50} = 4.119$ L/ha The NOER = 0.75 L/ha Study accepted.
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Reference: 10.3

Report An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Study code: B-78-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

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

Deviations: The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/28: ‘An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez.)’, other references given in section 9 and the SOP’s listed in section 10 of the report and the Study Plan. There were no deviations from mentioned documents. A deviation from the Study Plan concerning study completion date occurred. According to the Study Plan the study should be completed in November – December 2023. However, completion of the study is delayed beyond its scheduled date. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses will not be carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1. Those deviations had no impact on the quality, integrity and final results of the study

GLP: Yes

Acceptability:

Duplication
(if vertebrate study) No

Materials and methods

Test item:

CHR/H/AKO 600 SC
Batch number: CHR/02/2023
Production date: 23.03.2023
Expiry date: not determined by the Sponsor
Active substance: 607.8 g/L of aclonifen (CAS No. 74070-46-5)

Biological test system:

the parasitic wasp, *Aphidius rhopalosiphii* (De Stefani-Perez); Hymenoptera: Braconidae, Aphidinae
– age: imago (24 – 48 hours after emerging from mummies)
– source: breeding of the parasitic wasps at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from a commercial breeder (Katz Biotech AG)

Experimental design:

5 study groups:
– a control group (0.0 L/ha)
– test item at the rate of 0.75 L/ha
– test item at the rate of 1.5 L/ha
– test item at the rate of 3.0 L/ha
– reference item: dimethoate at the rate of 20.0 g/ha
mortality assessment: 6 replicates/group, 5 females/replicate
fecundity assessment: 15 replicates/group, 1 female/replicate

Test conditions:

– temperature: 20 – 21°C
– relative air humidity: 67 – 73%
– photoperiod: 16 hours light : 8 hours dark
– light intensity: mortality and oviposition assessment: 3463 lx fecundity phase: 4093 lx

Statistical analysis:

Fecundity:

– Probit analysis using linear max. likelihood regression,
– Chi2 2x2 Table Test with Bonferroni Correction,
– Shapiro-Wilk's Test on Normal Distribution,
– Levene's Test on Variance Homogeneity,
– Multiple Sequentially-rejective Welsh-t-test After Bonferroni Holm

Repellency:

– Shapiro-Wilk's Test on Normal Distribution,

- Levene's Test on Variance Homogeneity,
- STUDENT-t test Homogeneous Variances,
- Dunnett's Multiple t-test Procedure

Endpoints:

- wasp mortality after 48 hours of exposure
- LR50 and the NOERMortality
- ER50 and the NOERfecundity
- reduction in fecundity (Pr) of the surviving female wasps exposed to test item, 12 days after the oviposition period

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/AKO 600 SC on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphii*. On the basis of the results of the preliminary range-finding non-GLP test it was decided to use three rates of the test item in the definitive test. These were 0.75, 1.5 and 3.0 L/ha. Adult wasps were exposed to the test item applied to barley plants. Observations of settling behavior were made during the initial 3 hours of exposure. The aims were to determine repellent effects of CHR/H/AKO 600 SC and to check if the test insects had contact with barley plants sprayed with the test item. Settling behavior of wasps from each replicate was observed five times. Mortality was determined 2, 24 and 48 hours after the introduction of the wasps to the test arenas. Females from all the groups treated with the test item which survived the 48-hour exposure to test item and the ones from the control group were subjected to fecundity assessments. Fifteen female wasps from the five groups treated with the test item and the control were individually introduced into the fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded. Mortality after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints. To verify the sensitivity of the biological test system and the precision of the test procedure, dimethoate, which is an insecticide, was used as a reference item. The rate of the reference item was 20.0 g/ha. The control group was treated with distilled water.

In the definitive test, after 48 hours, mortality of the control wasps was 0.0%. After 48 hours of the exposure to CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha, the percentages mortality of *A. rhopalosiphii*, were 3.3, 0.0 and 0.0% respectively for each group.

Based on the obtained mortality results it could be assumed that the LR50 is higher than 3.0 L/ha and the NOERMortality is higher than or equal to 3.0 L/ha.

The mortality of the wasps exposed to dimethoate at the rate of 20.0 g/ha was 86.7% after 48 hours. Therefore, the validity criterion specified in the method description was met [6]. The results showed that the test organisms were sensitive to dimethoate.

At the significance level of 0.05, there were no statistically significant differences in the mean percentages of wasps settled on the plants between the test item groups at the rates of 0.75, 1.5 and 3.0 L/ha and the control group (Dunnett's Multiple t-test Procedure, $|t| > |t^*|$). The NOER is higher than or equal 3.0 L/ha.

The fecundity assessment showed that the mean number of mummies per female in the control group was 35.8 (after 12 days after oviposition). As for the wasps treated with test item at the rates of 0.75, 1.5 and 3.0 L/ha the mean number of mummies per female were 32.8, 25.0 and 20.5, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.75, 1.5 and 3.0 L/ha were 8.4, 30.2 and 42.6%, respectively.

At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the rates of 1.5 and 3.0 L/ha and the control group (Multiple Sequentially-rejective Welsh-t-test After Bonferroni- Holm).

Based on the obtained fecundity results it could be assumed that the ER50 value is 4.119 L/ha (95% con-

fidence limits: 2.777 – 13.637) and the NOERfecundity is 0.75 L/ha.

Parametr (endpoint)						
Mortality after 48 hours			Fecundity			
Test item [L/ha]	Total [%]	LR ₅₀ [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER ₅₀ [L/ha]
Control	0.0	–	Control	35.8	–	–
0.75	3.3	> 3.0	0.75	32.8	8.4	4.119 (95% confidence limits: 2.777 – 13.637)
1.5	0.0		1.5 ⁺	25.0	30.2	
3.0	0.0		3.0 ⁺	20.5	42.6	
NOER _{mortality}	≥ 3.0 L/ha		NOER _{fecundity}	0.75 L/ha		
Reference item: dimethoate						
Rate [g/ha]	Total mortality [%]		Fecundity			
20.0	86.7		not assessed			

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

Conclusions

On the basis of the obtained repellency results it can be concluded that CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha has no repellent properties.

On the basis of the obtained mortality results it can be concluded that CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha has no adverse effect on the mortality of the wasps, *A. rhopalosiphi*.

On the basis of the obtained fecundity results it can be concluded that CHR/H/AKO 600 SC at the rates of 1.5 and 3.0 L/ha has adverse effect on the fecundity of the wasps, *A. rhopalosiphi*.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- after 48 hours, mortality of the group treated with the reference item at the rate of 20.0 g/ha was 86.7% (criterion: a minimum of 50%),
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- the mean number of mummies per female in the control group was 35.8 (criterion: a minimum of 5.0 mummies/female),
- all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

A 2.3.d *Chrysoperla carnea*

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles. The methodology followed established international guidelines, including ESCORT 1, ESCORT 2, and specific guidelines for <i>C. carnea</i> from the IOBC, BART, and EPPO Joint Initiative (Vogt H. et al., 2000). The study successfully met all four of its pre-defined validity criteria as stipulated by the testing guideline. • Pre-imaginal mortality in the control group was 16.7% (criterion: $\leq 20.0\%$). • Mortality in the reference item group was 100.0% (criterion: $\geq 50\%$). • The mean number of eggs per female per day in the control group was 19.3 (criterion: ≥ 15.0). • The mean hatching rate in the control group was 88.8% (criterion: $\geq 70\%$). One deviations was noted, the use of leaf discs as the exposure surface instead of plastic discs. This was described in the Study Plan and internal SOPs. LR₅₀ >3.0 L/ha Study is accepted.
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Reference: 10.3

Report An extended laboratory test for evaluating the effects of CHR/H/AKO 600 SC on the green lacewing, *Chrysoperla carnea*; Study code: B-79-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

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

Deviations: The study was conducted according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Vogt H. et al., 2000), SOP/B/62 and other procedures related to the study and the Study Plan. In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/62. The deviations is due to the type of study ordered by the Sponsor. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses was not be carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1

GLP: Yes

Acceptability:

Duplication No

(if vertebrate study)

Materials and methods

Test item:	CHR/H/AKO 600 SC Content: 607.8 g/L of aclon- ifen Batch number: CHR/02/2023 Manufacturing date: 23.03.2023 Appearance: suspension 1 Expiry date: not specified by Sponsor
Biological test system:	the green lacewing, <i>Chrysoperla carnea</i> (Steph.), Neuroptera: Chrysopidae – age: first instars' larvae (3 days old) – source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was ob- tained from a commercial breeder (Katz Biotech AG, Germany), [SOP/B/78]
Experimental design:	5 study groups: – a control group (0.0 L/ha) – CHR/H/AKO 600 SC at the rates of: – 0.75 L/ha – 1.5 L/ha – 3.0 L/ha – dimethoate at the rate of 15.0 g/ha number of replicates: 30 replicates/group number of larvae: 1 larva of <i>Chrysoperla carnea</i> /replicate
Test conditions:	– temperature: 23.0 – 27.0°C – relative air humidity: 60.0 – 81.9% – photoperiod: 16 hours light : 8 hours dark – light intensity 1640 lux
Statistical analysis:	Probit analysis using linear max. likelihood regres- sion, Chi2 2x2 Table Test with Bonferroni Correc- tion
Endpoints:	– cumulative mortality of larvae, pupae, and adults after emergence – LR50 value – reproduction of the lacewings: – fecundity (mean number of eggs/female/day) – fertility (mean hatching rate)

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/AKO 600 SC on mortality and reproductive capacity of the green lacewing, *Chrysoperla carnea*. In a definitive test, three test item application rates were used. These were 0.75, 1.5 and 3.0 L/ha. To assess mortality, 3-day-old larvae of *Chrysoperla carnea* were exposed to dry residues of the test item on leaf discs. Eggs of the mill moth *Ephestia kuehniella* were offered as food. After emergence of adults, total mortality was calculated on the basis of the numbers of dead larvae, pupae, and adults which died during emergence. There were 30 replicates of each treated group. Each of them contained 1 larva of *Chrysoperla carnea*. To determine possible adverse effects of the test item on fecundity and fertility of the lacewings, reproductive

performance was conducted. Total mortality of the lacewings, the mean number of eggs laid per female lacewing per day, and the mean hatching rate were the endpoints. To control the sensitivity of the biological test system, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 15.0 g/ha. Control lacewings had contact with leaf discs sprayed with distilled water.

The effects of the test item, CHR/H/AKO 600 SC on mortality and reproductive capacity the green lacewings, *Chrysoperla carnea* (Steph.) in the laboratory test are summarized below.

Study group [application rate]	Parameter (endpoints)						
	Mortality				Reproduction		
Test item: CHR/H/AKO 600 SC							
[L/ha]	[no.]	[%]	Corr. [%] ^a	LR ₅₀	Mean number of eggs/ female/day [no.]	Mean hatching rate [%]	Reduction [%]
				[L/ha]			
Control (0.0)	5	16.7	-	> 3.0	19.3	88.8	-
0.75	5	16.7	0.0		16.7	89.7	(-1.1)*
1.5	7	23.3	8.0		15.3	73.8	16.9
3.0	7	23.3	8.0		18.2	87.0	2.0
NOER _{mortality}	≥ 3.0 [L/ha]						
Reference item: Dimethoate							
[g/ha]	[%]			Reproduction			
15.0	100.0			not assessed			

^a: Mortality corrected according to the Abbott formula [10]

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

Conclusions

The validity criterion concerning mortality was met, because mortality of the green lacewings, *Chrysoperla carnea* (Steph.) in the control group was 16.7%. The percentages of mortality of the green lacewings exposed to the test item at the rates of 0.75, 1.5 and 3.0 L/ha of the test item, after Abbott correction [10], were 0.0, 8.0 and 8.0% respectively.

There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.75, 1.5 and 3.0 L/ha in comparison to the control group (Chi2 2x2 Table Test with Bonferroni Correction, $p(z) > \text{Alpha} \cdot \text{sign}$, (Alpha=0.05)).

On the basis of the obtained results it can be concluded that the LR50 value is higher than 3.0 L/ha. The NOER_{mortality} value is higher than or equal to 3.0 L/ha. The percentage of mortality of *Chrysoperla carnea* (Steph.) exposed to dimethoate at rate of 15.0 g/ha was 100.0%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.

The mean number of eggs/female/day in the control group was equal to 19.3 (criterion: ≥ 15.0). The mean numbers of eggs/female/day in the groups treated with the test item at the rates of 0.75, 1.5 and 3.0 L/ha were equal to 16.7, 15.3 and 18.2, respectively. The mean hatching rate in the control group was 88.8% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of 0.75, 1.5 and 3.0 L/ha were 89.7, 73.8 and 87.0%, respectively. Fecundity reduction (Pr) in the group treated with

the test item at the rates 0.75, 1.5 and 3.0 L/ha were (-1.1), 16.9 and 2.0%, respectively. The negative value indicates that hatching rate in the group treated with the test item was higher than in the control group.

Based on the results it can be stated that CHR/H/AKO 600 SC at the rates of 0.75, 1.5 and 3.0 L/ha has no adverse effect on mortality of the tested organisms. The test item at the rates of 0.75, 1.5 and 3.0 L/ha has no adverse effect on the mean number of layed eggs by green lacewings and no adverse effect on mean hatching rate at all tested rates, i.e. 0.75, 1.5 and 3.0 L/ha.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- pre-imaginal mortality of the control group was 16.7% (criterion: a maximum of 20.0%),
- mortality of the reference item group was 100.0% (criterion: a minimum of 50%),
- the mean number of eggs per female per day in the control group (fecundity) was 19.3 (criterion: ≥ 15.0),
- the mean hatching rate in the control group (fertility) was 88.8 (criterion: $\geq 70\%$).

A 2.3 e Aged-residue extended laboratory test

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles , following guidelines from the IOBC, BART, EPPO Joint Initiative, and ESCORT 2. The study successfully met all of its mandatory validity criteria for all four laboratory bioassays (0, 14, 28, and 42 DAT) : • Mortality in the control group was between 3.0% and 4.0% (criterion: $<20\%$) . • Mortality in the reference item group was between 94.0% and 96.0%, confirming the sensitivity of the test population (criterion: $>50\%$) . • The cumulative mean number of eggs per female in the control group was between 5.2 and 5.3 (criterion: ≥ 4) . Deviation: • Minor temperature and humidity fluctuations were noted in the laboratory phases but did not compromise the validity criteria. The study concluded by demonstrating that the test item caused less than 50% mortality and less than 50% reduction in reproduction in the two consecutive bioassays at 28 and 42 DAT. Study is acceptable.
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Reference: 10.3

Report: Aged-residue extended laboratory test to determine the effects of the test item CHR/H/AKO 600 SC on the *Typhlodromus pyri* Scheuten according to the IOBC, BART and EPPO Joint Initiative, Blümel S. et al. (2000) and ESCORT 2, Candolfi M.P. et al. (2001)

Guideline(s): The study was conducted according to the IOBC, BART and EPPO Joint Initiative, Blümel S et al., (2000) and ESCORT 2, Candolfi M.P. et al. (2001) using corn leaves (*Zea mays* L.) as a test unit.

Deviations: 1. The environmental conditions during ageing period were obtained from nearest weather station Poznań – Marcellin, those measurements were performed outside the GLP system. 2. During the study initiated at 0 DAT, the temperature lowered to minimum 22.5°C and increased to maximum 27.5°C with average temperature 24.9°C and the humidity lowered to minimum 56.7% with average humidity 71.7% (requirements: temperature 25±2°C, humidity: 60-90% RH). During the study initiated at 14 DAT, the temperature lowered to minimum 22.6°C and increased to maximum 27.1°C with average temperature 24.6°C and the humidity lowered to minimum 53.0% with average humidity 75.7% (requirements: temperature 25±2°C, humidity: 60-90% RH). During the study initiated at 28 DAT, the temperature lowered to minimum 22.2°C with average temperature 24.8°C and the humidity lowered to minimum 57.0% with average humidity 69.9% (requirements: temperature 25±2°C, humidity: 60-90% RH). During the study initiated at 42 DAT, the temperature lowered to minimum 21.3°C with average temperature 24.1°C and the humidity lowered to minimum 41.7% and increased to maximum 99.9 with average humidity 74.3% (requirements: temperature 25±2°C, humidity: 60-90% RH). Deviations from the Study plan was found: 3. Experimental part of the study ended in September 2024 instead of planned October 2024. Above deviations did not affect the course of the study and the reliability of the study

GLP: Yes

Acceptability:

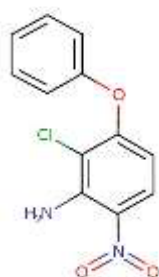
Duplication
(if vertebrate study) No

Materials and methods

Test item:

Name: CHR/H/AKO 600 SC
Type of packaging material: HDPE
Appearance: yellow suspension
Date of receipt: 10.05.2024
Batch No. CHR/02/2023
Name of the active substance: Aclonifen
IUPAC name of active substance: 2-chloro-6-nitro-3-phenoxyanilin
Content of active substance: 607.8 g/L
CAS of active substance: 74070-46-5
Molecular weight of active substance: 264.66 g/mol

Molecular structure of active substance:



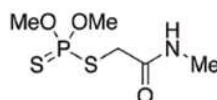
Molecular formula of active substance: $C_{12}H_9ClN_2O_3$
Production date: 23.03.2023

Water solubility, other solvents solubility
ability to form emulsion, solution, dispersion
forms suspension: forms suspension
The storage conditions temperature: 10-30°C
Reference number: LBS/26/24
Certificate of analysis: Appendix 1

Reference item:

Name: Dimethoate
Producer, batch no.: TRC Canada, 1-CGS-8-1
CAS: 60-51-5
Purity: 98%
IUPAC nomenclature: 2-Dimethoxy-phosphinothioylthio-N-methylacetamide
Molecular weight: 229.26 g/mol

Molecular structure:



Molecular formula: $C_5H_{12}NO_3PS_2$
Retest date: 26.09.2027
Certificate of analysis: Appendix 2

The course of the study:

The study was conducted on the basis of the IOBC, BART and EPPO Joint Initiative, Blümel S. et al. (2000) and ESCORT 2, Candolfi M.P. et al. (2001) and in accordance with the procedure SPB-E/69 using corn leaves (*Zea mays* L. cv. Vistula).

Solubility test was not performed. According to the information provided by the Sponsor the test item forms suspension (according to point 2.1).

Stability test was not performed. The test item solution was prepared shortly before application.

The test item was sprayed onto the plants, adaxial surface at BBCH 33 growth stage at 0 DAT (for reference item in all observed DAT). Leaves for the assessment of mortality and reproduction were sampled at 0, 14, 28, 42 DAT, until no negative impact (i.e. >50%) was observed in the two consecutive bioassays. The study started on each day after treatment was consist of two stages:

– Mortality assessment:

The test item and control were applied once (time of application corresponding to 0 DAT), and the reference item was applied before each bioassay. The freshly applied treatments were left to dry (max. 1.5 h after application) before excision of leaves and preparation of the units for introduction of protonymphs. The mortality of the organisms was assessed on the day 3 and day 7.

– Reproduction assessment:

The reproduction assessment was performed when mortality did not exceed 50%. The surviving mites were left for the next 7 days for egg-laying. After 10, 12, 14 days from the start of the test, the number of eggs per female in both the test item and control were checked

Preparation and application of control, test item and reference item:

The spray solutions were applied to the potted plants using a spray chamber T&B Masters S.C. The test item and reference item were diluted with deionized water prior to the spraying and the deionized water was used as the control. The application volume was 200 L of water/h. Spray chamber was calibrated on the day of application of test or reference item.

The test item was applied once in the study, at the date corresponding to the 0 DAT bioassay, the reference item and control were applied prior to each consecutive bioassay. The order of spray application at 0 DAT was as follows: control, 3 L of the test item/200 L of water/ha and the reference item and in between applications the nozzle was rinsed using deionized water.

Parameter of spraying:

- work pressure: 2.0 bar
- flat fan nozzles: Lechler, type 652.307.

Those parameters ensured a target volume of the applied solutions that correspond to the rate of test item, control and reference item/200L of water/ha (with an accuracy of $\pm 10\%$).

Details of the spray chamber calibration are presented as follows:

- 0 DAT for the test item, reference item and control in Table 25
- 14 DAT for the reference item in Table 26
- 28 DAT for the reference item in Table 27
- 42 DAT for the reference item in Table 28.

Preparation of the test item solution for the test is presented in Table 3.

Preparation of the reference item for the reference item treatments is presented in Table 4.

Results and discussions

Treatment	DAT	Mortality assessment		Reproduction assessment		
		Mortality [%]	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Reproduction reduction [%]	Significance*
Control	0	4.0		not tested		
	14	3.0		5.2	not applicable	not applicable
	28	3.0		5.3		
	42	4.0		5.3		
3 L of the test item/200 L of water/ha	0	54.0	52.1	not tested		
	14	37.0	35.1	2.5	52.2	+
	28	28.0	25.8	4.4	16.3	+
	42	23.0	19.8	4.7	12.4	+
Reference item (6 g of dimethoate/ 200 L of water/ha)	0	95.0	94.8	not tested		
	14	96.0	95.9			
	28	94.0	93.8			
	42	96.0	95.8			

* value calculated using ToxRat using test with significance level $p = 0.05$

In the bioassay initiated at 0 DAT, 4.0% mortality was observed in control. Mortality in rate 3 L of the test item/200 L of water/ha was 54.0%. Mortality in the reference item treatment was 95.0%. In the bioassay initiated at 14 DAT, 3.0% mortality was observed in control. Mortality in rate 3 L of the test item/200 L of water/ha was 37.0%. Mortality in the reference item treatment was 96.0%. In the bioassay initiated at 28 DAT, 3.0% mortality was observed in control. Mortality in rate 3 L of the test item/200 L of water/ha was 28.0%. Mortality in the reference item treatment was 94.0%. In the bioassay initiated at 42 DAT, 4.0% mortality was observed in control. Mortality in rate 3 L of the test item/200 L of water/ha was 23.0%. Mortality in the reference item treatment was 96.0%. The results of mortality are presented in Table 5 (0 DAT), Table 6 (14 DAT), Table 7 (28 DAT), Table 8 (42 DAT).

Reproduction assessment at 0 DAT was not performed due to mortality of >50% of the introduced organisms.

In the bioassay initiated at 14 DAT, the mean number of eggs and larvae per female was 5.2 in control compared with 2.5 in the rate 3 L of the test item/200 L of water/ha. The reproduction reduction was 52.2%.

In the bioassay initiated at 28 DAT, the mean number of eggs and larvae per female was 5.3 in control compared with 4.4 in the rate 3 L of the test item/200 L of water/ha. The reproduction reduction was 16.3%.

In the bioassay initiated at 42 DAT, the mean number of eggs and larvae per female was 5.3 in control compared with 4.7 in the rate 3 L of the test item/200 L of water/ha. The reproduction reduction was 12.4%.

Detailed results of the reproduction assessment are presented in the Table 12 (14 DAT), Table 13 (28 DAT), Table 14 (42 DAT).

Conclusion

The impact of the freshly-dried and field-aged residues of the test item on the *Typhlodromus pyri* was evaluated under laboratory test conditions in the following bioassays: 0 DAT, 14 DAT, 28 DAT and 42 DAT. Reproduction assessment on 0 DAT was not performed due to mortality $\geq 50\%$ of introduced organisms.

The test item caused both $<50\%$ mortality and $<50\%$ reduction in reproductive performance (relative to the control) in two subsequent bioassays at 28 DAT and 42 DAT.

The final results of the test are presented in Table 1.

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.1.1 *Apis mellifera* L.

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles and in accordance with OECD Guideline No. 213 and EU Method C.16. The report provides sufficient data to confirm that the study was conducted according to its stated methodology and that its results are valid. Validity criteria as stipulated by the OECD 213 guideline are met. A waiver for the chemical analysis of the test material's concentration, homogeneity, and stability was reported as a deviation from OECD GLP principles. The study director concluded this did not influence the study's course or results. LD₅₀/48h > 200.0 µg of test item/bee. LD₅₀/48h > 99.8 µg of the active ingredient acclonifen/bee.
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Reference: 10.3.1.1.1.1

Report CHR/H/AKO 600 SC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test; Study code: B-80-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)

Deviations: The test was performed according to the OECD Guideline for the Testing of Chemicals No. 213 (1998): 'Honeybees, acute oral toxicity test, the EU Method C.16 (2008): 'Honeybees, acute oral toxicity test, other references given in section 9, the SOP's listed in section 10 of the report and the Study Plan. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses will not be carried out. The waiver of these analyses constitutes a deviation

from the OECD Principles on Good Laboratory Practice Number 1

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item: CHR/H/AKO 600 SC content: 607.8 g/L of aclon-
ifen1 (CAS No. 74070-46-5)
batch no.: CHR/02/2023
density: 1.218 g/mL
production date: 23.03.2023
expiry date: not specified by Sponsor

Biological test system: the honeybee, *Apis mellifera* L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research
Network – Institute of Industrial Organic Chemis-
try, Branch Pszczyna [SOP/B/14]

Test design: the test item:
– exposure duration: 48 hours
– number of doses: 5 doses and a control
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate
the reference item:
– exposure duration: 24 hours
– number of doses: 3 doses
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate

Test item doses: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee
and a control (0.0 µg/bee)

Reference item doses: 0.1, 0.2 and 0.4 µg a.i./bee

Test conditions: – temperature: 24 – 26°C
– relative air humidity: 63 – 65%

Place: dark room

Statistical analysis: Probit analysis using linear max. likelihood regres-
sion

Endpoints: – honeybee mortality after 24 and 48 hours of the
exposure,
– the oral LD50/24 h of the reference item (dime-
thoate).

Results and discussions

Mortality of honeybees, *Apis mellifera*, exposed to CHR/H/AKO 600 SC was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0

µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of 50% sucrose solution, containing the test item at the doses mentioned above, using a micropipette. During the entire experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute oral toxicity test finished after the 48-hour observation.

The acute oral toxicity study of the test item, CHR/H/AKO 600 SC on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD ₅₀	
		Total		Test item [µg/bee]	Aclonifen [µg a.i./bee]
		[no.]	[%]		
0.0 (Control)	30	0	0.0	>200.0	> 99.8
12.5	30	0	0.0		
25.0	30	1	3.3		
50.0	30	0	0.0		
100.0	30	1	3.3		
200.0	30	5	16.7		

Conclusions

The median lethal doses LD₅₀/24 h and LD₅₀/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee, i.e. > 99.8 µg of aclonifen/bee.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- the LD₅₀/24 h of the reference item (dimethoate) was 0.119 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.1.2 *Bombus* spp.

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles and in accordance with OECD Guideline No. 247 (2017) . The report provides sufficient data to confirm that the study was conducted according to its stated methodology and that its results are valid. Validity criteria are met. A deviation from the guideline was noted where glass pipettes were used for dosing instead of plastic syringes. The report states this was done to allow for more precise administration and visual control of consumption and did not negatively
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	impact the study's quality or results . LD₅₀/48h > 200.0 µg of test item/bumblebee . LD₅₀/48h > 99.8 µg of the active ingredient aclonifen/bumblebee.
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Reference: 10.3.1.1.1.1

Report CHR/H/AKO 600 SC Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Study code: B-74-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline for Testing of Chemicals No. 247 (2017)

Deviations: The test was performed according to the OECD Guideline for the Testing of Chemicals No. 247 (2017): Bumblebee, Acute Oral Toxicity Test, 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 with consumption controlled by weight. In the experiment they was replaced by glass calibrated pipettes. The use of glass pipettes provides a more precise administration of the contaminated diet in a given volume and allows for visual real-time control of consumption during exposure. This deviations had no impact on the quality, integrity and final results of the study.

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item: CHR/H/AKO 600 SC content: 607.8 g/L of aclonifen (CAS No. 74070-46-5)
batch no.: CHR/02/2023
production date: 23.03.2023
expiry date: unspecified

Biological test system: species: bumblebee, *Bombus* spp.
source: Koppert Polska sp. z o.o. (a commercial supplier)
age: adult worker bumblebees

Experimental design: – a control (50% (w/v) aqueous sucrose solution)
number of replicates: 50;
number of insects: 1 insect/replicate;
– test item: number of doses: 1, number of replicates: 50;
number of insects: 1 insect/replicate;
– the reference item: number of doses: 1,
number of replicates: 30;
number of insects: 1 insect/replicate

Test item doses: 200.0 µg test item/bumblebee

Reference item doses:	4.0 µg/bumblebee
Exposure duration:	48 hours
Test conditions:	temperature: 24 – 26 (required: 25 ± 2°C) relative air humidity: 60 – 65% (required: 60 ± 20%)
Place	dark climate room
Statistical analysis:	statistical analysis was not needed due to the lack of mortality.
Endpoints:	– bumblebee mortality after 48 hours of exposure, – LD50 after 48 hours of exposure

Results and discussions

The study was conducted to determine the acute oral toxicity of CHR/H/AKO 600 SC to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 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 non-GLP preliminary range-finding test results. The bumblebees were exposed to the test item distributed in a 50% (w/v) 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, 24 and 48 hours after the test/ reference item administration. The acute oral toxicity test finished after the 48-hour observation.

The median lethal doses (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee i.e. > (99.8 µg acclonifen/bumblebee).

Dose		Number of tested bumblebees [no.]	Mortality after 48 h		LD ₅₀ /48 h	
Test item [µg/ bumblebee]	Ac lonifen [µg a.i. / bumblebee]		[no.]	[%]	Test item [µg/ bumblebee]	Ac lonifen [µg a.i. / bumblebee]
Control		50	0	0.0	> 200.0	> 99.8
200.0	99.8	50	0	0.0		
Reference item: dimethoate						
Dose [µg/ bumblebee]	4.0	30	29	96.7	–	

TEST VALIDITY CRITERIA

The following validity criteria were met:

- Mortality of the control group was 0.0% at the end of the test (criterion: $\leq 10\%$).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 96.7% (criterion: $\geq 50\%$).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.2.1 *Apis mellifera* L.

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles and in accordance with OECD Guideline 214 and EU Method C.17. The report provides sufficient data to confirm that the study was conducted according to its stated methodology and that its results are valid. Validity criteria as stipulated by the OECD 214 guideline are met. A deviation from the guideline was noted where mechanical immobilization was used instead of CO₂ anesthesia; this was pre-defined in the Study Plan and internal SOPs . A waiver for the chemical analysis of the test material was also reported as a deviation from OECD GLP principles. The study director concluded these did not affect the results . LD₅₀/48h > 200.0 µg of test item/bee LD₅₀/48h > 99.8 µg of the active ingredient acclonifen/bee
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Reference: 10.3.1.1.2

Report CHR/H/AKO 600 SC Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test; Study code: B-81-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)

Deviations: The study was performed according to the the OECD Guideline No. 214/EU Method C.17., references given in section 9, SOP's listed in section 10 of the report, and the study plan. 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. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses did not be carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1

GLP: Yes

Acceptability:

Duplication No

(if vertebrate study)

Materials and methods

Test item:	CHR/H/AKO 600 SC content: 607.8 g/L of aclon- ifen (CAS No. 74070-46-5) batch no.: CHR/02/2023 production date: 23.03.2023 expiry date: unspecified
Biological test system:	the honeybee, <i>Apis mellifera</i> L., strain: carnica – age: approximately 3 weeks – source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemis- try, Branch Pszczyna
Experimental design:	the test item: – exposure duration: 48 hours – number of doses: 5 doses with surfactant and two controls: water control and control with surfactant (0.1% Triton(R)X – 100) – number of replicates: 3 replicates – number of bees: 10 bees/replicate the reference item: – exposure duration: 24 hours – number of doses: 3 doses with surfactant – number of replicates: 3 replicates – number of bees: 10 bees/replicate
Test item doses:	12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and water control (0.0 µg/bee), 0.1% Triton control (0.0 µg/bee)
Reference item doses:	0.1, 0.2 and 0.4 µg a.i./bee
Test conditions:	– temperature: 24 – 26 °C – relative air humidity: 63 – 65%
Place	dark room
Statistical analysis:	Probit analysis using linear max. likelihood regres- sion
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, – the contact LD50/24 h of the reference item (di- methoate).

Results and discussions

Mortality of honeybees, *Apis mellifera*, exposed to CHR/H/AKO 600 SC was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used

to verify the sensitivity of the honeybees and the precision of the test procedure. After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

The acute contact toxicity study of the test item, CHR/H/AKO 600 SC on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD ₅₀	
		Total		Test item [µg/bee]	Aclonifen [µg a.i./ bee]
		[no.]	[%]		
0.0 (water control)	30	0	0.0		
0.0 (0.1% Triton control)	30	0	0.0		
12.5 + 1% Triton	30	0	0.0		
25.0 + 1% Triton	30	0	0.0	>200.0	>99.8
50.0 + 1% Triton	30	0	0.0		
100.0 + 1% Triton	30	1	3.3		
200.0 + 1% Triton	30	0	0.0		

Conclusions

The median lethal doses LD₅₀/24 h and LD₅₀/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee, i.e. > 99.8 µg of aclonifen/bee.

TEST VALIDITY CRITERIA

The following validity criteria were met:

- the mortality for the controls was 0.0% after 48 h (criterion: it must not exceed 10.0%),
- the LD₅₀/24 h of the reference item (dimethoate) was 0.115 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee)

A 2.3.1.1.2.2 *Bombus spp.*

Comments of zRMS:	The study was conducted in compliance with Good Laboratory Practice (GLP) principles and in accordance with OECD Guideline No. 246 (2017) . The report provides sufficient data to confirm that the study was conducted according to its stated methodology and that its results are valid. Validity criteria as stipulated by the OECD 246 guideline are met. Reported Deviations: A deviation from the guideline was noted where mechanical immobilization was used instead of CO₂ or cold anesthesia; this was predefined in the Study Plan and internal SOPs, and the Study Director concluded it did not influence the results .
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	LD ₅₀ /48h > 200.0 µg of test item/bumblebee . LD ₅₀ /48h > 99.8 µg of the active ingredient aclonifen/bee. Study accepted.
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Reference: 10.3.1.1.2

Report CHR/H/AKO 600 SC Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test; Study code: B-75-23 Study Director: A. Wojciech, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline for Testing of Chemicals No. 246 (2017)

Deviations: The test was performed according to the OECD Guideline for Testing of Chemicals No. 246 (2017): Bumblebee, Acute Contact Toxicity Test, 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. This deviation had no impact on the quality, integrity and final results of the study

GLP: Yes

Acceptability:

Duplication No
(if vertebrate study)

Materials and methods

Test item: CHR/H/AKO 600 SC content: 607.8 g/L of aclonifen (CAS No. 74070-46-5)
batch no.: CHR/02/2023
production date: 23.03.2023
expiry date: unspecified

Biological test system: species: bumblebee, *Bombus* spp.
source: Koppert Polska sp. z o.o. (a commercial supplier)
age: adult worker bumblebees

Experimental design: – control with surfactant (distilled water with 0.1% of Triton(R) X-100)
number of replicates: 50; number of insects: 1 insect/replicate;
– test item with surfactant 0.1% Triton(R) X-100
number of doses: 1,
number of replicates: 50;
number of insects: 1 insect/replicate;
– the reference item with surfactant 0.1% Triton(R)X-100:
number of doses: 1,
number of replicates: 30;
number of insects: 1 insect/replicate

Test item doses: 200.0 µg test item/bumblebee

Reference item doses:	10.0 µg/bumblebee
Test conditions:	temperature: 24 – 25°C relative air humidity: 60 – 63% place: a dark climate room
Statistical analysis:	Chi2-Contingency test
Endpoints:	– bumblebee mortality after 48 hours of exposure, – LD50 after 48 hours of exposure

Results and discussions

The study was conducted to determine the acute contact toxicity of CHR/H/AKO 600 SC to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 µg test item/bumblebee, plus the control with surfactant 0.1% Triton(R) X-100 and one dose of the reference item with surfactant 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 0.1% surfactant Triton(R) 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.

The median lethal doses (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee i.e. > 99.8 µg aclonifen/bumblebee.

Dose		Number of tested bumble-bees [no.]	Mortality after 48 h		LD ₅₀ /48 h	
Test item [µg/ bumble-bee]	Aclonifen [µg a.i. / bumblebee]		[no.]	[%]	Test item [µg/ bumble-bee]	Aclonifen [µg a.i. / bumblebee]
Control + 0.1% surfactant		50	0	0.0	> 200.0	> 99.8
200.0 + 0.1% surfactant	99.8	49	1	2.0		
Reference item: dimethoate + 0.1% surfactant						
Dose [µg/ bumblebee]	10.0	30	28	93.3	–	

TEST VALIDITY CRITERIA

The following validity criteria were met:

- Mortality of the control groups was 0.0% at the end of the test (criterion: $\leq 10\%$).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 93.3% (criterion: $\geq 50\%$)

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 *Apis mellifera* L. – chronic oral toxicity

Comments of zRMS:	The study was conducted in accordance with OECD 245. All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the control was 0 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %. Several minor deviations in temperature and humidity were noted but were assessed by the Study Director as not impacting the study's validity. It should be noted that the statistical software appendix incorrectly cites the validity criteria from a different guideline (OECD 213) and erroneously flags the study as "NOT VALID" on that basis. However, the main body of the report correctly identifies and meets the applicable OECD 245 criteria . LDD₅₀ (10-day lethal daily dose): $> 93.58 \mu\text{g}/\text{bee}/\text{day}$ NOEDD (No Observed Effect Daily Dose): $\geq 93.58 \mu\text{g}/\text{bee}/\text{day}$ Study is acceptable.
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Reference: 10.3.1.1.2

Report Honey bee, chronic oral toxicity test of test item CHR/H/AKO 600 SC according to OECD 245 Guideline, Study code: 0038/0164/E, Study director: Zaworska K. 2023, SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland

Guideline(s): according to OECD 245 Guideline,

Deviations:

1. During the stability test under the test conditions ($33 \pm 2^\circ\text{C}$), the temperature lowered to minimum 25.3°C and increased to maximum 43.1°C with average temperature 34.0°C and the humidity lowered to minimum 45.2 % with average humidity 62.3% (requirements: temperature $33 \pm 2^\circ\text{C}$; humidity 50-70%).
2. During the stability test under storage conditions ($6 \pm 2^\circ\text{C}$), the temperature increased to maximum 8.6°C with average temperature 6.5°C (requirements: $6 \pm 2^\circ\text{C}$).
3. During the acclimatization for the definitive test, the temperature lowered to minimum of 27.0°C and increased to maximum 43.1°C with average temperature of 36.8°C and the humidity lowered to minimum 45.2% with average humidity 50.6% (requirements: temperature $33 \pm 2^\circ\text{C}$; humidity 50-70%).
4. During the acclimatization for the range-finding test, the temperature lowered to minimum of 23.2°C and increased to maximum 37.3°C with average temperature of 34.1°C and the humidity lowered to minimum 47.1% with average humidity 53.5% (requirements: temperature $33 \pm 2^\circ\text{C}$; humidity 50-70%).
5. During the range-finding test, the temperature increased to maximum 35.8°C with average temperature 33.6°C (requirements: temperature

33±2°C).

GLP:

Yes

Acceptability:

Duplication
(if vertebrate study)

No

Materials and methods

Test item:

Name: CHR/H/AKO 600 SC

Type of packaging material: HDPE

Appearance: yellow suspension

Date of receipt: 03.08.2023

Batch No. CHR/02/2023

Name of active substance: Aclonifen

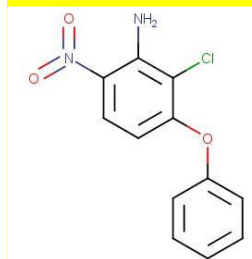
IUPAC name of active substance: 2-Chloro-6-nitro-3-phenoxyaniline

Content of active substance: 600 g/L

CAS of active substance: 74070-46-5

Molecular weight of active substance: 264.66 g/mol

Molecular structure of active substance:



Molecular formula of active substance:

C₁₂H₉ClN₂O₃

Production date: 23.03.2023

Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion: forms suspension

The storage conditions: temperature: 10-30°C

Reference number: LBS/65/23

Reference item:

Name: dimethoate

Batch No. 1-CGS-8-1, TRC Canada

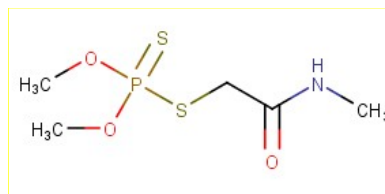
CAS: 60-51-5

Purity: 98%(w/w)

IUPAC nomenclature: 2-Dimethoxy-phosphinothiolythio-N-methylacetamide

Molecular formula: C₅H₁₂NO₃PS₂

Structural formula:



Molecular weight: 229.26 g/mol
Expiry date: 26.09.2027

Experimental design:

Stability test:

To determine the stability of the test item under storage conditions, i.e. temperature $\leq -18^{\circ}\text{C}$, darkness and temperature $6\pm 2^{\circ}\text{C}$, darkness and test conditions, i.e. temperature $33\pm 2^{\circ}\text{C}$, humidity 50-70%, darkness, test vessels containing the test item solution in the appropriate concentration and in parallel without the addition of test item (control) were stored under the above-mentioned conditions. Then, analytical determinations were made.

The following concentrations of the test item in a 50% (w/v) sucrose solution were used in the stability test: 2500 mg of the test item/kg of food and control (0 mg of the test item/kg of food). Test item concentration and control were prepared in one replicate for storage (temperature $\leq -18^{\circ}\text{C}$ and temperature $6\pm 2^{\circ}\text{C}$) and for study conditions. Every day syringes with solutions of the test item in study conditions were placed from the storage conditions and analyzed after 24 hours.

Definitive test:

The definitive test was performed for one concentration of test item: 2500 mg of the test item/kg of food and control (0 mg of test item/kg of food). Concentration and control were prepared in five replicates, 10 bees per replicate. The treatment with the reference item was performed for one concentration 0.75 mg of the reference item/kg of food. Concentration was prepared in three replicates, 10 bees per replicate.

Test conditions:

Stability test:

Parameters for study conditions ($33\pm 2^{\circ}\text{C}$, 50-70% relative humidity):

material subject to stability test: starting solution, after 24, 48, 72 hours of stability test in storage conditions; duration: 24 hours; test vessels: plastic containers of 100 mL volume; lighting: darkness; temperature: average 34.0°C (minimum 25.3°C ;

maximum 43.1°C); humidity: average 62.3% (minimum 45.2%; maximum 68.7%)¹.

Parameters for storage conditions in temperature 6±2°C:

test duration: 72 hours; test vessels: plastic containers of 100 mL volume; lighting: darkness; temperature: average 6.5°C (minimum 5.3°C; maximum 8.6°C).

Parameters for storage conditions in temperature ≤-18°C

test duration: 96 hours; test vessels: plastic containers of 100 mL volume; lighting: darkness; temperature: average -19.7°C (minimum -20.8°C; maximum -18.3°C).

Definitive test:

Acclimatization: time: 24 hours, average temperature 36.8°C (minimum 27.0°C; maximum 43.1°C); average humidity 50.6% (minimum 45.2%; maximum 64.2%)³; Exposure time: 10 days; Replicates per concentration: 5 - control and test item concentration, 3 - treatment with reference item; Number of honey bees per replicate: 10 individuals; Test vessels: well-ventilated, plastic cages, size 18 cm × 12 cm × 7.5 cm with food dispensers; Temperature: average 33.4°C (minimum 31.8°C; maximum 34.5°C); Humidity: average 67.0% (minimum 63.4%; maximum 69.3%); Lighting: darkness; Feeding: the food was fed with a feeder, e.g. using 2 mL plastic syringes (the tip of the syringe has been removed); feeding was carried out by changing the feeders every 24±2 hours.

Results and discussions

Stability test:

The stability of the test item under the conditions of the test was confirmed on the basis of the results of chemical analysis of the solutions at the beginning of the study and daily (for 96 hours) after 24 hours for tested concentration and control.

The stability of the test item in storage conditions (6±2°C) was confirmed on the basis of the results of chemical analysis of the solutions at the beginning of the test, after 24, 48 and 72 hours for tested concentration and control.

The stability of the test item under storage conditions (≤-18°C) was confirmed on the basis of the results of chemical analysis of the solutions at the beginning of the test, after 72 hours under the study conditions and after 96 hours for tested concentration and control.

The concentration of the test item did not differ by more than ±20% from the initial concentration. On the

basis of the results obtained in the study, the test item was found to be stable under the conditions of the test and under storage conditions.

Food with appropriate test item concentration or without (control) was prepared on day 0, 4 and 8 during the definitive test. Between this days the test item solutions were stored under $6\pm 2^{\circ}\text{C}$.

Definitive test:

During the test, no statistically significant honey bee mortality was observed for the tested concentration compared to the control.

Food consumption data was presented as:

- average food consumption per bee for each day (mg of the test item/bee) in terms of the number of alive honey bees before feeding
- overall average daily food consumption for each dose over the test period (mg of the test item/bee/day)
- overall average daily food consumption per replicate over the test period (mg of the test item/bee/day)
- average amount of test item consumed per bee per day (μg of the test item/bee/day)
- accumulated uptake of test item per bee over the test period (μg or ng of test item/bee).

TEST VALIDITY CRITERIA

The test met the validity criteria of the experiment according to OECD 245 guideline:

- bee mortality in control after 10 days was 0.0% (required: $\leq 15\%$)
- bee mortality in the reference test after 10 days was 100.0% (required: $\geq 50\%$)

Final results

The test item in the course of this test did not show any apitoxic effect on the mortality of honey bees after 10 days of the study.

Based on the analysis of the results, the LC10, LC20, LC50, LOEC and NOEC values were determined as ≥ 2500 mg of the test item/kg food, the LDD10, LDD20, LDD50, LOEDD and NOEDD values were determined as ≥ 93.58 μg of the test item/bee/day.

Bee mortality in the reference item treatment after 10 days was 100.0% (required: $\geq 50\%$).

The final results of the study are presented in Table 1.

Table 1. Final results of the study

Final results			
Parameter	Concentration [mg of the test item/kg of food]	Parameter	Dose [µg of the test item/bee/day]
LC ₁₀	>2500*	LDD ₁₀	>93.58*
LC ₂₀	>2500*	LDD ₂₀	>93.58*
LC ₅₀	>2500*	LDD ₅₀	>93.58*
NOEC	≥2500*	NOEDD	≥93.58*
LOEC	>2500*	LOEDD	>93.58*
Reference item treatment			
Concentration [mg of the reference item/kg of food]		Mortality [%]	
0.75		100.0	

n.d. impossible to determine for mathematical reasons
 LC₁₀ test item concentration causing mortality of 10% population
 LC₂₀ test item concentration causing mortality of 20% population
 LC₅₀ test item concentration causing mortality of 50% population
 NOEC the highest test item concentration not causing statistically significant differences in relations to the control
 LOEC the lowest test item concentration causing statistically significant differences in relations to the control
 LDD₁₀ test item dose causing mortality of 10% population
 LDD₂₀ test item dose causing mortality of 20% population
 LDD₅₀ test item dose causing mortality of 50% population
 NOEDD the highest test item dose not causing statistically significant differences in relations to the control
 LOEDD the lowest test item dose causing statistically significant differences in relations to the control
 * values determined based on the analysis of the results

A 2.3.1.2.2 *Apis mellifera* L.- Honey bee larval toxicity

Comments of zRMS:	The study was conducted in accordance with OECD 239. The validity criteria with regards to control larval mortality on D8, control adult emergence on D22 and toxicity of the reference item were met. Minor deviations in temperature and humidity were reported. However, the Study Director concluded these did not affect the study's integrity, a conclusion supported by the fact that all validity criteria were successfully met . Study is acceptable. EC₅₀ = 374.27 mg/kg of diet ED₅₀ = 57.59 µg/larva NOEC = 72.22 mg/kg of diet NOED = 11.11 µg/larva
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Reference: 10.3.1.1.2

Report

Honey bee larval toxicity test following repeated exposure of the test item CHR/H/AKO 600 SC according to OECD GD 239 ENV/JM/MONO(2016)34; Study code: 0038/0162/E; Woźniak A. 2024; SORBOLAB Research Laboratory LLC, Zaniemska Street 11, 61-029 Poznań, Poland

Guideline(s): according to OECD GD 239 ENV/JM/MONO(2016)34

Deviations: Deviations from the Study plan and OECD GD 239 ENV/JM/MONO(2016)34 guideline were found:
 1. During the definitive test the decrease in temperature during larval stage

(to 33.3°C) and pre-pupal stage (to 30.0°C) and increase in temperature during pupal/imago stage (to 36.8°C). Additionally increase in average humidity 88.0%RH and in maximum humidity to 97.8% was observed during pre-pupal stage and increase in average humidity 85.9% and minimum 81.8% and maximum 88.3% humidity was observed during pupal/imago stage.

Requirements: temperature: 34.5±0.5°C, humidity: larvae 95±5% RH, pre-pupae 80±5% RH, pupae/imago 50-80% RH.

3. During range-finding test decrease in temperature during larval stage (to 32.6°C), pre-pupal stage 33.7°C and in humidity during larval stage (to 85.6 % RH), pre-pupal stage (to 58.5%RH) and pupal/imago stage (to 35.6 % RH) and pupae/imago to average 82.6% with observed min. 80.1% and max. 83.6%.

Requirements: temperature: 34.5±0.5°C, humidity: larvae 95±5% RH, pre-pupae 80±5% RH, pupae/imago 50-80% RH.

Deviations from the Study plan were found:

2. During stability test the temperature increased to maximum 8.6°C. Requirements: 6.0±2.0°C.

GLP:

Yes

Acceptability:

Duplication

(if vertebrate study)

No

Materials and methods

Test item:

Name: CHR/H/AKO 600 SC

Type of packaging material: HDPE

Appearance: yellow suspension

Date of receipt: 03.08.2023

Batch No. CHR/02/2023

Name of active substance: Aclonifen

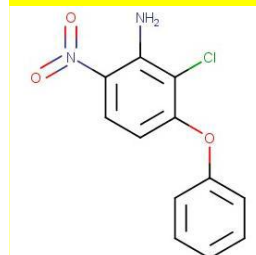
IUPAC name of active substance: 2-Chloro-6-nitro-3-phenoxyaniline

Content of active substance: 600 g/L

CAS of active substance: 74070-46-5

Molecular weight of active substance: 264.66 g/mol

Molecular structure of active substance:



Molecular formula of active substance:

C₁₂H₉ClN₂O₃

Production date: 23.03.2023

Water solubility, other solvents solubility, ability

to form emulsion, solution, dispersion: forms suspension

The storage conditions: temperature: 10-30°C

Reference number: LBS/65/23

Reference item:

Name: Dimethoate

Producent: TRC Canada

Batch No. 1-CGS-8-1

Purity: 98%

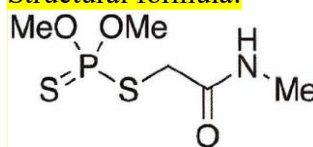
CAS: 60-51-5

IUPAC nomenclature: 2-Dimethoxyphosphinothioylthio-N-Methylacetamide

Molecular formula: $C_5H_{12}NO_3PS_2$

Molecular weight: 229.26 g/mol

Structural formula:



Expiry date: 26.09.2027

Storage conditions: temperature: 2-8°C

Process of the study:

The test was carried out based on the OECD GD 239 ENV/JM/MONO(2016)34 Guideline and in accordance with the SPB-E/53 procedure.

On day 1, the frames with freshly brooded larvae were transferred from the hive to laboratory in the temperature optimal for larvae (definitive test and reference item treatment 27.2°C). Frames were placed under the inactive laminar-flow hood heated to 31.2°C during definitive test and reference item treatment. For the study, larvae, which has not yet formed C-shape or the ones laying on the top of royal jelly were chosen. A. To ensure the minimum number of larvae (36 larvae, i.e. 12 larvae from 3 colonies) needed to commence exposure to the test item or reference item on day 3, larvae were collected in excess.

The larvae were carefully placed in the same position at the bottom of queen-cell cup filled with diet A placed in breeding plate's well (Wuxi NEST Biotechnology Co., Ltd.). Plates were placed in desiccators (Laboplay, series E13PP), in which, during 1-8 days, humidity was maintained on level of 95±5% using saturated solution of K₂SO₄ placed in the dish at the bottom of desiccator.

On day 8, larvae were transferred to the fresh plates with pieces of absorption paper placed at the bottom of each well. Plates with larvae were placed in the desiccator, in which humidity was maintained at the level of 80±5% with saturated

solution of NaCl placed at the bottom of desiccator. Desiccators were placed in test room.

On day 15, plated were transferred to plastic cages with *ad libitum* access to 50% (w/v) sucrose solution and pine pollen. Cages were placed in test room of humidity 50-80% maintained with humidifier

Experimental design:

Stability test:

The stability of the test item under storage conditions was determined (temperature $6\pm 2^{\circ}\text{C}$, darkness). Due to the highly complex composition of the diet for the larvae (royal jelly), the stability of the test item in the diet was not tested under the experimental conditions.

During the stability test, following concentrations of test item were used: 0.05 g of test item/L of deionized water corresponding to a concentration of 0.65 mg of test item/kg of diet, 50.00 g of test item/L of deionized water corresponding to a concentration of 650.00 mg of test item/kg of diet, and control. All test item concentrations and control groups were prepared in one replicate.

Definitive test:

In definitive test, test item was administrated in larvae diet in proper concentration during 4-days exposition. During the test, mortality observations and behavioral changes were performed.

The definitive test was performed using six concentrations of the test item and control.

- control (0.00 mg of test item/kg of diet), corresponding to 0.00 μg of test item/larva (0.00 g of test item/L of deionized water)
- 8.02 mg of test item/kg of diet, corresponding to 1.23 μg of test item/larva (0.62 g of test item/L of deionized water)
- 24.07 mg of test item/kg of diet, corresponding to 3.70 μg of test item/larva (1.85 g of test item/L of deionized water)
- 72.22 mg of test item/kg of diet, corresponding to 11.11 μg of test item/larva (5.56 g of test item/L of deionized water)
- 216.67 mg of test item/kg of diet, corresponding to 33.33 μg of test item/larva (16.67 g of test item/L of deionized water)

- 650.00 mg of test item/kg of diet, corresponding to 100.00 µg of test item/larva (50.00 g of test item/L of deionized water).

Test item during the range-finding test affected more significantly on the larvae mortality, on this basis, dimethoate was used as the reference item during the reference item treatment.

One concentration of reference item was used:

- 48 mg of reference item/kg of diet, corresponding to 7.39 µg/larva (0.528 g of reference item/L of deionized water).

Each concentration and control consisted of 36 larvae i.e. 12 larvae from 3 different colonies (breedings) located on the same plate.

Test conditions:

Stability test:

Test duration: 72 h.

- Test vessels: volumetric flasks of 100 mL volume.

- Lighting: darkness.

- Temperature: average temperature 6.5°C (min. 5.3°C, max 8.6°C)

Definitive test:

The definitive test and reference item treatment was conducted in the following conditions:

- Time of exposure: 4 days.

- Number of bee larvae in one plate: 36.

- Test vessels: up to day 15 – 48-well culture plates with queen cells, placed in a desiccator and incubator; after the 15th day – transparent plastic boxes, placed in the test room.

- Temperature and humidity¹:

- for larval stage (day 1-8):

average temperature 34.2°C (min. 33.3°C, max. 34.4°C)

average relative humidity 99.9% (min. 98.6%, max. 99.9%)

- for pre-pupal stage (day 8-15):

average temperature 34.2°C (min. 30.0°C, max. 34.4°C)

average relative humidity 88.0% (min. 83.1%, max. 97.8%)

- for pupal/imago stage (day 15-22):

average temperature 34.4°C (min. 31.4°C, max. 36.8°C)
average relative humidity 85.9% (min. 81.8%, max. 88.3%).
-Lightning: darkness (excluding observations).
-Feeding: diet according to Table 2.

Results and discussions

Stability test:

Test item stability in storage conditions was confirmed based on results solutions chemical analysis at the test beginning and after 24, 48 and 72 hours for all tested concentrations and control.
Test item concentrations differed by no more than $\pm 20\%$ from initial concentrations for 72 hours.
Based on the results, test item was found to be stable for 72 h in storage conditions.
Test item concentration determination results during stability test are shown in.

Definitive test:

The mortality data of the following development stages of the honey bee was calculated using the ToxRat Professional (version 3.3.0) statistical analysis software and presented as NOEC values for:

- larval mortality from day 4 to day 8
- pupal mortality as pre-pupal mortality from day 8 to day 15 and the number of non-emerged pupae on day 22
- emergence rate on day 22.

The NOEC and NOED values and ECx (in mg of test item or μg of test item/kg of diet) and EDx (in μg of the test item/larva) values were calculated for the emergence rate on day 22.

The statistical calculations were made in accordance with OECD 54.

TEST VALIDITY CRITERIA

The study met the validity criteria (acc. to OECD GD 239 ENV/JM/MONO(2016)34):

- larval mortality in control on days 3-8 was 8.33% in all replicates, (required: $\leq 15\%$),
- the adults emergence rate in control on day 22 was 91.67% in replicate 1 and 75.00% in replicate 2 and 83.33% in replicate 3 (required: $\geq 70\%$),
- for dimethoate as reference item, the larval mortality on day 8 was 100.00% (required: $\geq 50\%$),

Final results

During definitive test, statistically significant effect on larval mortality was observed on day 8 at a concentration 650.00 mg test item/kg of diet.

The test item showed statistically significant effect on the mortality of pupae on day 22 at a concentration 216.67 mg test item/kg of diet and 650.00 mg test item/kg of diet.

For emerged adults, a statistically significant effect was observed at the 216.67 mg test item/kg of diet and 650.00 mg test item/kg of diet.

The final results of the study are presented in Table 1.

Table 1. Final results of the study

Larval mortality results				
Concentration [mg of test item/kg of diet]	Time [day]			
	8			
	Mortality [%]	Statistical significance ^{*)}	LOEC	NOEC
			[mg of test item/kg of diet]	
Control	8.33	-	650.00	216.67
8.02	8.33	-		
24.07	13.89	-		
72.22	13.89	-		
216.67	27.78	-		
650.00	41.67	+		
Concentration [mg of reference item/kg of diet]	Time [day]			
	8			
	Mortality [%]	Statistical significance ^{*)}	LOEC	NOEC
			[mg of reference item/kg of diet]	
48.00	100.00	+	not applicable	

- statistically insignificant

+ statistically significant

NOEC the highest test item concentration not causing statistically significant differences in relations to the control

LOEC the lowest test item concentration causing statistically significant differences in relations to the control

^{*)} values calculated using ToxRat Professional using Fisher's Test after Bonferroni-Holm correction with significance level $p=0.05$

Pupal mortality results					
Concentration [mg of test item/kg of diet]	Time [day]				
	15		22		
	Mortality [%]	Statistical significance ^{*)}	Mortality [%]	Statistical significance ^{**)}	LOEC [mg of test item/kg of diet]
Control	6.06	not applicable	9.09	not applicable	216.67
8.02	6.06	-	18.18	-	
24.07	9.68	-	12.90	-	
72.22	9.68	-	22.58	-	
216.67	15.38	-	30.77	+	
650.00	19.05	-	47.62	+	

- statistically insignificant

NOEC the highest test item concentration not causing statistically significant differences in relations to the control

LOEC the lowest test item concentration causing statistically significant differences in relations to the control

^{*)} values calculated using ToxRat Professional using Chi2 2x2 Table with Bonferroni correction test after Bonferroni-Holm correction with significance level $p=0.05$

^{**) values calculated using ToxRat Professional using step-down Cochran-Armitage test after correction with significance level $p=0.05$}

Emergence results						
Concentration [mg of test item/kg of diet]	Dose [µg of test item/larva]	Time [day]				
		22				
		Mortality [%]	Statistical significance ^{*)}	LOED [µg of test item/larva]	NOED [µg of test item/larva]	LOEC [mg of test item/kg of diet]
Control	Control	16.67	not applicable	33.33 ^{**)}	11.11	216.67 ^{**)}
8.02	1.23	25.00	-			
24.07	3.70	25.00	-			
72.22	11.11	33.33	-			
216.67	33.33	50.00	+			
650.00	100.00	69.44	+			

- statistically insignificant

+ statistically significant

^{*)} values calculated using ToxRat Professional using Fisher test after Bonferroni-Holm correction with significance level $p=0.05$

^{**) values determined based on the analysis of the results}

NOEC the highest test item concentration not causing statistically significant differences in relations to the control

LOEC the lowest test item concentration causing statistically significant differences in relations to the control

NOED the highest test item dose not causing statistically significant differences in relations to the control

LOED the lowest test item dose causing statistically significant differences in relations to the control

Emergence results			
Parameter	Concentration [mg of test item/kg of diet]	Parameter	Dose [µg of test item/larva]
EC ₁₀	16.39	ED ₁₀	2.52
EC ₂₀	47.96	ED ₂₀	7.37
EC ₅₀	374.27	ED ₅₀	57.59

EC₁₀ test item concentration causing effect on 10% population
EC₂₀ test item concentration causing effect on 20% population
EC₅₀ test item concentration causing effect on 50% population
ED₁₀ test item dose causing effect on 10% population
ED₂₀ test item dose causing effect on 20% population
ED₅₀ test item dose causing effect on 50% population

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

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 *Eisenia andrei*

Comments of zRMS:	The study was performed according to OECD TG 222 and principles of GLP. The validity criteria are met: For the control group: - Adult mortality: ≤ 10 % (being 2.5 %) - Number of juveniles per replicate: ≥ 30 (being 70 to 94) - Coefficient of variation of reproduction: ≤ 30 % (being 8.8 %). No deviation to the study plan was recorded. The study is considered acceptable and suitable for the risk assessment.
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Reference: 10.4.1.1

Report CHR/H/AKO 600 SC Earthworm reproduction test (*Eisenia andrei*); Study code: G-35-23 Study Director: A. Gierbuszewska, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline for Testing of Chemicals No. ~~246 (2017)~~ 222 (2016)

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

Materials and methods

Test item: CHR/H/AKO 600 SC
batch no.: CHR/02/2023

Active substance: aclonifen – 607.8 g/L (49.9% w/w)

Artificial soil: 10% sphagnum peat, 20% kaolin clay, 69.775% air-dried quartz sand, 0.225% calcium carbonate;

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

Test design: test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate

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

Test conditions: temperature: 19.0 – 22.0°C;
pH at the beginning of the experiment: 5.77 – 5.87;
pH at the end of the experiment: 5.51 – 5.58;
soil moisture content at the beginning of the experiment: 24.8 – 26.0% (50.1 – 52.5% of the maximum water holding capacity); soil moisture content at the end of the experiment: 22.5 – 24.0% (45.4 – 48.5% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 733.3 – 780.1 lux
light intensity at the end of the experiment: 729.4 – 738.1 lux

Statistical analysis: EC10, EC20, EC50 – nonlinear regression using the 4-parameter logistic
LC10, LC20, LC50 – probit analysis using linear max. likelihood regression
NOEC (reproduction): - Shapiro-Wilk's Test on Normal Distribution, - Bartlett's Test Procedure on Variance Homogeneity, - Williams Multiple Se-

quential t-test Procedure,
NOEC (survival): - Fisher's Exact Binomial Test
with Bonferroni Correction
LOEC: a values suggested by the ToxRat Profes-
sional 2.10 statistical computer software

Endpoints:

EC10, EC20, EC50, NOEC, LOEC (reproduction)
LC50, NOEC, LOEC (survival)

Results and discussions

The aims of the study were to assess the impact of CHR/H/AKO 600 SC on reproduction of the earthworm, *Eisenia andrei* and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. TeN concentrations of the test item were: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

At concentrations ranging from 5.6 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 15.0%. In the control group mortality of the adult earthworms was equal to 2.5%. The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is higher than 1000 mg/kg dry weight of the artificial soil (i.e. 499.01 mg of aclonifen/kg dry weight of the artificial soil). No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed. After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between -13.2 and 8.9%. As for the control group, the body weight decrease was equal to 1.2%. After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 24.5 and 81.8 per replicate. The mean number of juveniles in the control group was equal to 80.3 per replicate. After 8 weeks of the experiment, it was concluded that CHR/H/AKO 600 SC had a statistically significant impact on reproduction of the earthworms at the concentration equal to 1000.0 mg/kg dry weight of the artificial soil.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of aclonifen/kg dry weight of artificial soil]
EC₁₀	889.96	444.10
EC₂₀	921.05	459.62
EC₅₀	974.62	486.35
NOEC (reproduction)	560.0	279.45
LOEC (reproduction)	1000.0	499.01
LC₁₀	> 1000.0	> 499.01
LC₂₀	> 1000.0	> 499.01
LC₅₀	> 1000.0	> 499.01
NOEC (survival)	≥ 1000.0	≥ 499.01
LOEC (survival)	> 1000.0	> 499.01

TEST VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced from 70 to 94 juveniles (80.3 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 8.8% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 *Folsomia candida*

Comments of zRMS:	The study was performed according to OECD TG 232 and principles of GLP. The validity criteria are met: For the control group: Mean adult mortality: ≤ 20 % (observed: 5%) Mean number of juveniles per test vessel: ≥ 100 (observed:352.1) Coefficient of variation for the mean number of juveniles: < 30 % (observed: 18.4%). The following deviations from the study plan were noted:
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	According to the OECD Guideline No. 232 (2016) in order to calculate EC_x and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations was used. At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) These deviations did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.
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Reference: 10.4.2

Report CHR/H/AKO 600 SC Collembolan (*Folsomia candida*) Reproduction Test; Study code: G-36-23 Study Director: M. Górka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 232 (2016)

Deviations: Deviation from the OECD Guideline No. 232 (2016) and SOP/G/87: According to the OECD Guideline No. 232 (2016) in order to calculate EC_x and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations will be used

Deviation from the OECD Guideline No. 232 (2016)

At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016)

These deviations did not affect the results of the study

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item: CHR/H/AKO 600 SC
batch no.: CHR/02/2023

Active substance: aclonifen – 49.9% (w/w) 607.8 g/L

Artificial soil: 5% sphagnum peat, 20% kaolin clay, 74.88% air-dried industrial sand and 0.12% calcium carbonate,

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

Test design: test duration: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control;
number of collembolans: 10 / replicate

Test item doses:	a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil
Test conditions:	temperature: 19.0 – 21.3°C; pH at the beginning of the test: 5.56 – 5.89; pH at the end of the test: 5.51 – 5.58; soil moisture content at the beginning of the test: 16.3 – 16.8% (45.3 – 46.7 % of the maximum water holding capacity); soil moisture content at the end of the test: 15.4 – 16.3% (43.4 – 45.3% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 729.8 – 736.6 lux; light intensity at the end of the experiment: 729.9 – 736.6 lux;
Statistical analysis:	EC10, EC20, EC50 – Probit analysis using linear max. likelihood regression LC10, LC20, LC50 – Probit analysis using linear max. likelihood regression NOEC (number of juveniles): - Shapiro-Wilk's Test on Normal Distribution, - Levene's Test on Variance Homogeneity (with Residuals), - Trend analysis by Contrasts (Monotonicity of Concentration/Response), - Williams Multiple Sequential t-test Procedure, NOEC (survival): - Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response) - Tarone's Test Procedure - Step-down Cochran-Armitage Test Procedure
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC

Results and discussions

The aims of the study were to assess the impact of CHR/H/AKO 600 SC on reproduction of the collembolans, *Folsomia candida* and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These were 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous suspension was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 2.5 to 67.5%. As for the control group, it was equal to 5.0%. The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of aclonifen/kg dry weight of the artificial soil]
LC₁₀	144.08 (106.43 – 195.06)	71.90 (53.11 – 97.34)
LC₂₀	235.07 (185.94 – 297.19)	117.30 (92.79 – 148.30)
LC₅₀	599.66 (466.19 – 771.33)	299.24 (232.64 – 384.91)
NOEC	100.00	49.90

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 14.8 – 384.8 per replicate. As for the control group, the number of juveniles was equal 352.1 per replicate. The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of aclonifen/kg dry weight of the artificial soil]
EC₁₀	103.42 (69.04 – 154.92)	51.61 (34.45 – 77.31)
EC₂₀	150.85 (111.57 – 203.95)	75.28 (55.68 – 101.77)
EC₅₀	310.57 (256.46 – 376.10)	154.98 (127.98 – 187.68)
NOEC	100.00	49.90

TEST VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- mean adult mortality: 5.0% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 352.1 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 18.4% (criterion: $\leq 30\%$).

A 2.4.2.2 *Hypoaspis (Geolaelaps) aculeifer*

Comments of zRMS:	The study was performed according to OECD TG 226 and principles of GLP. The validity criteria are met: For the control group: - Mean mortality of adult females: $\leq 20\%$ (observed: 10%) - Mean number of juveniles per replicate: ≥ 50 (observed: 83.4) - Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (ob-
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	served: 11.3%). Following deviations to the study plan were recorded: 1. According to the OECD Guideline No. 226 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations will be used (Chapter 3.5.2). 2. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test. 3. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution. 4. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. These deviations did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.
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Reference: 10.4.2

Report CHR/H/AKO 600 SC Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil; Study code: G-37-23 Study Director: M. Górską, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 226 (2016)

Deviations: There are four deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: According to the OECD Guideline No. 226 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations will be used. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites.

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item: CHR/H/AKO 600 SC
batch no.: CHR/02/2023

Active substance: aclonifen – 49.9% (w/w) 607.8 g/L

Artificial soil:	5% sphagnum peat, 20% kaolin clay, and 74.88% air-dried industrial sand, 0.12% calcium carbonate
Test system:	the predatory mites, <i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i> (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.
Test design:	exposure time: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Concentrations of the test item:	a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg test item/kg dry weight of the artificial soil
Test conditions:	temperature: 19.0 – 21.4°C pH at the beginning of the test: 5.52 – 5.89 pH at the end of the test: 5.51 – 5.85 soil moisture content at the beginning of the test: 15.6 – 17.2% (43.4 – 47.8% of the maximum water holding capacity) soil moisture content in the middle of the test: 15.3 – 16.8% (42.5 – 46.7% of the maximum water holding capacity) soil moisture content at the end of the test: 14.8 – 16.5% (41.2 – 45.9% of the maximum water holding capacity) light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 718.4 – 727.1 lux light intensity at end of the test: 724.3 – 733.1 lux
Statistical analysis:	EC10, EC20, EC50 – probit analysis using linear max. likelihood regression. LC10, LC20, LC50 – logit analysis using linear max. likelihood regression. NOEC: - offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Non-parametric Trend analysis by Contrasts (Monotonicity of Concentration/Response), Step-down Jonckheere-Terpstra Test Procedure. - survival – Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response), Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure.
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC

Results and discussions

The aims of the study were to assess the impact of CHR/H/AKO 600 SC on reproduction of the predatory mite, *Hypoaspis* (*Geolaelaps*) *aculeifer* and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These included: 5.6, 10.0 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of aqueous suspension was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil was between 7.5% and 45.0%. Mortality of the control group was equal to 10.0%. After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 38.8 and 84.5 per replicate. The mean number of juveniles in the control group was equal to 83.4 per replicate

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days of exposure		
		No.	%	
control	80	8	10.0	83.4
5.6	40	3	7.5	83.3
10.0	40	4	10.0	84.5
18.0	40	3	7.5	84.3
32.0	40	6	15.0	80.5
56.0	40	5	12.5	80.0
100.0	40	6	15.0	79.3
180.0	40	7	17.5	79.5
320.0	40	9	22.5	64.8
560.0	40	11	27.5	47.0
1000.0	40	18	45.0	38.8

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

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of aclonifen/kg dry weight of the artificial soil]
EC₁₀	172.46 (125.34 - 237.31)	86.06 (62.55 - 118.42)
EC₂₀	293.36 (237.42 - 362.48)	146.39 (118.48 - 180.88)
EC₅₀	810.51 (678.15 - 968.70)	404.46 (338.41 - 483.40)
NOEC (reproduction)	180.00	89.82
LC₁₀	201.62 (132.28 - 307.31)	100.61 (66.01 - 153.35)
LC₂₀	457.62 (314.86 - 665.09)	228.36 (157.12 - 331.89)
LC₅₀	> 1000.00	> 499.01
NOEC (survival)	180.00	89.82

TEST VALIDITY CRITERIA

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

- mean adult mortality: 10.0% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 83.4 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 11.3% (criterion: $\leq 30\%$).

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1

Comments of zRMS:	The study was conducted according to OECD guideline 216 and principles of GLP. The validity criterion is met: the variation between the replicate control samples did not exceed the validity criterion of 15% throughout the study. There following deviation to the study plan was recorded: According the Guide-line, 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 ex-traction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. This deviation did not affect the results of the study. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	10.5
Report	CHR/H/AKO 600 SC Soil Microorganisms: Nitrogen Transformation Test; Study code: G-38-23 Study Director: P. Pieczka, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland
Guideline(s):	according to the OECD Guideline No. 216 (2000)/EU Method C.21
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 (point 3.4.4.4.). These deviation did not affect the results of the study
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	No

Materials and methods

Test item:	CHR/H/AKO 600 SC batch no.: CHR/02/2023
Active substance:	aclonifen – 49.9% (w/w) 607.8 g/L
Soil:	Agricultural soil collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.
Test design:	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. After adding the deionized water, every portion was divided into three replicates (3 x 545 g). Exposure period: 28 days.
Concentrations of the test item:	control; - PEC: 4.87 mg test item/kg dry weight of soil, - 5 x PEC: 24.36 mg test item/kg dry weight of soil.
Test conditions:	temperature: 21.1 – 22.0°C, soil moisture: 44.7 – 49.3% of the maximum water holding capacity, incubation in darkness
Statistical analysis:	- Shapiro-Wilk's test on Normal Distribution - Levene's Test on Variance Homogeneity (with Residuals) -tTrend analysis by Contrasts (Monotonicity of ttRate/Response) - Williams Multiple Sequential t-test Procedure
Endpoints:	The concentration of nitrate [mg/kg dry soil] after

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

Results and discussions

The aim of the study was to detect long-term adverse effects of CHR/H/AKO 600 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: 4.87 mg test item/kg dry weight of soil, - 5 x PEC: 24.36 mg test item/kg dry weight of soil. The treated and the control soils were divided into three replicates. On days 0, 7, 14 and 28 of incubation, soil samples were collected to determine the quantities of nitrate. The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using de-ionised water. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used. The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

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: 4.87 mg test item/kg dry weight of soil and 5 x PEC: 24.36 mg test item/kg dry weight of soil did not exceed 25% on 28 day of analysis.

The nitrate ions concentrations on 0, 7, 14 and 28 day of incubation are shown in Tables 5 – 8. Nitrate formation rates [mg nitrate/kg dry weight soil/day] for selected time intervals, i.e. 0 – 7, 0 – 14, 0 – 28 days are given in Table 9. Deviations from the control based on nitrogen ions formation rates are shown in Table 10.

Table 5. Concentration of the nitrate ions on day 0 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	95.291	98.541	96.261	97.491	87.981	98.891	100.291	94.161	100.891
Nitrate ion concentration [mg/kg of dry soil]	476.46	492.71	481.31	487.46	439.91	494.46	501.46	470.81	504.46
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	483.49 ± 8.34			473.94 ± 29.68			492.24 ± 18.62		
CV	1.7			6.3			3.8		

* - values adjusted for the value of the blank sample

Table 6. Concentration of the nitrate ions on day 7 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	239.594	256.994	265.394	250.794	254.394	255.994	250.594	267.494	258.694
Nitrate ion concentration [mg/kg of dry soil]	1197.97	1284.97	1326.97	1253.97	1271.97	1279.97	1252.97	1337.47	1293.47
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	1269.97 ± 65.80			1268.64 ± 13.32			1294.64 ± 42.26		
CV	5.2			1.0			3.3		

* - values adjusted for the value of the blank sample

Table 7. Concentration of the nitrate ions on day 14 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	257.775	273.575	270.675	273.175	263.175	273.675	279.375	279.975	265.875
Nitrate ion concentration [mg/kg of dry soil]	1288.88	1367.88	1353.38	1365.88	1315.88	1368.38	1396.88	1399.88	1329.38
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	1336.71 ± 42.05			1350.04 ± 29.62			1375.38 ± 39.87		
CV	3.1			2.2			2.9		

* - values adjusted for the value of the blank sample

Table 8. Concentration of the nitrate ions on day 28 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	349.375	380.675	366.875	375.175	360.075	358.675	371.175	377.975	375.375
Nitrate ion concentration [mg/kg of dry soil]	1746.88	1903.38	1834.38	1875.88	1800.38	1793.38	1855.88	1889.88	1876.88
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	1828.21 ± 78.43			1823.21 ± 45.74			1874.21 ± 17.16		
CV	4.3			2.5			0.9		

* - values adjusted for the value of the blank sample

Table 9. Nitrate formation rate* [mg nitrate/kg dry weight of soil/day] for selected time intervals.

Time interval [d]	Control				PEC				5xPEC			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 – 7	102.069	114.497	120.497	112.355 ± 9.40	111.433	114.005	115.147	113.528 ± 1.90	108.676	120.747	114.462	114.628 ± 6.04
0 – 14	57.528	63.170	62.135	60.944 ± 3.00	63.710	60.138	63.888	62.579 ± 2.12	64.617	64.831	59.795	63.081 ± 2.85
0 – 28	45.121	50.710	48.246	48.026 ± 2.80	50.069	47.373	47.123	48.188 ± 1.63	48.701	49.916	49.451	49.356 ± 0.61

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' days; 'a' = 7, 14 and 28 day

Table 10. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	-1.04	-2.02
0 – 14	-2.68	-3.51
0 – 28	-0.34	-2.77

*- obtained values are higher than the ones for the control group

Conclusions

On the basis of the results, it was concluded that CHR/H/AKO 600 SC at the concentrations corresponding to the PEC: 4.87 mg test item/kg dry weight of soil and 5 x PEC: 24.36 mg test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

TEST VALIDITY CRITERIA

The coefficients of variation (CV) in the control group were 1.7, 5.2, 3.1 and 4.3%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Seedling Emergence and Seedling Growth – Part 1/2

Comments of zRMS:	On request of zRMS the Applicant submitted the Amendment No. 1 to the final report (March 25, 2025), the aim of which was to correct the names of the plant species that appear in many sections of the report. The original document mistakenly contained the name corn (<i>Zea mays</i>) instead of oats (<i>Avena sativa</i>). This correction did not affect the results and conclusions presented in the report. The study was conducted according to OECD guideline 208 and principles of GLP. All the validity criterion are met: - Seedling emergence in the control: ≥ 70 % (actual 90.5% - 100%), - mean survival of emerged control seedlings: ≥ 90 % (actual 100 %), - the control seedlings did not exhibit any visible phytotoxic effects,
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	environmental conditions for all plants of the same species were identical. The following deviation to the study plan was recorded: According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 201.0 and 270.0 $\mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The study is considered to be reliable and suitable for the risk assessment.
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Reference: 10.6.2

Report CHR/H/AKO 600 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Study code: G-40-23 Study Director: P. Pieczka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 208 (2006)

Deviations: According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 201.0 and 270.0 $\mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item

CHR/H/AKO 600 SC
batch number: CHR/02/2023
active substance: aclonifen – 607.8 g/L (49.9 % w/w)

Test species

sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea* var. *capitata*), pea (*Pisum sativum*), carrot (*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*)

Soil

sandy loam.

Study design:

Plant species	Number of replicates per rate	Number of seeds per replicate	Total number of seeds per rate
Sunflower	7	3	21
Cabbage	7	3	21
Pea	7	3	21
Carrot	4	5	20
Onion	4	5	20
Oats	4	5	20

Application rates:

Application rates [mL/ha]
0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3, 1000.0, 3000.0

Exposure termination:

14 days after the emergence of 50% of the control seedlings;

Test conditions

temperature: 21.7 – 25.1°C, humidity: 46.7 – 70.5%, lighting: 16 h light : 8 h dark; light intensity: 201.0 – 270.0 $\mu\text{E}/\text{m}^2/\text{s}$; carbon dioxide concentration: 338 – 370 ppm;

Statistical analysis:

The ER10, ER25 and ER50 values for the emergence of plants (on the basis of maximum emergence of plants) were determined with: probit analysis using linear max. likelihood regression.

The ER10, ER25 and ER50 values for the survival of cabbage, oats plants were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression. Because plant mortality was not observed in cultivation of onion, carrot and pea, no computations were performed.

The ER10, ER25 and ER50 values for shoot length were determined with probit analysis using linear max. likelihood regression or 3-param. normal CDF or 4-param. normal CDF.

The ER10, ER25 and ER50 values for shoot dry weight were determined with probit analysis using linear max. likelihood regression or 3-param. normal CDF or 4-param. normal CDF.

The ER50 for visual phytotoxicity effects (on the basis of the results obtained at the end exposure period) were determined with: probit analysis using linear max. likelihood regression or 3-param. normal CDF.

NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on seedling emergence and seedling growth.

In order to determine the NOER values, the following tests were used: The emergence of plants (on the basis of maximum emergence of plants): Tarone's Test Procedure, Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Chi2 2x2 Table Test with Bonferroni Correction, Fisher's Exact Binomial Test with Bonferroni Correction.

The survival of plants:

Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Fisher's Exact Binomial Test with Bonferroni Correction, Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure Because no plants mortality was observed in cultivation of carrot, onion and pea, no computations were performed.

The shoot length:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Dunnett's Multiple t-test Procedure, Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm.

The plant dry weight:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Dunnett's Multiple t-test Procedure.

Results and discussions

The study, aimed at evaluating the effect of CHR/H/AKO 600 SC on seedling emergence and seedling growth of 6 terrestrial plants. The study was conducted on 4 dicotyledonous and 2 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 3 (sunflower, pea, cabbage), 5 (carrot, onion, oats) 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 60°C, and weighed. The results concerning the emergence, survival, the shoot length, and the dry weight were statistically analyzed in order to determine the ER10, ER25, ER50, and NOER. Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period.

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

In the table below, instead of corn should be oats according to Amendment No. 1 to the final report (March 25, 2025).

	Sunflower <i>Helianthus annuus</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Pea <i>Pisum sativum</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Corn <i>Zea mays</i>
Emergence of plants						
ER₅₀	> 3000.00	> 3000.00	> 3000.00	> 3000.00	> 3000.00	> 3000.00
NOER	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00
Survival of plants						
ER₅₀	> 3000.00	> 3000.00	> 3000.00	> 3000.00	> 3000.00	2358.90 (1418.80 – > 3000.00)
NOER	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	333.30
Shoot length						
ER₅₀	> 3000.00	1010.14 (699.63 – 1586.95)	> 3000.00	> 3000.00	> 3000.00	484.52 (261.34 – 869.71)
NOER	≥ 3000.00	37.00	≥ 3000.00	≥ 3000.00	333.30	333.30
Shoot dry weight						
ER₅₀	> 3000.00	94.98 (69.21 – 131.15)	> 3000.00	> 3000.00	> 3000.00	569.21 (430.38 – 745.51)
NOER	≥ 3000.00	1.40	≥ 3000.00	333.30	333.30	111.10
Plant damages						
ER₅₀	> 3000.00	116.15 (91.02 – 148.21)	> 3000.00	> 3000.00	> 3000.00	850.97 (725.22 – 909.63)

Conclusions

On the basis of the obtained results it was proved that the test item i.e. CHR/H/AKO 600 SC had varied impact on the process of growth of selected plant species. Mortality of plants was observed in cultivation of sunflower, cabbage, carrot, onion. Delayed emergence of plants in comparison to the control group was noticed in cultivation of carrot. On the basis of NOER, ER10, ER25 and ER50 values determined from the plant emergence during exposure period it was proved that the test item had no impact on seedling emergence of all tested species. On the basis of NOER, ER10, ER25 and ER50 values determined from the survival of plants it was proved that the test item had an impact on survival of cabbage and oats. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of growth of cabbage, carrot, onion and oats. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot dry weight it was proved that the test item inhibited the process of growth of cabbage, carrot, onion and oats. During the exposure period, the phytotoxic symptoms of the test item were observed in cultivation of cabbage, sunflower, carrot, onion and oats.

VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CHR/H/AKO 600 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:
90.5% – sunflower, 95.2% – cabbage, 100.0% – pea, 100.0% – carrot, 100.0% – onion, 95.0% – corn,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

A 2.6.2.2 Seedling Emergence and Seedling Growth – Part 2/2

Comments of zRMS:	The study was conducted according to OECD guideline 208 and principles of GLP. All the validity criterion are met: - Seedling emergence in the control: ≥ 70 % (actual 90% - 100%), - mean survival of emerged control seedlings: ≥90 % (actual 100 %), - the control seedlings did not exhibit any visible phytotoxic effects, - environmental conditions for all plants of the same species were identical.
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	The following deviation to the study plan was recorded: According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 200.6 and 263.0 $\mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The study is considered to be reliable and suitable for the risk assessment.
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Reference: 10.6.2

Report CHR/H/AKO 600 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Study code: G-09-24 Study Director: P. Pieczka, MSc; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 208 (2006)

Deviations: According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 200.6 and 263.0 $\mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials and methods

Test item

CHR/H/AKO 600 SC

batch number: CHR/02/2023

active substance: aclonifen – 607.8 g/L (49.9 % w/w)

Test species

sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), corn (*Zea mays*)

Soil

sandy loam.

Study design:

Plant species	Number of replicates per rate	Number of seeds per replicate	Total number of seeds per rate
sugar beet	10	2	20
oilseed rape	7	3	21
corn	10	2	20

Application rates:

Application rates [mL/ha]
0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3, 1000.0, 3000.0

Exposure termination:

14 days after the emergence of 50% of the control seedlings;

Test conditions

temperature: 21.7 – 25.1°C, humidity: 46.7 – 70.5%, lighting: 16 h light : 8 h dark; light intensity: 200.6 – 263.0 $\mu\text{E}/\text{m}^2/\text{s}$; carbon dioxide concentration: 338 – 370 ppm;

Statistical analysis:

The ER10, ER25 and ER50 values for the emergence of plants (on the basis of maximum emergence of plants) were determined with: probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.

The ER10, ER25 and ER50 values for the survival of oilseed rape and sugar beet plants were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression. Because plant mortality was not observed in cultivation of corn, no computations were performed.

The ER10, ER25 and ER50 values for shoot length were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.

The ER10, ER25 and ER50 values for shoot dry weight were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.

The ER50 for visual phytotoxicity effects (on the basis of the results obtained at the end exposure period) were determined with: probit analysis using linear max. likelihood regression.

NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on seedling emergence and seedling growth.

In order to determine the NOER values, the following tests were used: The emergence of plants (on the basis of maximum emergence of plants): Tarone's Test Procedure, Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Fisher's Exact Binomial Test with Bonferroni Correction.

The survival of plants:

Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Fisher's Exact Binomial Test with Bonferroni Correction, Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure Because no plants mortality was observed in cultivation of corn, no computations were performed.

The shoot length:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure

The plant dry weight:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Dunnett's Multiple t-test Procedure.

Results and discussions

The study, aimed at evaluating the effect of CHR/H/AKO 600 SC on seedling emergence and seedling growth of 3 terrestrial plants. The study was conducted on 2 dicotyledonous and 1 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 2 (sugar beet and corn) or 3 (oilseed rape) 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 60°C, and weighed. The results concerning the emergence, survival, the shoot length, and the dry weight were statistically analyzed in order to determine the ER10, ER25, ER50, and NOER. Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period.

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

	Sugar beet <i>Beta vulgaris</i>	Oilseed rape <i>Brassica napus</i>	Corn <i>Zea mays</i>
Emergence of plants			
ER₅₀	> 3000.00	> 3000.00	> 3000.00
NOER	≥ 3000.00	≥ 3000.00	≥ 3000.00
Survival of plants			
ER₅₀	187.88 (152.51 – 232.34)	> 3000.00	> 3000.00
NOER	111.1	333.30	≥ 3000.00
Shoot length			
ER₅₀	327.87 (152.95 – 702.86)	623.15 (484.65 – 801.23)	> 3000.00
NOER	37.00	37.00	≥ 3000.00
Shoot dry weight			
ER₅₀	114.10 (82.94 – 157.53)	51.63 (39.69 – 67.17)	> 3000.00
NOER	37.0	1.40	≥ 3000.00
Plant damages			
ER₅₀	158.73 (153.25 – 165.84)	110.82 (99.60 – 123.31)	> 3000.00

Conclusions

On the basis of the obtained results it was proved that the test item i.e. CHR/H/AKO 600 SC had varied impact on the process of growth of selected plant species. Mortality of plants was observed in cultivation of sugar beet and oilseed rape. Delayed emergence of plants in comparison to the control group was not observed in cultivation of selected plant species. On the basis of NOER, ER10, ER25 and ER50 values determined from the plant emergence during exposure period it was proved that the test item had no impact on seedling emergence of sugar beet and oilseed rape. Slight effect was noticed in cultivation of corn. On the basis of NOER, ER10, ER25 and ER50 values determined from the survival of plants it was proved that the test item had an impact on survival of sugar beet and oilseed rape. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of growth of sugar beet and oilseed rape. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot dry weight it was proved that the test item inhibited the process of growth of sugar beet and oilseed rape. During the exposure period, the phytotoxic symptoms of the test item were observed in cultivation of all selected plant species.

VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CHR/H/AKO 600 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:
90.0% – sugar beet, 100.0% – oilseed rape, 95.0% – corn,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

A 2.6.2.3 Vegetative Vigour Test – Part 1/2

Comments of zRMS:	The study was conducted according to OECD guideline 227 and principles of GLP. All the validity criterion are met: - seedling emergence: $\geq 70\%$ (actual 92.9 - 100 %). - mean plant survival for the duration of the study: $\geq 90\%$ (actual 100 %), - the control plants did not exhibit any visible phytotoxic symptoms - environmental conditions for all plants belonging to the same species were identical The following deviation from the study plan was noted: According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 $221 - 290 \mu\text{E}/\text{m}^2/\text{s}$. 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.. The study is considered to be reliable and suitable for the risk assessment.
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Reference: 10.6.2

Report CHR/H/AKO 600 SC Terrestrial Plant Test: Vegetative Vigour Test; Study code: G-39-23 Study Director: A. Gierbuszewska, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 227 (2006)

Deviations: According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 $221 - 290 \mu\text{E}/\text{m}^2/\text{s}$. 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:

Duplication (if vertebrate study) No

Materials and methods

Test item

CHR/H/AKO 600 SC
batch number: CHR/02/2023
active substance: aclonifen – 607.8 g/L (49.9% w/w)

Test species

pea (*Pisum sativum*), sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea* var. *capitata*), carrot

(*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*)

Soil

Sandy loam

Study design

Plant species	Number of replicates per rate	Number of plants per replicate	Total number of plants per rate
pea	7	3	21
sunflower	7	3	21
cabbage	7	3	21
carrot	4	5	20
onion	4	5	20
oats	4	5	20

Exposure termination

21 days after spraying

Application rates

0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3, 1000.0, 3000.0 mL/ha In case of each species, there was one untreated control group. volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha

Test conditions

temperature: 17.1 – 23.3°C, humidity: 48.7 – 79.7%, lighting: 16 h light : 8 h dark; light intensity: 221 – 290 $\mu\text{E}/\text{m}^2/\text{s}$; carbon dioxide concentration: 338 – 365 ppm

Statistical analysis

Due to the lack of mortality of plants during the exposure period, no computations have been performed for the plant number at the end of the experiment for pea, sunflower, carrot, onion and oats.

The ER10, ER25 and ER50 values were determined by the following tests:

- the plant number: probit analysis using linear max. likelihood regression
- the shoot length: 3-param. normal CDF, logit analysis using linear max. likelihood regression
- the dry weight: probit analysis using linear max. likelihood regression, 3-param. normal CDF
- plant damages: probit analysis using linear max. likelihood regression, logit analysis using linear max. likelihood regression.

NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on vegetative vigour

In order to determine the NOER values, the following tests were used:

- for the plant number: Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Chi2 2x2 Table Test with Bonferroni Correction.
- for the shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homoge-

neity (with Residuals), Multiple Sequentially-rejective t-test After Bonferroni-Holm.

- for the plant shoot 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.

Results and discussions

The study, aimed at evaluating the effect of CHR/H/AKO 600 SC on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea, sunflower and cabbage; 10 seeds/pot for carrot, onion and oats). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. The pot is defined as a replicate. The test item was sprayed onto the plants. 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 60°C to a constant weight and weighed. The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER10, ER25, ER50 and NOER. Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results after 21 days of the experiment.

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

	Pea <i>Pisum sativum</i>	Sunflower <i>Helianthus annuus</i>	Cabbage <i>Brassica oleracea</i> var. <i>capitata</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment						
ER₅₀	> 3000.00	> 3000.00	> 3000.00	> 3000.00	> 3000.00	> 3000.00
NOER	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00	≥ 3000.00
Shoot length						
ER₅₀	> 3000.00	> 3000.00	794.31 (481.49 – 1310.36)	> 3000.00	> 3000.00	1595.78 (1150.87 – 2212.68)
NOER	≥ 3000.00	≥ 3000.00	37.00	1000.00	333.30	333.30
Shoot dry weight						
ER₅₀	> 3000.00	> 3000.00	100.92 (40.66 – 247.02)	> 3000.00	1820.74 (548.45 – > 3000.00)	403.61 (291.80 – 558.25)
NOER	≥ 3000.00	≥ 3000.00	0.50	1000.00	37.00	37.00
Plant damages						
ER₅₀	> 3000.00	> 3000.00	130.15 (112.69 – 150.31)	> 3000.00	2347.81 (2287.15 – 2410.08)	1084.76 (1009.01 – 1166.19)

Conclusions

The test item, i.e. CHR/H/AKO 600 SC, had a varied impact on vegetative vigour of all tested plant species. On the basis of NOER, ER10, ER25 and ER50 values determined from the plant number at the end of the experiment it was proved that the test item inhibited the process of growth of cabbage. No influence was noticed in cultivation of pea, sunflower, carrot, onion and oats. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of

growth of cabbage, onion and oats. It slightly inhibited the process of growth of pea and carrot. The test item did not inhibit the process of growth of sunflower. On the basis of NOER, ER10, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of pea, cabbage, carrot, onion and oats. The test item did not inhibit the process of growth of sunflower. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of all tested plant species

VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CHR/H/AKO 600 SC, on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
 90.5 – 95.2% – pea, 83.3 – 92.9% – sunflower, 85.7 – 92.9% – cabbage, 90.0 – 97.5% – carrot, 87.5 – 100.0% – onion, 90.0 – 97.5% – oats
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical

A 2.6.2.4 Vegetative Vigour Test – Part 2/2

Comments of zRMS:	The study was conducted according to OECD guideline 227 and principles of GLP. All the validity criterion are met: - seedling emergence: $\geq 70\%$ (actual 83.3 – 92.9 %). - mean plant survival for the duration of the study: $\geq 90\%$ (actual 100 %), - the control plants did not exhibit any visible phytotoxic symptoms - environmental conditions for all plants belonging to the same species were identical The following deviation from the study plan was noted: According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 $207.4 - 253.2 \mu\text{E}/\text{m}^2/\text{s}$. 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. The study is considered to be reliable and suitable for the risk assessment.
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Reference: 10.6.2

Report CHR/H/AKO 600 SC Terrestrial Plant Test: Vegetative Vigour Test; Study code: G-10-24 Study Director: A. Gierbuszewska, MSc Eng; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland

Guideline(s): according to the OECD Guideline No. 227 (2006)

Deviations: According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. 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 $207.4 - 253.2 \mu\text{E}/\text{m}^2/\text{s}$. 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.

tion did not affect the results of the experiment.

GLP: Yes

Acceptability:

Duplication No
(if vertebrate study)

Materials and methods

Test item

CHR/H/AKO 600 SC
batch number: CHR/02/2023
active substance: aclonifen – 607.8 g/L (49.9% w/w)

Test species

oilseed rape (Brassica napus), sugar beet (Beta vulgaris), corn (Zea mays)

Soil

Sandy loam

Study design

Plant species	Number of replicates per rate	Number of plants per replicate	Total number of plants per rate
oilseed rape	7	3	21
sugar beet	10	2	20
corn	10	2	20

Exposure termination

21 days after spraying

Application rates

0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3, 1000.0, 3000.0 mL/ha In case of each species, there was one untreated control group. volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha

Test conditions

temperature: 17.1 – 23.3°C, humidity: 48.7 – 79.7%, lighting: 16 h light : 8 h dark; light intensity: 207.4 – 253.2 µE/m² /s; carbon dioxide concentration: 329 – 365 ppm

Statistical analysis

Due to the lack of mortality of plants during the exposure period, no computations have been performed for the plant number at the end of the experiment for oilseed rape and corn.

The ER10, ER25 and ER50 values were determined by the following tests

- the plant survival: probit analysis using linear max. likelihood regression
- the shoot length: Weibull analysis using linear max. likelihood regression
- the dry weight: logit analysis using linear max. likelihood regression, 4-param. normal CDF.
- plant damages: probit analysis using linear max. likelihood regression

NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on vegetative vigour:

In order to determine the NOER values, the following tests were used

- for the plant survival: Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Rao-Scott-Cochran-Armitage Test Procedure
- for the shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Dunnett's Multiple t-test Procedure
- for the plant shoot dry weight: Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure

Results and discussions

The study, aimed at evaluating the effect of CHR/H/AKO 600 SC on vegetative vigour of 3 terrestrial plants, was conducted on 2 dicotyledonous and 1 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (4 seeds/pot for sugar beet and corn; 6 seeds/pot for oilseed rape). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. The pot is defined as a replicate. The test item was sprayed onto the plants. 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 60°C to a constant weight and weighed. The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER10, ER25, ER50 and NOER. Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results after 21 days of the experiment.

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

	Oilseed rape <i>Brassica napus</i>	Sugar beet <i>Beta vulgaris</i>	Corn <i>Zea mays</i>
Plant number at the end of the experiment			
ER₅₀	> 3000.00	192.43 (147.47 – 251.08)	> 3000.00
NOER	≥ 3000.00	37.00	≥ 3000.00
Shoot length			
ER₅₀	> 3000.00	194.19 (151.52 – 248.88)	> 3000.00
NOER	4.10	4.10	≥ 3000.00
Shoot dry weight			
ER₅₀	27.67 (15.32 – 48.97)	14.39 (9.47 – 21.55)	2957.90 (1913.29 – > 3000.00)
NOER	4.10	4.10	4.10
Plant damages			
ER₅₀	106.88 (95.19 – 120.01)	39.43 (36.04 – 43.14)	> 3000.00

Conclusions

The test item, i.e. CHR/H/AKO 600 SC, had a varied impact on vegetative vigour of all tested plant species. On the basis of NOER, ER10, ER25 and ER50 values determined from the plant number at the end of the experiment it was proved that the test item inhibited the process of growth of sugar beet. No influence was noticed in cultivation of oilseed rape and corn. On the basis of NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of growth of oilseed rape and sugar beet. No influence was noticed in cultivation of corn. On the basis of NOER, ER10, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of all tested plant species. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of oilseed rape and sugar beet.

VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CHR/H/AKO 600 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% – oilseed rape, 85.0 – 92.5% – sugar beet, 85.0 – 87.5% – corn;
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%)
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

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

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

A 2.8 KCP 10.8 Monitoring data