FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 123000 A

Product name: AZA

Chemical active substance:

Azadirachtin, 10 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: October 2020

MS Finalisation date: February 2022, July 2022

Version history

When	What
December 2021	Applicant update
February 2021	Finalisation of the assessment by zRMS
July 2022	Final version of evaluation prepared by zRMS.

Table of Contents

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-pods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	**Tomato**	F	*Aleuroids, Thrips, Aphids*	Foliar Spray	Apply at pest presence BBCH 12-85	a) 2 b) 2	7-10	a) 3.0 b) 6.0	a) 0.03 b) 0.06	750-1000	3	DE: Plant height until 50 cm 2 l/ha in 600 l/ha, from 50 to 125 cm 2.5 l/ha in 800 l/ha, over 125 cm 3 l/ha in 1000 l/ha							
2	CEU	**Potato**	F	Collorado beetle *(Lep-tinotarsa decemlineata)*	Foliar Spray	Apply at pest presence BBCH 12-91	a) 2 b) 2	7-10	a) 2.5 b) 5.0	a) 0.025 b) 0.05	500-1000	3								
3	CEU	**Ornamentals**	F	*Aleuroids, Thrips, Aphids*	Foliar Spray	Apply at pest presence BBCH 12-89	a) 2 b) 2	7-10	a) 3.0 b) 6.0	a) 0.03 b) 0.06	750-1000	3								

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional

and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

9.1.1 Overall conclusions

zRMS comment:

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

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

Birds:
According to the screening tier assessments, a low acute and chronic risk are expected for birds following the intended uses of AZA for all the intended uses assessed in the framework of this application. No risk for birds was identified via drinking water exposure and secondary poisoning via the food chain can be excluded, following the intended uses of AZA, due to the low log Pow values below 3 of the two major fractions Azadirachtin A and B. Moreover, no risk of biomagnification is terrestrial food chain was identified.

Mammals:
According to the screening and first-tier assessments, a low acute and chronic risk are expected for mammals following the intended uses of AZA for all the intended uses assessed in the framework of this application, except for the uses on Ornamentals and fruiting vegetables (Tomato) for which a chronic risk was identified regarding the small herbivorous mammal "vole". However, according to the higher-tier assessment, a low chronic risk is expected for mammals for the intended uses on ornamentals and fruiting vegetables. No risk for mammals was identified via drinking water exposure and secondary poisoning via the food chain can be excluded following the intended uses of AZA, due to the low log Pow values below 3 of the two major fractions Azadirachtin A and B. Moreover, no risk of biomagnification is terrestrial food chain was identified.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

For all the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for sediment dwelling organisms as characterised by a NOEC for *Chironomus riparius* of 1.6 µg/L in connection with an assessment factor of 10) in several or all FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

After the FOCUS step 4 calculations, risk unacceptable is still detected in R3 and R4 stream scenarios in potatoes. Therefore, a further refinement is needed. A refinement based on a geomean value of **0.29 µg a.s./L** was used. This value was obtained from five chronic studies on *Chironomus* with different representative formulations and with technical from Monograph. After the FOCUS step 4 calculations and refinement based on geomean value for *Chironomus*, the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch, R1 stream and R2 stream; 5 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip

- R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip

Potato

- D6 ditch; 5 m no-spray buffer.
- R1 stream and R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D6 ditch and R2 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- D4 stream, R1 stream and R4 stream: 5 m no-spray buffer.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip with 50% of nozzles reduction or 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch and R2 stream: 5 m no-spray buffer.
- D6 ditch: risk unacceptable. However, this scenario is not relevant under CEU conditions.
- R1 stream and R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

The results obtained for each intended use with the relevant mitigation measures are proposed below:

- **Fruiting Vegetables (Tomato):** 20 m no-spray buffer and 20 m vegetative filter strip
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 20m to surface water bodies.*

- **Potato:** 10 m no-spray buffer and 10 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 10m to surface water bodies*

- **Ornamentals >50cm:** 5 m no-spray buffer and 5 m vegetative filter strip with 50% of nozzles reduction or 10 m no-spray buffer and 10 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies with 50% of nozzles reduction*
  Or
  Spe3 *- To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 10m to surface water bodies.*

- **Ornamentals <50cm:** 20 m no-spray buffer and 20 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 20m to surface water bodies.*

After the FOCUS step 4 calculations and RAC of 0.16 microgram/L the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch; 5 m no-spray buffer.
- R1 stream: 20 m no-spray buffer and 20 m vegetative filter strip
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip
- **R3 stream and R4 stream: risk unacceptable.** Therefore, a further refinement is needed.

Potato

- D6 ditch; 5 m no-spray buffer and 5 m vegetative filter strip.
- R1 stream: 15 m no-spray buffer and 15 m vegetative filter strip.
- R3 stream: 20 m no-spray buffer and 20 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D4 stream, D6 ditch, R1 stream and R4 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- R2 stream: 5 m no-spray buffer with 75% of nozzles reduction or 10 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch; 5 m no-spray buffer.
- D6 ditch, R1 stream and R4 stream: risk unacceptable. Therefore, a further refinement is needed.
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip.
- R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip

The final risk mitigation measures should be decided at MSs level.

9.1.1.3 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to the product AZA is expected according to the proposed intended uses. According to EU Reg. 284 /2009, the chronic toxicity test for adult bees, the chronic test for larvae should be provided for authorization of plant protection product. However, the final decision of the date of submission these studies by the Applicant should be considered at MSs level.

9.1.1.4 First-tier assessments indicate that no unacceptable risk for bees exposed to the product AZA is expected according to the proposed intended uses.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

No in-field and off-field risk to non-target arthropods is expected after the application of AZA according to the proposed GAP.

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

The TER_{lt} value for AZA formulation is higher than the Annex VI trigger value of 5, indicating a low long-term risk to earthworms. The TER calculated is far above the trigger and, therefore, the Applicant considers that an acceptable risk to *Folsomia candida* and *Hypoaspis aculeifer* can be concluded on the basis that low risks to other soil macro-organisms was concluded.

Risk assessments conducted with relevant PEC_{soil} for the active substance Azadirachtin and AZA formulation indicated a low risk to soil microorganisms.

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

~~According to the preliminary screening data using a limit test at a rate of 30.9 g a.s./ha, no phytotoxicity was observed following the use of Azadirachtin. Since the application rates of the intended uses are lower than those used in the preliminary screening data, it can be considered that Azadirachtin poses a low risk to non-target plants according to the intended uses.~~

~~In addition, no unacceptable off-field risk on non-target plants was obtained with AZA formulation~~.

The TER_{LT} values form seedling and vegetative vigour test for non - target plants were achieved the trigger value of 5 indicating acceptable risk. No mitigation measures are required to non-crop area.

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

No inhibition of respiration of activated sewage sludge of >10 % was observed up to the highest tested concentration of 1000 mg Neem Azal (34% Azadirachtin A)/L. NOEC is therefore >1000 mg/L for Azadirachtin. It is not expected that Azadirachtin reaches biological sewage treatment plants at higher concentrations. Therefore, the risk to biological methods of sewage treatment is expected to be low from the intended uses.

9.1.2 Grouping of intended uses for risk assessment

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

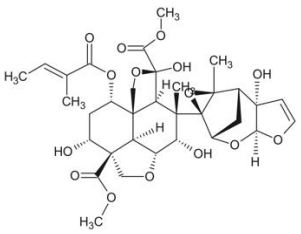
Table 9.1-2: Critical use pattern of AZA grouped according to application rate, number of application and drift value

Grouping according to application rate			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
All crops	Fruiting vegetables (including Tomato, Strawberry and Melon uses), Citrus and Potato	Same application rate (3 L prod./ha), same number of applications (2)	Highest application rate for assessment of drinking water for birds and mammals, for assessment of bees.
Fruitting vegetables	Tomato	Maximum application rate of 2 x 3.0 L f.p./ha (equivalent to 2 x 30 g a.s./ha)	Focal species for birds and mammals. Aquatic organisms. Dirft rate (7.23%) for off-field non-target arthropods and non-target plants
Potato	Potato	Maximum application rate of 2 x 2.5 L f.p./ha (equivalent to 2 x 25 g a.s./ha)	Focal species for birds and mammals. Aquatic organisms. Dirft rate (2.38%) for off-field non-target arthropods and non-target plants
Ornamentals >50cm	Ornamentals	Maximum application rate of 2 x 3.0 L f.p./ha (equivalent to 2 x 30 g a.s./ha)	Focal species for birds and mammals. Aquatic organisms. Dirft rate (7.23%) for off-field non-target arthropods and non-target plants
Ornamentals <50cm	Ornamentals	Maximum application rate of 2 x 3.0 L f.p./ha (equivalent to 2 x 30 g a.s./ha)	Focal species for birds and mammals. Aquatic organisms. Dirft rate (2.38%) for off-field non-target arthropods and non-target plants

9.1.3 Consideration of metabolites

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

Table 9.1-3 Metabolites of Azadirachtin

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Azadirachtin H		678.9	Soil: 63% Water/Sediment: No data available	No assessment performed. No endpoints are available.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of AZA were not evaluated as part of the EU assessment of Azadirachtin.

However, the provision of further data on the AZA is not considered essential, because active substance data on toxicity to birds can be used and additional formulation data are not considered essential.

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

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	a.s. NeemAzal technical (Trifolio M-GmbH)	Oral 1 d Acute	LD_{50} >1000 mg azadirachtin A./kg bw LD_{50} >4000 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Colinus virginianus	a.s. NPI-720 (Mitsui)	Oral 1 d Acute	LD_{50} >225 mg azadirachtin A./kg bw LD_{50} >2250 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Colinus virginianus	a.s. Azadirachtin technical (Sipcam)	Oral 1 d Acute	**LD_{50} >320 mg azadirachtin A./kg bw** LD_{50} >2000 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Colinus virginianus	a.s. NeemAzal technical (Trifolio M-GmbH)	Dietary 5 d Short-term	LC_{50} >269.5 mg azadirachtin A./kg bw LC_{50} >1078 mg extract/kg bw LC_{50} >1300 mg azadirachtin A./kg feed LC_{50} >5200 mg extract/kg feed	EFSA Journal 2018;16(4):5234
Colinus virginianus	a.s. NPI-720 (Mitsui)	Dietary 5 d Short-term	LC_{50} >139.9 mg azadirachtin A./kg bw LC_{50} >1398.8 mg extract/kg bw LC_{50} >562 mg azadirachtin A./kg feed LC_{50} >5620 mg extract/kg feed	EFSA Journal 2018;16(4):5234
Colinus virginianus	a.s. Azadirachtin technical (Sipcam)	Dietary 22 weeks	**NOEC =8.4 mg azadirachtin A./kg bw** NOEC =71.2 mg extract/kg bw	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
		Long-term Reproductive toxicity	NOEC =118 mg azadirachtin A./kg feed NOEC =1000 mg extract/kg feed	

9.2.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of AZA in Ornamentals

Intended use	**Ornamentals**				
Active substance/product	Azadirachtin				
Application rate (g/ha)	2 × 30				
Acute toxicity (mg/kg bw)	>320				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Ornamentals	Indicator focal species for screening Small insectivorous bird	46.8	1.4	1.97	162.8
Reprod. toxicity (mg/kg bw/d)	8.4				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Ornamentals	Indicator focal species for screening Small insectivorous bird	18.2	1.6 x 0.53	0.46	18.1

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

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of AZA in Fruiting vegetables

Intended use	**Fruiting vegetables**
Active substance/product	Azadirachtin

Application rate (g/ha)	2 × 30				
Acute toxicity (mg/kg bw)	>320				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Fruiting vegetables	Indicator focal species for screening Small omnivorous bird	158.8	1.4	6.67	48.0
Reprod. toxicity (mg/kg bw/d)	8.4				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Fruiting vegetables	Indicator focal species for screening Small omnivorous bird	64.8	1.6 x 0.53	1.65	5.1

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

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of AZA in Potatoes

Intended use	**Potatoes**				
Active substance/product	Azadirachtin				
Application rate (g/ha)	2 × 25				
Acute toxicity (mg/kg bw)	>320				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes	Indicator focal species for screening Small omnivorous bird	158.8	1.4	5.56	57.6
Reprod. toxicity (mg/kg bw/d)	8.4				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Potatoes	Indicator focal species for screening Small omnivorous bird	64.8	1.6 x 0.53	1.37	6.1

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

zRMS comments:

The risk assessment is considered acceptable. 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). Safe use of Azadirachtin for birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

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

Puddle scenario

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

With a K(f)oc of 75.2 L/kg (median, N=7, EFSA Journal 2018;16(4):5234), Azadirachtin belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for birds from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	=	30	AR x 1.6	48
Acute toxicity (mg/kg bw)	=	>320	quotient =	0.15
Reprod. toxicity (mg/kg bw/d)	=	8.4	quotient =	5.71

With a K(f)oc of 75.2 L/kg, Azadirachtin belongs to the group of less sorptive substances. Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50 for all uses scenarios, a quantitative risk assessment (calculation of TER values) is not necessary.

zRMS comments:

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

Evaluation of exposing for birds through the drinking water Puddle scenario for the active substances, demonstrate that the acceptable risk for birds for proposed use pattern of AZA.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Azadirachtin A and Azadirachtin B amounts to 0.99 and 1.29 respectively, and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

zRMS comments:

The log P_{ow} of Azadirachtin A and Azadirachtin B amounts to 0.99 and 1.29 respectively, and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, pills or treated seed

Not relevant.

9.2.4 Overall conclusions

According to the screening tier assessments, a low acute and chronic risk are expected for birds following the intended uses of AZA for all the intended uses assessed in the framework of this application. No risk for birds was identified via drinking water exposure and secondary poisoning via the food chain can be excluded, following the intended uses of AZA, due to the low log Pow values below 3 of the two major fractions Azadirachtin A and B. Moreover, no risk of biomagnification is terrestrial food chain was identified.

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

Effects on mammals of AZA were not evaluated as part of the EU assessment of Azadirachtin. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

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

process.

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

Species	Substance	Exposure System	Results	Reference
Mouse	a.s.: NeemAzal technical (Trifolio M-GmbH)	Oral 1 d Acute	LD_{50} > 841 mg azadirachtin A./kg bw LD_{50} > 3365 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Rat	a.s.: Azadirachtin technical Fortune Aza (Sipcam)	Oral 1 d Acute	**LD_{50} > 330 mg azadirachtin A./kg bw** LD_{50} > 5000 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Rat	a.s.: Azadirachtin technical Fortune Aza (Sipcam)	Oral 90 d Short-term	LC_{50} > 3.4 mg azadirachtin A./kg bw LC_{50} > 33 mg extract/kg bw	EFSA Journal 2018;16(4):5234
Rat	a.s.: NeemAzal technical (Trifolio M-GmbH)	Long-term Reproductive toxicity	NOEC ≥ 13.7 mg azadirachtin A./kg bw NOEC ≥ 50.0 mg extract/kg bw NOEC ≥ 206 mg azadirachtin A./kg feed NOEC ≥ 750 mg extract/kg feed	EFSA Journal 2018;16(4):5234
Rat	ATI-720	Long-term Teratogenicity	**NOAEL ≥ 8.3 mg azadirachtin A./kg bw**	EFSA Journal 2018;16(4):5234

9.3.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AZA in Ornamentals

Intended use	Ornamentals				
Active substance/product	Azadirachtin				
Application rate (g/ha)	2 × 30				
Acute toxicity (mg/kg bw)	> 330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Ornamentals	Indicator focal species for screening Small herbivorous mammal	136.4	1.4	5.73	57.6

Reprod. toxicity (mg/kg bw/d)	≥ 8.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Ornamentals Application to plant – exposure to underlying ground	Small insectivorous mammal “shrew”. 100% ground arthropods	1.9	1.6 x 0.53	0.05	171.7
Ornamentals/Nursery BBCH 40-49	Small herbivorous mammal "vole”. 100% grass	72.3	1.6 x 0.53	1.84	**4.5**
Ornamentals/Nursery BBCH ≥ 50	Small herbivorous mammal "vole”. 100% grass	36.1	1.6 x 0.53	0.92	9.0
Ornamentals/nursery Application crop directed BBCH 10 - 49	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	1.6 x 0.53	0.20	41.8
Ornamentals/nursery Application crop directed BBCH ≥ 50	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.6 x 0.53	0.10	83.7

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

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AZA in Fruiting vegetables

Intended use	Fruiting vegetables				
Active substance/product	Azadirachtin				
Application rate (g/ha)	2 × 30				
Acute toxicity (mg/kg bw)	> 330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Fruiting vegetables	Indicator focal species for screening Small herbivorous mammal	136.4	1.4	5.73	57.6
Reprod. toxicity (mg/kg bw/d)	≥ 8.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Fruiting vegetables BBCH 10 - 19	Small insectivorous mammal “shrew” ground dwelling invertebrates without interception 100% ground arthropods	4.2	1.6 x 0.53	0.11	77.7
Fruiting vegetables BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods	1.9	1.6 x 0.53	0.05	171.7
Fruiting vegetables BBCH 10 - 49	Small herbivorous mammal "vole Grass + cereals 100% grass	72.3	1.6 x 0.53	1.84	**4.5**
Fruiting vegetables BBCH ≥ 50	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.6 x 0.53	0.55	15.0

Fruiting vegetables BBCH 10 - 49	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	1.6 x 0.53	0.20	41.8
Fruiting vegetables BBCH ≥ 50	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.6 x 0.53	0.06	141.9
Fruiting vegetables Fruit stage BBCH 71-89	Frugivorous mammal "rat" Gourds 100% fruit	25.2	1.6 x 0.53	0.64	12.9

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

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AZA in Potatoes

Intended use	Potatoes				
Active substance/product	Azadirachtin				
Application rate (g/ha)	2 × 25				
Acute toxicity (mg/kg bw)	> 330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes	Indicator focal species for screening Small herbivorous mammal	118.4	1.4	4.14	79.6
Reprod. toxicity (mg/kg bw/d)	≥ 8.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Potatoes	Indicator focal species for screening Small herbivorous mammal	48.3	1.6 x 0.53	1.02	8.1

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

9.3.2.2 Higher-tier risk assessment

Higher tier risk assessment for Azadirachtin regarding the small herbivorous mammal "vole" in orchards and fruiting vegetables

Deposition factor (DF)

Since grass will be covered by the crop, an interception by the crop has to be taken into account.

For ornamentals, onions and vines can be used as surrogates crops for ornamentals <50 and >50 cm, respectively. Regarding ornamentals <50cm, scenario BBCH 40-49 corresponds with the flowering, and according to the interception values of EFSA (2014)[1], an interception factor of 40% for onion should be considered as highest worst case. Therefore, for the refinement of the risk a deposition factor of 0.6 should be applied. Regarding ornamentals >50cm, an interception factor of 50% for vines corresponding to leaf development should be considered as highest worst case. Therefore, for the refinement of the risk a deposition factor of 0.5 should be applied

[1] EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil.

For tomato, scenario BBCH 40-49 corresponds with the flowering, and according to the interception values of EFSA (2014), an interception factor of 80% should be considered as highest worst case. Therefore, for the refinement of the risk a deposition factor of 0.2 should be applied.

Table 9.3-5: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of AZA in ornamentals <50cm – refined parameters (*) are further described and justified in the text

Intended use	Ornamentals (<50 cm)						
Active substance/product	Azadirachtin						
Application rate (g/ha)	2 × 30						
Reprod. toxicity (mg/kg bw/d)	≥ 8.3						
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_{lt}**
Common vole (*Microtus arvalis*) Ornamentals/Nursery BBCH 40-49	Grass+Cereals	1.33	54.2[1] × 0.6	1.6 × 0.53	1.0	1.10	7.5

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1] According to Appendix A of EFSA/2009/1438.

Table 9.3-6: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of AZA in ornamentals >50cm – refined parameters (*) are further described and justified in the text

Intended use	Ornamentals (>50 cm)						
Active substance/product	Azadirachtin						
Application rate (g/ha)	2 × 30						
Reprod. toxicity (mg/kg bw/d)	≥ 8.3						
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_{lt}**
Common vole (*Microtus arvalis*) Ornamentals/Nursery BBCH 40-49	Grass+Cereals	1.33	54.2[1] × 0.5	1.6 × 0.53	1.0	0.92	9.1

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1] According to Appendix A of EFSA/2009/1438.

Table 9.3-7: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of AZA in Fruiting vegetables – refined parameters (*) are further described and justified in the text

Intended use	Fruiting vegetables
Active substance/product	Azadirachtin

Application rate (g/ha)	2 × 30						
Reprod. toxicity (mg/kg bw/d)	≥ 8.3						
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_{lt}**
Common vole (*Microtus arvalis*) Fruiting vegetables BBCH 10 - 49	Grass+Cereals	1.33	54.2[1] × 0.2	1.6 × 0.53	1.0	0.37	22.6

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1] According to Appendix A of EFSA/2009/1438.

Weight of evidence approach

In addition, a monitoring study was conducted looking at the utilization of tomato fields by small mammals (*Barfknecht R (2003a). Attractiveness of Tomato fields for herbivorous mammals and birds; Field monitoring in Lombardia*). In this study, small mammals were monitored within and around tomato fields in the region of Lombardia, in Italy. In this study, voles (or herbivores) were only found occasionally in tomato fields (0% tomato field in home range), whereas the omnivorous wood mouse was the only mammal species that had its home range in tomato fields (73-86% tomato field in home range). Therefore, it can be considered that the primary source of food for the vole is outside tomato crops.

RMS comments:

The risk assessment at Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). The refinement of the risk for vole was provided according to DF included EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil.

Safe use of for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective

application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 75.2 L/kg (median, N=7, EFSA Journal 2018;16(4):5234),, Azadirachtin belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for mammals from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	=	30	AR x 1.6		48
Acute toxicity (mg/kg bw)	=	330	quotient	=	0.15
Reprod. toxicity (mg/kg bw/d)	=	13.7	quotient	=	3.50

With a K(f)oc of 75.2 L/kg, Azadirachtin belongs to the group of less sorptive substances. Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50 for all uses scenarios, a quantitative risk assessment (calculation of TER values) is not necessary.

zRMS comments:

The log P_{ow} of Azadirachtin A and Azadirachtin B amounts to 0.99 and 1.29 respectively, and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Azadirachtin A and Azadirachtin B amounts to 0.99 and 1.29 respectively, and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, pills or treated seed

Not relevant.

9.3.4 Overall conclusions

According to the screening and first-tier assessments, a low acute and chronic risk are expected for mammals following the intended uses of AZA for all the intended uses assessed in the framework of this application, except for the uses on Ornamentals and fruiting vegetables (Tomato) for which a chronic risk was identified regarding the small herbivorous mammal "vole". However, according to the higher-tier

assessment, a low chronic risk is expected for mammals for the intended uses on ornamentals and fruiting vegetables. No risk for mammals was identified via drinking water exposure and secondary poisoning via the food chain can be excluded following the intended uses of AZA, due to the low log Pow values below 3 of the two major fractions Azadirachtin A and B. Moreover, no risk of biomagnification is terrestrial food chain was identified.

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

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 Azadirachtin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

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

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

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	a.s. NeemAzal (Trifolio)	96 h, ff	EC_{50} > 2.219 mg Azadirachtin A/L_{mm} EC_{50} > 6.18 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	a.s. NPI-720 (Mitsui)	96 h, ff	**EC_{50} = 0.048 mg Azadirachtin A/L_{mm}** EC_{50} = 0.48 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	a.s. Fortune Aza tech. (Sipcam)	96 h, s	EC_{50} = 0.086 mg Azadirachtin A/L_{mm} EC_{50} = 0.73 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	a.s. azadirachtin techn. (Sipcam)	28 d, ff	**$NOEC_{growth}$ = 0.0047 mg Azadirachtin A/L_{nom}** $NOEC_{growth}$ = 0.04 mg extract/L_{nom}	EFSA Journal 2018;16(4):5234
Danio rerio	a.s. NeemAzal (Trifolio M- GmbH)	174 d FLC, ff	Not valid $NOEC_{growth}$ = 1.9 mg Azadirachtin A/L_{mm} $NOEC_{growth}$ = 6.4 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	Preparation NeemAzal-TS (Trifolio M- GmbH)	96 h, ss	EC_{50} = 1.41 mg Azadirachtin A/L_{mm} EC_{50} = 141 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	Preparation Oikos (Sipcam)	96 h, s	EC_{50} = 0.077 mg Azadirachtin A/L_{mm} EC_{50} = 2.96 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Oncorhynchus mykiss	Preparation NeemAzal-TS (Trifolio M- GmbH)	28 d, ff	$NOEC_{growth}$ = 0.712 mg Azadirachtin A/L_{mm} $NOEC_{growth}$ = 63.6 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Daphnia magna	a.s. NeemAzal (Trifolio M- GmbH)	48 h, s	EC_{50} = 3.54 mg Azadirachtin A/L_{mm} EC_{50} = 10.6 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Daphnia magna	a.s. NPI-720 (Mitsui)	48 h, ff	**EC_{50} = 1 mg Azadirachtin A/L_{mm}** EC_{50} = 10 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Daphnia magna	a.s. NeemAzal (Trifolio M- GmbH)	21 d, ss	$NOEC_{reproduction}$ = 0.615 mg Azadirachtin A/L_{mm} $NOEC_{reproduction}$ = 1.84 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Daphnia magna	a.s. Azadirachtin techn. (Sipcam)	21 d, ss	$NOEC_{reproduction}$ = 0.27 mg Azadirachtin A/L_{mm} $NOEC_{reproduction}$ = 2.3 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Daphnia magna	Preparation NeemAzal-TS (Trifolio M- GmbH)	48 h, s	EC_{50} > 8 mg Azadirachtin A/L_{mm} EC_{50} > 800 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Daphnia magna	Preparation NeemAzal-TS (Trifolio M- GmbH)	21 d, ss	**$NOEC_{reproduction}$ = 0.038 mg Azadirachtin A/L_{mm}** $NOEC_{reproduction}$ = 3.4 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	NeemAzal batch 134	28 d, s	NOEC = 0.0037 mg Azadirachtin A/L_{mm} NOEC = 0.011 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	Azatin Technical-grade Active Ingredient AZ/148/06-07	28 d, s	**NOEC = 0.0016 mg Azadirachtin A/L_{mm}** NOEC = 0.01 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	Fortune 11004062007	28 d, s	NOEC = 0.0033 mg Azadirachtin A/L_{mm} NOEC = 0.0245 mg extract/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	OIKOS, batch G249	28 d, s	NOEC = 0.0036 mg Azadirachtin A/L_{mm} NOEC = 0.144 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	NeemAzal-T/S batch 240707M	28 d, s	NOEC = 0.0029 mg Azadirachtin A/L_{mm} NOEC = 0.262 mg product/L_{mm}	EFSA Journal 2018;16(4):5234
Chironomus riparius	Azadirachtin A	96 h (screening test)	EC_{50} = 0.844 mg/L_{nom}	EFSA Journal 2018;16(4):5234
Chironomus riparius	Azadirachtin B	96 h (screening test)	EC_{50} = 0.391 mg/L_{nom}	EFSA Journal 2018;16(4):5234
Chironomus riparius	14, 15-epoxy-azadiradione	96 h (screening test)	EC_{50} = 0.716 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	Salannin	96 h (screening test)	EC_{50} = 2.99 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	3-deacetyl- salannin	96 h (screening test)	EC_{50} = 1.82 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	Substance 8 (see Vol. 4)	96 h (screening test)	EC_{50} > 50.0 mg/L*	EFSA Journal 2018;16(4):5234
Chironomus riparius	Azadiradione	96 h (screening test)	EC_{50} = 1.46 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	Nimbin	96 h (screening test)	EC_{50} = 1.24 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	6-desacetyl- nimbin	96 h (screening test)	EC_{50} = 1.38 mg/L	EFSA Journal 2018;16(4):5234
Chironomus riparius	11-epi- azadirachtin D	96 h (screening test)	EC_{50} < 0.37 mg/L**	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Chironomus riparius	12-decarbo-methoxy-azadirachtin	96 h (screening test)	EC_{50} = 1.96 mg/L	EFSA Journal 2018;16(4):5234
Pseudokirchneriella subcapitata	a.s. Azadirachtin techn. (Sipcam)	72 h, s	$E_bC_{50\ Biomass}$ > 5.76 mg Azadirachtin A/L $_{mm}$ **$E_rC_{50\ Growth\ rate}$ > 36 mg extract/L $_{mm}$**	EFSA Journal 2018;16(4):5234
Desmodesmus subspicatus	Preparation NeemAzal-TS (Trifolio M- GmbH)	72 h, s	$E_bC_{50\ Biomass}$ > 27.4 mg Azadirachtin A/L $_{ini\ nom}$ $E_rC_{50\ Growth\ rate}$ > 2494 mg product/L $_{ini\ nom}$	EFSA Journal 2018;16(4):5234
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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
*data should be treated with caution since the test item precipitated in the stock solution
**3 11-epi-azadirachtin D: 70 % average immobilisation at the lowest test concentration of 0.37 mg/L after 96 hours. As more than 50 % effect appeared at the lowest test concentration, the endpoint has to be treated with care and can be considered as rough estimation only.

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	AZA	96 h, ss	**LC_{50} > 100 mg f.p./L** (equivalent to >1.04 mg a.s./L) $_{nom}$	KCP 10.2.1-01 ……. 2019 W/67/17
Daphnia magna	AZA	48 h, s	**EC_{50} > 100 mg f.p./L** (equivalent to >1.06 mg a.s./L) $_{nom}$	KCP 10.2.1-02 Czarnecka, M. 2019 W/69/17
Pseudokirchneriella subcapitata	AZA	72 h, s	**E_rC_{50} > 1000 mg f.p./L $_{nom}$** (equivalent to >6.52 mg a.s./L $_{geomean}$) E_yC_{50} = 32.34 mg f.p./L $_{nom}$ (equivalent to 0.13 mg a.s./L $_{geomean}$)	KCP 10.2.1-02 Czarnecka, M. 2019 W/68/17
Higher-tier studies (micro- or mesocosm studies)				
No data available				

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

9.5.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to AZA formulation.

According to R (EU) nº 284/2013: "*Possible effects on aquatic species (fish, aquatic invertebrates, algae and in the case of herbicides and plant growth regulators, aquatic macrophytes) shall be investigated except where the possibility that aquatic species will be exposed can be ruled out*". AZA is a insecticide therefore the Applicant considers that studies on macrophytes with formulation is not mandatory.

9.5.2 Risk assessment

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

AZA

The relevant global maximum PEC_{SW} for risk assessments with AZA formulation covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AZA for each organism group for the use of AZA in potato and ornamentals <50cm (single/multiple application)

Group				**Fish acute**	**Inverteb. acute**	**Algae**
Test species				***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint				LC_{50}	EC_{50}	E_rC_{50}
(µg/L)				>100000	>100000	>1000000
AF				100	100	10
RAC (µg/L)				>1000	>1000	>100000
Distance	**% Drift**	**Nozzles reduction (%)**	**PEC $_{gl\text{-}max}$ (µg/L)**			
1m	2.77 / 2.38	None	26.614 / 45.734	0.027/0.046	0.027/0.046	<0.001/<0.001

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AZA for each organism group for the use of AZA in tomato and ornamentals >50cm (single/multiple application)

Group				**Fish acute**	**Inverteb. acute**	**Algae**
Test species				***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint				LC_{50}	EC_{50}	E_rC_{50}
(µg/L)				>100000	>100000	>1000000
AF				100	100	10
RAC (µg/L)				>1000	>1000	>100000
Distance	**% Drift**	**Nozzles reduction (%)**	**PEC $_{gl\text{-}max}$ (µg/L)**			
1m	8.02 / 7.23	None	77.056 / 138.932	0.077/0.139	0.077/0.139	0.001/0.001

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

Azadirachtin A

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Azadirachtin A for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of AZA in Fruiting Vegetables (tomato) (single/multiple application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		48	4.7	1000	38	36000	1.6
AF		100	10	100	10	10	10
RAC (µg/L)		0.48	0.47	10	3.8	3600	0.16
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)***						
Step 1							
	9.29/18.58	**19.354/38.708**	**19.766/39.532**	0.929/**1.858**	**2.445/4.889**	0.003/0.005	**58.063/116.125**
Step 2							
S-Europe	1.56/82.08	**3.250/171.000**	**3.319/174.638**	0.156/**8.208**	0.411/**21.600**	0.000/0.023	**9.750/513.000**
N-Europe	0.89 / 1.20	**1.854/1.854**	**1.894/1.894**	0.089/0.089	0.234/0.234	<0.001/<0.001	**5.563/5.563**
Step 3							
D3/ditch	0.157 / 0.137	0.327/0.285	0.334/0.291	0.016/0.014	0.041/0.036	<0.001/<0.001	0.981/0.856
D4/pond	0.006 / 0.007	0.013/0.015	0.013/0.015	0.001/0.001	0.002/0.002	<0.001/<0.001	0.038/0.044
D4/stream	0.134 / 0.115	0.279/0.240	0.285/0.245	0.013/0.012	0.035/0.030	<0.001/<0.001	0.838/0.719
D6/ditch	0.187 / 0.164	0.390/0.342	0.398/0.349	0.019/0.016	0.049/0.043	<0.001/<0.001	**1.169/1.025**
R1/pond	0.015 / 0.043	0.031/0.090	0.032/0.091	0.002/0.004	0.004/0.011	<0.001/<0.001	0.094/0.269
R1/stream	0.244 / 0.536	0.508/**1.117**	0.519/**1.140**	0.024/0.054	0.064/0.141	<0.001/<0.001	**1.525/3.350**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
R2/stream	0.174 / 0.187	0.363/0.390	0.370/0.398	0.017/0.019	0.046/0.049	<0.001/<0.001	**1.088/1.169**
R3/stream	0.690 / 0.690	**1.438/1.438**	**1.468/1.468**	0.069/0.069	0.182/0.182	<0.001/<0.001	**4.313/4.313**
R4/stream	0.894 / 0.930	**1.863/1.938**	**1.902/1.979**	0.089/0.093	0.235/0.245	<0.001/<0.001	**5.588/5.813**

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Azadirachtin A for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of AZA in Potato (single/multiple application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. sub-capitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		48	4.7	1000	38	36000	1.6
AF		100	10	100	10	10	10
RAC (µg/L)		0.48	0.47	10	3.8	3600	0.16
FOCUS Scenar-io	**PEC $_{gl\text{-}max}$ (µg/L)***						
Step 1							
	7.74/15.48	**16.125/32.250**	**16.468/32.936**	0.774/**1.548**	**2.037/4.074**	0.002/0.004	**48.375/96.750**
Step 2							
S-Europe	1.45/1.93	**3.021/4.021**	**3.085/4.106**	0.145/0.193	0.382/0.508	<0.001/0.001	**9.063/12.063**
N-Europe	0.82/1.10	**1.708/1.708**	**1.745/1.745**	0.082/0.082	0.216/0.216	<0.001/<0.001	**5.125/5.125**
Step 3							
D3/ditch	0.131/0.114	0.273/0.238	0.279/0.243	0.013/0.011	0.034/0.030	<0.001/<0.001	0.819/0.713
D4/pond	0.005/0.006	0.010/0.013	0.011/0.013	0.001/0.001	0.001/0.002	<0.001/<0.001	0.031/0.038
D4/stream	0.112/0.096	0.233/0.200	0.238/0.204	0.011/0.010	0.029/0.025	<0.001/<0.001	0.700/0.600

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
D6/ditch (1st)	0.146/0.182	0.304/0.379	0.311/0.387	0.015/0.018	0.038/0.048	<0.001/<0.001	0.913/1.138
D6/ditch (2nd)	0.129/0.112	0.269/0.233	0.274/0.238	0.013/0.011	0.034/0.029	<0.001/<0.001	0.806/0.700
R1/pond	0.012/0.035	0.025/0.073	0.026/0.074	0.001/0.004	0.003/0.009	<0.001/<0.001	0.075/0.219
R1/stream	0.202/0.444	0.421/0.925	0.430/0.945	0.020/0.044	0.053/0.117	<0.001/<0.001	**1.263/2.775**
R2/stream	0.145/0.160	0.302/0.333	0.309/0.340	0.015/0.016	0.038/0.042	<0.001/<0.001	0.906/**1.000**
R3/stream	0.479/0.479	0.998/0.998	**1.019/1.019**	0.048/0.048	0.126/0.126	<0.001/<0.001	**2.994/2.994**

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

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Azadirachtin A for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of AZA in Vines late application (worst case for bush ornamentals) (single/multiple application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. sub-capitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		48	4.7	1000	38	36000	1.6
AF		100	10	100	10	10	10
RAC (µg/L)		0.48	0.47	10	3.8	3600	0.16
FOCUS Scenar-io	**PEC gl-max (µg/L)***						
Step 1							
	9.82/19.63	**20.458/40.896**	**20.894/41.766**	0.982/**1.963**	**2.584/5.166**	0.003/0.005	**61.375/122.688**
Step 2							
S-Europe	1.70/2.34	**3.542/4.875**	**3.617/4.979**	0.170/0.234	0.447/0.616	<0.001/0.001	**10.625/14.625**
N-Europe	1.16/1.64	**2.417/2.417**	**2.468/2.468**	0.116/0.116	0.305/0.305	<0.001/<0.001	**7.250/7.250**
Step 3							

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
D3/ditch	0.511/0.452	**1.065**/0.942	**1.087**/0.962	0.051/0.045	0.134/0.119	<0.001/<0.001	**3.194/2.825**
D4/pond	0.018/0.022	0.038/0.046	0.038/0.047	0.002/0.002	0.005/0.006	<0.001/<0.001	0.113/0.138
D4/stream	0.447/0.417	0.931/0.869	0.951/0.887	0.045/0.042	0.118/0.110	<0.001/<0.001	**2.794/2.606**
D6/ditch	0.506/0.458	**1.054**/0.954	**1.077**/0.974	0.051/0.046	0.133/0.121	<0.001/<0.001	**3.163/2.863**
R1/pond	0.018/0.029	0.038/0.060	0.038/0.062	0.002/0.003	0.005/0.008	<0.001/<0.001	0.113/0.181
R1/stream	0.374/0.330	0.779/0.688	0.796/0.702	0.037/0.033	0.098/0.087	<0.001/<0.001	**2.338/2.063**
R2/stream	0.497/0.439	**1.035**/0.915	**1.057**/0.934	0.050/0.044	0.131/0.116	<0.001/<0.001	**3.106/2.744**
R3/stream	0.529/0.468	**1.102**/0.975	**1.126**/0.996	0.053/0.047	0.139/0.123	<0.001/<0.001	**3.306/2.925**
R4/stream	0.374/0.330	0.779/0.688	0.796/0.702	0.037/0.033	0.098/0.087	<0.001/<0.001	**2.338/2.063**

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Azadirachtin A for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of AZA in Bulb Vegetables (herbaceous ornamentals use) (single/multiple application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. sub-capitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		48	4.7	1000	38	36000	1.6
AF		100	10	100	10	10	10
RAC (µg/L)		0.48	0.47	10	3.8	3600	0.16
FOCUS Scenar-io	**PEC $_{gl\text{-}max}$ (µg/L)***						
Step 1							
	9.29/18.58	**19.354/38.708**	**19.766/39.532**	0.929/1.858	**2.445/4.889**	0.003/0.005	**58.063/116.125**
Step 2							

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
S-Europe	1.83/2.43	**3.813/5.063**	**3.894/5.170**	0.183/0.243	0.482/0.639	0.001/0.001	**11.438/15.188**
N-Europe	1.02/1.37	**2.125/2.125**	**2.170/2.170**	0.102/0.102	0.268/0.268	<0.001/<0.001	**6.375/6.375**
Step 3							
D3/ditch	0.190/0.166	0.396/0.346	0.404/0.353	0.019/0.017	0.050/0.044	<0.001/<0.001	**1.188/1.038**
D4/pond	0.007/0.010	0.015/0.021	0.015/0.021	0.001/0.001	0.002/0.003	<0.001/<0.001	0.044/0.063
D4/stream	0.146/0.126	0.304/0.263	0.311/0.268	0.015/0.013	0.038/0.033	<0.001/<0.001	0.913/0.788
D6/ditch (1st)	0.191/0.170	0.398/0.354	0.406/0.362	0.019/0.017	0.050/0.045	<0.001/<0.001	**1.194/1.063**
D6/ditch (2nd)	0.605/1.565	**1.260/3.260**	**1.287/3.330**	0.061/0.157	0.159/0.412	<0.001/<0.001	**3.781/9.781**
R1/pond	0.016/0.047	0.033/0.098	0.034/0.100	0.002/0.005	0.004/0.012	<0.001/<0.001	0.100/0.294
R1/stream	0.282/0.920	0.588/**1.917**	0.600/1.957	0.028/0.092	0.074/0.242	<0.001/<0.001	**1.763/5.750**
R2/stream	0.180/0.191	0.375/0.398	0.383/0.406	0.018/0.019	0.047/0.050	<0.001/<0.001	**1.125/1.194**
R3/stream	0.176/0.309	0.367/0.644	0.374/0.657	0.018/0.031	0.046/0.081	<0.001/<0.001	**1.100/1.931**
R4/stream	0.828/1.102	**1.725/2.296**	**1.762/2.345**	0.083/0.110	0.218/0.290	<0.001/<0.001	**5.175/6.888**

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

For the intended uses on Fruiting vegetables (Tomato), calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for sediment dwelling organisms as characterised by a NOEC for *Chironomus riparius* of 1.6 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

For the intended uses on Potato, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for sediment dwelling organisms as characterised by a NOEC for *Chironomus riparius* of 1.6 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

For the intended uses on ornamentals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for sediment dwelling organisms as characterised by a NOEC for *Chironomus riparius* of 1.6 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-9: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for lowest endpoint for fish and sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Fruiting Vegetables (Tomato) (single/multiple application)

Intended use **Active substance** **Application rate (g/ha)**		Fruiting Vegetables Azadirachtin 2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D6 ditch	0.051/0.04	-/-	-/-	-/-	-/-
	R1 stream	0.244/0.53	0.157/0.349	-/0.244	-/0.187	-/0.128
	R2 stream	0.174/0.18	0.113/0.120	-/-	-/-	-/-
	R3 stream	0.690/0.69	0.449/0.449	0.313/0.313	0.240/0.240	0.163/0.163
	R4 stream	0.894/0.93	0.583/0.605	0.407/0.421	0.312/0.323	0.213/0.220
RAC 0.47		**PEC/RAC ratio**				
None	D6 ditch	0.109/0.09	-/-	-/-	-/-	-/-
	R1 stream	0.519/1.14	0.334/0.743	-/0.519	-/0.398	-/0.272
	R2 stream	0.370/0.39	0.240/0.255	-/-	-/-	-/-
	R3 stream	**1.468/1.46**	0.955/0.955	0.666/0.666	0.511/0.511	0.347/0.347
	R4 stream	**1.902/1.97**	**1.240/1.287**	0.866/0.896	0.664/0.687	0.453/0.468
RAC 0.16		**PEC/RAC ratio**				
None	D6 ditch	0.319/0.26	-/-	-/-	-/-	-/-
	R1 stream	1.**525/3.35**	0.981/**2.181**	**-/1.525**	**-/1.169**	-/0.800
	R2 stream	**1.088/1.16**	0.706/0.750	-/-	-/-	-/-

Intended use		Fruiting Vegetables				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
	R3 stream	**4.313/4.31**	**2.806/2.806**	**1.956/1.956**	**1.500/1.500**	**1.019/1.019**
	R4 stream	**5.588/5.81**	**3.644/3.781**	**2.544/2.631**	**1.950/2.019**	**1.331/1.375**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

Table 9.5-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Potato (single/multiple application)

Intended use		Potato				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 25				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D6 ditch stream	-/0.147	-/-	-/-	-/-	-/-
	R1 stream	0.202/0.444	0.131/0.290	-/0.202	-/0.155	-/-
	R3 stream	0.479/0.479	0.313/0.313	0.219/0.219	0.168/0.168	0.115/0.115
RAC 0.47		**PEC/RAC ratio**				
None	D6 ditch stream	-/0.313	-/-	-/-	-/-	-/-
	R1 stream	0.430/0.945	0.279/0.617	-/0.430	-/0.330	-/-
	R3 stream	**1.019/1.019**	0.666/0.666	0.466/0.466	0.357/0.357	0.245/0.245
RAC 0.16		**PEC/RAC ratio**				
None	D6 ditch stream	-/0.919	-/-	-/-	-/-	-/-
	R1 stream	**1.263/2.775**	0.819/**1.813**	-/**1.263**	-/0.969	-/-
	R3 stream	**2.994/2.994**	**1.956/1.956**	**1.369/1.369**	**1.050/1.050**	0.719/0.719

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

Table 9.5-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Vines late application (worst case for bush ornamentals use) (single/multiple application)

Intended use		Bush ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
None	D3 ditch	0.309/0.272	0.112/0.098	-/-	-/-	-/-
50 %		0.154/0.136	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D4 stream	0.271/0.253	0.098/0.091	-/-	-/-	-/-
50 %		0.136/0.126	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D6 ditch	0.307/0.278	0.112/0.103	-/-	-/-	-/-
50 %		0.154/0.141	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R1 stream	0.272/0.240	0.099/0.086	-/-	-/-	-/-
50 %		0.136/0.120	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R2 stream	0.362/0.319	0.131/0.115	-/-	-/-	-/-
50 %		0.181/0.159	-/-	-/-	-/-	-/-
75 %		0.091/-	-/-	-/-	-/-	-/-
None	R3 stream	0.412/0.412	0.412/0.412	0.386/0.340	0.186/0.186	0.142/0.142
50 %		0.412/0.412	-/-	0.268/0.268	0.186/0.186	-/-
75 %		-/-	-/-	0.268/0.268	-/-	-/-
None	R4 stream	0.273/0.240	0.099/0.086	-/-	-/-	-/-
50 %		0.136/0.120	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
RAC 0.47		**PEC/RAC ratio**				
None	D3 ditch	0.657/0.579	0.238/0.209	-/-	-/-	-/-
50 %		0.328/0.289	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D4 stream	0.577/0.538	0.209/0.194	-/-	-/-	-/-
50 %		0.289/0.268	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D6 ditch	0.653/0.591	0.238/0.219	-/-	-/-	-/-
50 %		0.328/0.300	-/-	-/-	-/-	-/-

Intended use **Active substance** **Application rate (g/ha)**		Bush ornamentals Azadirachtin 2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
75 %		-/-	-/-	-/-	-/-	-/-
None	R1 stream	0.579/0.511	0.211/0.183	-/-	-/-	-/-
50 %		0.289/0.255	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R2 stream	0.770/0.679	0.279/0.245	-/-	-/-	-/-
50 %		0.385/0.338	-/-	-/-	-/-	-/-
75 %		0.194/-	-/-	-/-	-/-	-/-
None	R3 stream	0.877/0.877	0.877/0.877	0.821/0.723	0.396/0.396	0.302/0.302
50 %		0.877/0.877	-/-	0.570/0.570	0.396/0.396	-/-
75 %		-/-	-/-	0.570/0.570	-/-	-/-
None	R4 stream	0.581/0.511	0.211/0.183	-/-	-/-	-/-
50 %		0.289/0.255	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
RAC 0.16		**PEC/RAC ratio**				
None	D3 ditch	**1.931/1.700**	0.700/0.613	-/-	-/-	-/-
50 %		0.963/0.850	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D4 stream	**1.694/1.581**	0.613/0.569	-/-	-/-	-/-
50 %		0.850/0.788	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D6 ditch	**1.919/1.738**	0.700/0.644	-/-	-/-	-/-
50 %		0.963/0.881	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R1 stream	**1.700/1.500**	0.619/0.538	-/-	-/-	-/-
50 %		0.850/0.750	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R2 stream	**2.263/1.994**	0.819/0.719	-/-	-/-	-/-
50 %		**1.131**/0.994	-/-	-/-	-/-	-/-
75 %		0.569/-	-/-	-/-	-/-	-/-
None	R3 stream	**2.575/2.575**	**2.575/2.575**	**2.413/2.125**	**1.163/1.163**	0.888/0.888
50 %		**2.575/2.575**	-/-	**1.675/1.675**	**1.163/1.163**	-/-
75 %		-/-	-/-	**1.675/1.675**	-/-	-/-
None	R4 stream	**1.706/1.500**	0.619/0.538	-/-	-/-	-/-
50 %		0.850/0.750	-/-	-/-	-/-	-/-

Intended use		Bush ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
75 %		-/-	-/-	-/-	-/-	-/-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

Table 9.5-12: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Bulb Vegetables (herbaceous ornamentals) (single/multiple application)

Intended use		Herbaceous ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.052/0.043	-/-	-/-	-/-	-/-
	D6 ditch	0.052/0.044	-/-	-/-	-/-	-/-
	D6 ditch	0.605/1.565	-/-	-/-	-/-	-/-
	R1 stream	0.282/0.920	0.173/0.599	0.116/0.417	-/0.320	-/0.218
	R2 stream	0.180/0.191	0.117/0.123	-/-	-/-	-/-
	R3 stream	0.092/0.309	-/0.202	-/0.141	-/-	-/-
	R4 stream	0.828/1.102	0.539/0.719	0.376/0.501	0.288/0.384	0.197/0.262
RAC 0.47		**PEC/RAC ratio**				
None	D3 ditch	0.111/0.091	-/-	-/-	-/-	-/-
	D6 ditch	0.111/0.094	-/-	-/-	-/-	-/-
	D6 ditch	**1.287/3.330**	-/-	-/-	-/-	-/-
	R1 stream	0.600/1.957	0.368/1.274	0.247/0.887	-/0.681	-/0.464
	R2 stream	0.383/0.406	0.249/0.262	-/-	-/-	-/-
	R3 stream	0.196/0.657	-/0.430	-/0.300	-/-	-/-
	R4 stream	**1.762/2.345**	**1.147/1.530**	0.800/1.066	0.613/0.817	0.419/0.557
RAC 0.16		**PEC/RAC ratio**				
None	D3 ditch	0.325/0.269	-/-	-/-	-/-	-/-
	D6 ditch	0.325/0.275	-/-	-/-	-/-	-/-

Intended use		Herbaceous ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
	D6 ditch	**3.781/9.781**	-/-	-/-	-/-	-/-
	R1 stream	**1.763/5.750**	**1.081/3.744**	0.725/**2.606**	**-/2.000**	**-/1.363**
	R2 stream	**1.125/1.194**	0.731/0.769	-/-	-/-	-/-
	R3 stream	0.575/**1.931**	**-/1.263**	-/0.881	-/-	-/-
	R4 stream	**5.175/6.888**	**3.369/4.494**	**2.350/3.131**	**1.800/2.400**	**1.231/1.638**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

After the FOCUS step 4 calculations the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch; 5 m no-spray buffer.
- R1 stream: 20 m no-spray buffer and 20 m vegetative filter strip
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip
- R3 stream and R4 stream: risk unacceptable. Therefore, a further refinement is needed.

Potato

- D6 ditch; 5 m no-spray buffer and 5 m vegetative filter strip.
- R1 stream: 15 m no-spray buffer and 15 m vegetative filter strip.
- R3 stream: 20 m no-spray buffer and 20 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D4 stream, D6 ditch, R1 stream and R4 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- R2 stream: 5 m no-spray buffer with 75% of nozzles reduction or 10 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch; 5 m no-spray buffer.
- D6 ditch, R1 stream and R4 stream: risk unacceptable. Therefore, a further refinement is needed.
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip.
- R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip.

Refinement of the risk assessment for the most sensitive group of aquatic organisms: sediment dwelling organisms

In the monograph of azadiracthrin, 5 chronic toxicity studies for the same species *Chironomus riparius* are available with different representative formulations and for technical. The Applicant wishes to consider that the geomean value of NOEC of 2.9 µg a.s./L from these five different studies with an assessment factor of 10 is more appropriate to use in the refinement of the risk. The value of the RAC obtained is **0.29 µg a.s./L**. In addition, the Applicant considers that the risk assessment on fish is covered by the risk assessment performed below with the obtained RAC of 0.29.

Test item	sediment dwelling organisms	NOEC (mg a.s./L)
NeemAzal batch 134	*Chironomus riparius*	0.0037
Azatin Technical-grade Active Ingredient AZ/148/06-07	*Chironomus riparius*	0.0016
Fortune 11004062007	*Chironomus riparius*	0.0033
OIKOS, batch G249	*Chironomus riparius*	0.0036
NeemAzal-T/S batch 240707M	*Chironomus riparius*	0.0029
Geomean (ug a.s./L)		**0.0029**

Table 9.5-13: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for geomean value sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Fruiting Vegetables (Tomato) (single/multiple application)

Intended use **Active substance** **Application rate (g/ha)**		Fruiting Vegetables Azadirachtin 2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D6 ditch	0.051/0.04	-/-	-/-	-/-	-/-
	R1 stream	0.244/0.53	0.157/0.349	-/0.244	-/0.157	-/0.125
	R2 stream	0.174/0.18	0.113/0.120	-/-	-/-	-/-
	R3 stream	0.690/0.69	0.449/0.449	0.313/0.313	0.240/0.240	0.163/0.163
	R4 stream	0.894/0.93	0.583/0.605	0.407/0.421	0.312/0.323	0.213/0.220
RAC 0.29		**PEC/RAC ratio**				
None	D6 ditch	0.176/0.14	-/-	-/-	-/-	-/-
	R1 stream	0.841/1.84	0.541/1.203	-/0.841	-/0.545	-/0.441
	R2 stream	0.600/0.64	0.390/0.414	-/-	-/-	-/-
	R3 stream	**2.379/2.37**	**1.548/1.548**	**1.079/1.079**	0.828/0.828	0.562/0.562
	R4 stream	**3.083/3.20**	**2.010/2.086**	**1.403/1.452**	**1.076/1.114**	0.734/0.759

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

Table 9.5-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for geomean value sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Potato (single/multiple application)

Intended use		Potato				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 25				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D6 ditch	-/0.147	-/-	-/-	-/-	-/-
	R1 stream	0.202/0.444	0.131/0.290	-/0.202	-/0.155	-/-
	R3 stream	0.479/0.479	0.313/0.313	0.219/0.219	0.169/0.169	0.115/0.115
RAC 0.29		**PEC/RAC ratio**				
None	D6 ditch	-/0.507	-/-	-/-	-/-	-/-
	R1 stream	0.697/**1.531**	0.452/**1.000**	-/0.697	-/0.534	-/-
	R3 stream	**1.652/1.652**	**1.079/1.079**	0.755/0.755	0.579/0.579	0.397/0.397

*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.
PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for geomean value sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Vines late application (worst case for bush ornamentals use) (single/multiple application)

Intended use		Bush ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
None	D3 ditch	0.310/0.273	0.112/0.098	-/-	-/-	-/-
50 %		0.155/0.137	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D4 stream	0.299/0.264	0.108/0.095	-/-	-/-	-/-
50 %		0.150/0.132	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-

Intended use **Active substance** **Application rate (g/ha)**		Bush ornamentals Azadirachtin 2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
None	D6 ditch	0.309/0.275	0.112/0.099	-/-	-/-	-/-
50 %		0.155/0.138	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R1 stream	0.383/0.383	-/-	0.274/0.247	0.171/0.171	0.131/0.131
50 %		-/-	-/-	0.247/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R2 stream	0.369/0.325	0.134/0.117	-/-	-/-	-/-
50 %		0.184/0.162	-/-	-/-	-/-	-/-
75 %		0.092/0.081	-/-	-/-	-/-	-/-
None	R3 stream	0.386/0.341	0.140/0.123	-/-	-/-	-/-
50 %		0.193/0.171	-/-	-/-	-/-	-/-
75 %		0.097/0.085	-/-	-/-	-/-	-/-
None	R4 stream	0.275/0.242	0.100/0.087	-/-	-/-	-/-
50 %		0.138/0.121	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
RAC 0.29		**PEC/RAC ratio**				
None	D3 ditch	**1.066**/0.938	0.386/0.338	-/-	-/-	-/-
50 %		0.531/0.469	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D4 stream	0.934/0.872	0.338/0.314	-/-	-/-	-/-
50 %		0.469/0.434	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	D6 ditch	**1.059**/0.959	0.386/0.355	-/-	-/-	-/-
50 %		0.531/0.486	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R1 stream	0.938/0.828	0.341/0.297	-/-	-/-	-/-
50 %		0.469/0.414	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-
None	R2 stream	**1.248/1.100**	0.452/0.397	-/-	-/-	-/-
50 %		0.624/0.548	-/-	-/-	-/-	-/-
75 %		0.314/-	-/-	-/-	-/-	-/-
None	R3 stream	**1.421/1.421**	**1.421/1.421**	**1.331/1.172**	0.641/0.641	0.493/0.493

Intended use		Bush ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None		5*	10	15**
	No spray buffer (m)	5	10	5	10	15
50 %	R4 stream	**1.421/1.421**	-/-	0.924/0.924	0.641/0.641	-/-
75 %		-/-	-/-	0.924/0.924	-/-	-/-
None		0.941/0.828	0.341/0.297	-/-	-/-	-/-
50 %		0.469/0.414	-/-	-/-	-/-	-/-
75 %		-/-	-/-	-/-	-/-	-/-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on FOCUS Step 4 calculations and toxicity data for geomean value sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Bulb Vegetables (herbaceous ornamentals) (single/multiple application)

Intended use		Herbaceous ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.052/0.043	-/-	-/-	-/-	-/-
	D6 ditch	0.052/0.044	-/-	-/-	-/-	-/-
	D6 ditch	0.605/1.565	-/-	-/-	-/-	-/-
	R1 stream	0.282/0.920	0.173/0.599	0.116/0.417	-/0.320	-/0.218
	R2 stream	0.180/0.191	0.117/0.123	-/-	-/-	-/-
	R3 stream	0.092/0.309	-/0.202	-/0.141	-/-	-/-
	R4 stream	0.828/1.102	0.539/0.719	0.376/0.501	0.290/0.384	0.197/0.262
RAC 0.29		**PEC/RAC ratio**				
None	D3 ditch	0.179/0.148	-/-	-/-	-/-	-/-
	D6 ditch	0.179/0.152	-/-	-/-	-/-	-/-
	D6 ditch	**2.086/5.397**	-/-	-/-	-/-	-/-

Intended use		Herbaceous ornamentals				
Active substance		Azadirachtin				
Application rate (g/ha)		2 × 30				
Nozzle reduction	Vegetative strip (m)	None	5*	10	15**	20
	No spray buffer (m)	5	5	10	15	20
	R1 stream	0.972/**3.172**	0.597/**2.066**	0.400/**1.438**	-/1.103	-/0.752
	R2 stream	0.621/0.659	0.403/0.424	-/-	-/-	-/-
	R3 stream	0.317/**1.066**	-/0.697	-/0.486	-/-	-/-
	R4 stream	**2.855/3.800**	**1.859/2.479**	**1.297/1.728**	0.993/1.324	0.679/0.903

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.
*The value used for reduction in run-off volume, run-off flux, erosion mass and erosion flux was 0.4, according to the Austrian Environmental Agency AGES.
**The values used for reduction in run-off volume and run-off flux was 0.7, and for erosion mass and erosion flux was 0.9, according to the Austrian Environmental Agency AGES.

After the FOCUS step 4 calculations and refinement based on geomean value for *Chironomus*, the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch, R1 stream and R2 stream; 5 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip
- R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip

Potato

- D6 ditch; 5 m no-spray buffer.
- R1 stream and R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D6 ditch and R2 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- D4 stream, R1 stream and R4 stream: 5 m no-spray buffer.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip with 50% of nozzles reduction or 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch and R2 stream: 5 m no-spray buffer.
- D6 ditch: risk unacceptable. However, this scenario is not relevant under CEU conditions.
- R1 stream and R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

zRMS comment:

zRMS does not agree with the refined risk for Chironomus riparius based on geomean approach based on calculation based on five chronic toxicity studies for the same species *Chironomus riparius* with different representative formulations and for technical. The lowest endpoint should be taken into account according to EFSA Conclusion 2018.

After the FOCUS step 4 calculations and RAC of 0.16 microgram/L the following risk mitigation measures is needed:

Fruiting Vegetables (Tomato)

- D6 ditch; 5 m no-spray buffer.
- R1 stream: 20 m no-spray buffer and 20 m vegetative filter strip
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip
- **R3 stream and R4 stream: risk unacceptable. Therefore, further refinement is needed.**

Potato

- D6 ditch; 5 m no-spray buffer and 5 m vegetative filter strip.
- R1 stream: 15 m no-spray buffer and 15 m vegetative filter strip.
- R3 stream: 20 m no-spray buffer and 20 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D4 stream, D6 ditch, R1 stream and R4 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- R2 stream: 5 m no-spray buffer with 75% of nozzles reduction or 10 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch; 5 m no-spray buffer.
- D6 ditch, R1 stream and R4 stream: risk unacceptable. **Therefore, further refinement is needed.**
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip.
- R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip

In conclusion:

Further refinement of the risk for Chironomus riparius for some scenarios for uses provided in the GAP is needed.
The final risk mitigation measures are needed at MSs level.

For PL for potato the 20 m no-spray buffer and 20 m vegetative filter strip is required to surface water bodies

***Remark from e-fate expert, Section 8**.

Evaluator agrees with modelling carried out by Applicant. Predicted concentrations of azadirachtin and its metabolites in surface water were calculated by the Applicant at Steps 1to 3 and 4 on the basis input parameters from EFSA Journal 2018;16(4):5234, EFSA Journal 2011;9(3):1858 and Addendum 08 (confirmatory data) to the Additional Report of azadirachtin, 2017.
The modelling approach according to the Austrian Environmental Agency AGES should be accepted at national level. Nevertheless, additional simulations may be required by the sMS that do not accept calculations performed using FOCUS models.

9.5.3 Overall conclusions

For all the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for sediment dwelling organisms as characterised by a NOEC for *Chironomus riparius* of 1.6 µg/L in connection with an assessment factor of 10) in several or all FOCUS

Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

After the FOCUS step 4 calculations, risk unacceptable is still detected in R3 and R4 stream scenarios in potatoes. Therefore, a further refinement is needed. A refinement based on a geomean value of **0.29 µg a.s./L** was used. This value was obtained from five chronic studies on *Chironomus* with different representative formulations and with technical from Monograph. After the FOCUS step 4 calculations and refinement based on geomean value for *Chironomus*, the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch, R1 stream and R2 stream; 5 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip
- R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip

Potato

- D6 ditch; 5 m no-spray buffer.
- R1 stream and R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D6 ditch and R2 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- D4 stream, R1 stream and R4 stream: 5 m no-spray buffer.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip with 50% of nozzles reduction or 10 m no-spray buffer and 10 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch and R2 stream: 5 m no-spray buffer.
- D6 ditch: risk unacceptable. However, this scenario is not relevant under CEU conditions.
- R1 stream and R4 stream: 20 m no-spray buffer and 20 m vegetative filter strip.
- R3 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

The results obtained for each intended use with the relevant mitigation measures are proposed below:

- **Fruiting Vegetables (Tomato):** 20 m no-spray buffer and 20 m vegetative filter strip
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 20m to surface water bodies.*

- **Potato:** 10 m no-spray buffer and 10 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 10m to surface water bodies*

- **Ornamentals >50cm:** 5 m no-spray buffer and 5 m vegetative filter strip with 50% of nozzles reduction or 10 m no-spray buffer and 10 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies with 50% of nozzles reduction*
  Or
  Spe3 *- To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 10m to surface water bodies.*

- **Ornamentals <50cm:** 20 m no-spray buffer and 20 m vegetative filter strip.
  Spe3 *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 20m to surface water bodies.*

After the FOCUS step 4 calculations and RAC of 0.16 microgram/L the following risk mitigation measures would be needed:

Fruiting Vegetables (Tomato)

- D6 ditch; 5 m no-spray buffer.
- R1 stream: 20 m no-spray buffer and 20 m vegetative filter strip
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip
- **R3 stream and R4 stream: risk unacceptable.** Therefore, a further refinement is needed.

Potato

- D6 ditch; 5 m no-spray buffer and 5 m vegetative filter strip.
- R1 stream: 15 m no-spray buffer and 15 m vegetative filter strip.
- R3 stream: 20 m no-spray buffer and 20 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D4 stream, D6 ditch, R1 stream and R4 stream; 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.
- R2 stream: 5 m no-spray buffer with 75% of nozzles reduction or 10 m no-spray buffer.
- R3 stream: 15 m no-spray buffer and 15 m vegetative filter strip.

Ornamentals <50 cm

- D3 ditch, D6 ditch; 5 m no-spray buffer.
- D6 ditch, R1 stream and R4 stream: risk unacceptable. Therefore, a further refinement is needed.
- R2 stream: 5 m no-spray buffer and 5 m vegetative filter strip.
- R3 stream: 10 m no-spray buffer and 10 m vegetative filter strip

The final risk mitigation measures should be considered at MSs level.

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

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

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Azadirachtin A	Oral	**LD_{50} > 8.1 µg Azadirachtin A/bee**	EFSA Journal 2018;16(4):5234
Apis mellifera	Azadirachtin A	Contact	**LD_{50} > 11.81 µg Azadirachtin**	EFSA Journal

Species	Substance	Exposure System	Results	Reference
			A/bee	2018;16(4):5234
Apis mellifera	Preparation NeemAzal-T/S	Oral	LD_{50} > 5.9 µg Azadirachtin A/bee	EFSA Journal 2018;16(4):5234
Apis mellifera	Preparation NeemAzal-T/S	Contact	LD_{50} > 21.0 µg Azadirachtin A/bee	EFSA Journal 2018;16(4):5234
Apis mellifera	AZA	Oral	**LD_{50} > 200 µg f.p./bee** (equivalent to >2.65 µg a.s./bee)	KCP 10.3.1.1.1 Parma, P. 2018 B/52/16
Apis mellifera	AZA	Contact	**LD_{50} > 200 µg f.p./bee** (equivalent to >2.65 µg a.s./bee)	KCP 10.3.1.1.2 Parma, P. 2018 B/53/16
Higher-tier studies (tunnel test, field studies)				
In a tunnel test NeemAzal-T/S applied during bee flight at a high rate of 6.0 L/ha had no harmful effects on the brood development and on adult honey bees. Therefore the risk to honey bees is acceptable.				

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to AZA formulation.

9.6.2 Risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for bees from all other intended uses (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 AZA in All crops

Intended use	All crops		
Active substance	Azadirachtin		
Application rate (g/ha)	2 × 30		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	8.1	30	3.70
Contact toxicity	11.81		2.54
Product	AZA		
Application rate (g/ha)	2 × 2882.4*		
Test design	**LD_{50} (lab.) (µg f.p./bee)**	**Single application rate (g f.p./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**

Oral toxicity	>200	2882.4	<14.41
Contact toxicity	>200		<14.41

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*Based on a density of 0.9608 g/mL

zRMS comments:

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

Therefore, the risk assessment has been conducted according to EPPO/OEPP (2003) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)).

Based on the acute risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values for adult bees from exposure of AZA are < 50, indicating un acceptable risk to adult bees. According to EU Reg. 284 /2009, the chronic toxicity test for adult bees, the chronic test for larvae should be provided for authorisation of plant protection product.

However, the final decision of the date of submission these studies by the Applicant should be considered at MSs level.

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

Not relevant.

9.6.3 Effects on bumble bees

No data available.

9.6.4 Effects on solitary bees

No data available.

9.6.5 Overall conclusions

First-tier assessments indicate that no unacceptable risk for bees exposed to the product AZA is expected according to the proposed intended uses.

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 Azadirachtin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

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

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

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	Preparation Azadirachtin 3% (Sipcam)	Laboratory test glass plates (2D) 14 d	**Mortality: Limit, 100 g azadirachtin A/ha: 50 % (corr.)** Reproduction red. : Limit, 100 g azadirachtin A/ha: 92.7 %	EFSA Journal 2018;16(4):5234
Aphidius rhopalosiphi (adults)	Preparation Azadirachtin 3% (Sipcam)	Laboratory test glass plates (2D) 14 d	Mortality: Limit, 100 g Azadirachtin A/ha: 65.7 % (corr.) Reproduction red. : Limit, 100 g azadirachtin A/ha: 54 %	EFSA Journal 2018;16(4):5234
Aphidius rhopalosiphi	Preparation NeemAzal-T/S 1% (Trifolio M-GmbH)	Laboratory test glass plates (2D) 14 d	Mortality: Limit, 57.6 g azadirachtin A/ha: 100 % (corr.)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation Azadirachtin 3% (Sipcam)	Laboratory test glass, 14 d, Limit (100 g/ha as initial)	Mortality corr. : Whole unit sprayed: 79 % Half unit sprayed, food on sprayed part: 63 % Half unit sprayed, food on clean part: 0 % (-21 %)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	NeemAzal 34 %	Extended laboratory test, 26 d, exposure on detached apple leaves, dose response test (6.4 – 150 g Azadirachtin A/ha) 2D	Mortality corr. : 100 % (total mortality) LR_{50} < 6.4g a.s./ha No reproduction assessment, because > 50 % of the adults died	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Azatin Technical-grade Active Ingredient 15.6 %	Extended laboratory test, 36 d, exposure on detached apple leaves, dose response test (6.4 – 150 g Azadirachtin A/ha) 2D	Mortality corr. : 100 % (total mortality) LR_{50} < 6.4g a.s./ha Reproduction (hatching rate) 14.1 g a.s./ha other conc. not tested: 9.11 % (compared to solvent control)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Fortune 13.6 %	Extended laboratory test, 36 d, exposure on detached apple leaves, dose response test (6.4 – 150 g Azadirachtin A/ha) 2D	Mortality corr. : 96.3 % (total mortality) LR_{50}< 6.4 g a.s./ha Reproduction (hatching rate) 6.40 g a.s./ha other conc. not tested : 28.56 % (compared to solvent control)	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea (larvae)	Preparation NeemAzal-T/S 1.09 %	Extended laboratory test, 34 d, exposure on detached apple leaves, dose response test (0.77 – 30 g Azadirach- tin A/ha) 2D	Mortality corr. : 100 % (for 1.9 a.s./ha) LR_{50} ~ 0.77 g a.s./ha Reproduction (hatching rate) 0.77 g a.s./ha other conc. not tested : - 0.12 % (compared to control, value unreproductible)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation NeemAzal-T/S 1.09 %	Extended laboratory test, 34 d, exposure on detached apple leaves, dose response test (0.05 – 1.9 g Azadirach- tin A/ha) 2D	Mortality corr. : 4.0 % (max. value**) LR_{50} > 1.9 g a.s./ha Reproduction (hatching rate): 0.05 g a.s./ha : - 0.1 % 0.12 g a.s./ha : - 7.8 % 0.30 g a.s./ha : - 2.6 % 0.76 g a.s./ha : - 8.8 % 1.90 g a.s./ha : - 7.0 % (compared to control)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation NeemAzal-T/S 1.09 %	Extended laboratory test, 38 d, exposure on detached sweet pepper leaves, freshly applied and aged residues 48.4 g Azadirach- tin A/ha 3D	Mortality corr. : 96.2 % (max. value*) Reproduction (reduction): 14 DAA : 45.3 % 28 DAA: 24.9 % (compared to control) freshly applied and 42 DAA not tested	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation NeemAzal-T/S 1.09 %	Extended laboratory test, 49 d, exposure on detached sweet pepper leaves, freshly applied and aged residues 20.1 g Azadirach- tin A/ha 3D	Mortality corr. : 57.7 % (max. value*) Reproduction (reduction): 21 DAA : - 12.8 % (compared to control) freshly applied not tested	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation OIKOS 2.53 %	Extended laboratory test, 34 d, exposure on detached apple leaves, dose response test 0.77 – 30.0 g Azadirachtin A/ha 2D	Mortality corr. : 100 % (max. value*) LR_{50}: 12.44 g a.s/ha Reproduction (hatching rate): 0.77 g a.s./ha: - 52.67 % 1.90 g a.s./ha: - 30.11 % 4.80 g a.s./ha: - 41.01 % (compared to control) 12 and 30 g a.is/ha not tested	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation OIKOS 2.9 %	Extended laboratory test, 34 d, exposure on detached apple leaves, dose response test 0.02 – 0.77 g Azadirachtin A/ha	Mortality corr. : - 11.5 % (max. value*) LR_{50} > 0.77 g a.s/ha Reproduction (hatching rate): 0.02 g a.s./ha: - 3.9 % 0.05 g a.s./ha: - 4.1 % 0.12 g a.s./ha: - 2.9 % 0.31 g a.s./ha: - 2.7 % 0.77 g a.s./ha: - 4.4 % (compared to control)	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea (larvae)	Preparation OIKOS 2.53 %	Extended laboratory test, 38 d, exposure on detached sweet pepper leaves, freshly applied and aged residues 48.4 g Azadirach- tin A/ha 3D	Mortality corr. : 84.6 % (max. value*) Reproduction (reduction): 14 DAA: not tested 28 DAA: - 16.6 % freshly applied and 42 DAA: 17.40 % (compared to control)	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae)	Preparation OIKOS 2.53 %	Extended laboratory test, 49 d, exposure on detached sweet pepper leaves, freshly applied and aged residues 20.1 g Azadirach- tin A/ha 3D	Mortality corr. : 57.7 % (max. value*) Reproduction (reduction): 21 DAA: 0 % (compared to control) freshly applied not tested	EFSA Journal 2018;16(4):5234
Poecilus cupreus (adults)	Preparation Azadirachtin 3 % (Sipcam)	Laboratory test sand, 14 d, Limit (100 g/ha as initial)	Mortality corr. : 0 %	EFSA Journal 2018;16(4):5234
Poecilus cupreus (adults)	Preparation NeemAzal-T/S 0.4 % [a] (Trifolio M-GmbH)	Laboratory test sand, 14 d, Limit (8 [a] g/ha as initial)	Mortality corr. : 3.3 %	EFSA Journal 2018;16(4):5234
Coccinella septempunctata (larvae)	Preparation NeemAzal-T/S 0.4 % [a] (Trifolio M-GmbH)	Laboratory test glass, 65 d, Limit (12 [a] g/ha as initial)	Mortality corr. : 10.2 % Reproduction reduction: 17 %	EFSA Journal 2018;16(4):5234
Coccinella septempunctata (larvae)	NeemAzal 34 %	Extended laboratory test, 42 d, exposure on detached sweet pepper leaves, dose response test (2.6 – 100 g Azadirach-tin A)/ha) 2D	Mortality corr. : 83.3 % (max. value*) LR_{50}: 15.8 g a.s./ha Reproduction reduction: 2.6 g a.s./ha: 62.6 % 6.4 g a.s./ha: 78.3 % other conc. not tested	EFSA Journal 2018;16(4):5234
Coccinella septempunctata (larvae)	Azatin 15 % Technical product	Extended laboratory test, 42 d, exposure on detached sweet pepper leaves, dose response test (2.6 – 100 g Azadirachtin A/ha) 2D	Mortality corr. : 76.6 % (max. value*) LR_{50}: 25.4 g a.s./ha Reproduction reduction: 2.6 g a.s./ha: 50.7 % 6.4 g a.s./ha: 60.6 % 16 g a.s./ha: 73.4 %	EFSA Journal 2018;16(4):5234
Coccinella septempunctata (larvae)	Fortune Aza Technical Powder 13.6 %	Extended laboratory test, 42 d, exposure on detached sweet pepper leaves, dose response test (2.6 – 100 g Azadirachtin A/ha) 2D	Mortality corr. : 73.3 % (max. value*) LR_{50}: 26.2 g a.s./ha Reproduction reduction: 2.6 g a.s./ha: 43.3 % 6.4 g a.s./ha: 49.7 % 16 g a.s./ha: 74 %	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Coccinella septempunctata (larvae)	Preparation NeemAzal-T/S 1.09 %	Extended laboratory test, 47 d, exposure on detached bean leaves, dose response test (0.08 – 7.00 g Azadirachtin A/ha) 3D	Mortality corr. : 100 % (max. value*) LR_{50}: 1.94 g a.s./ha Reproduction reduction: 0.08 g a.s./ha: 34.58 % 0.23 g a.s./ha: 18.12 % 0.73 g a.s./ha: - 79.53 % 2.26 g a.s./ha: - 108.71 % 7 g a.s./ha not tested	EFSA Journal 2018;16(4):5234
Coccinella septempunctata (larvae)	Preparation OIKOS 2.53 %	Extended laboratory test, 50 d, exposure on detached bean leaves, dose response test (0.15 – 14.00 g Azadirachtin A/ha) 3D	Mortality corr. : 53.33 % (max. value*) LR_{50}: 10.45 g a.s./ha Reproduction reduction: 0.15 g a.s./ha: 9.26 % 0.47 g a.s./ha: 45.37 % 1.46 g a.s./ha: - 2.08 % 4.52 g a.s./ha: - 13.59 % 14 g a.s/ha not tested	EFSA Journal 2018;16(4):5234
Aphidius rhopalosiphi (adults)	Preparation Oikos 2.16 % (Sipcam)	Extended laboratory test, barley, 2 d, 11.3 – 118.4 g a.s./ha	Mortality corr. : 6.9 % (max. value) Reproduction reduction: 12 % (max. value)	EFSA Journal 2018;16(4):5234
Aphidius rhopalosiphi (adults)	Preparation NeemAzal-T/S 1 % (Trifolio M-GmbH)	Extended laboratory test, oat, 2 d, Limit **(57.6 g/ha as initial)**	Mortality corr. : 15 % Reproduction reduction: 5 % beneficial capacity reduction : 19.3 %	EFSA Journal 2018;16(4):5234
Typhlodromus pyri (adults)	Preparation Oikos 2.16 % (Sipcam)	Extended laboratory test, apple leaves, 7 d, **11.3** – 118.4 **g as/ha**	Mortality corr. : 1.8 % (max. value) [b] NOEC Reproduction: 65.8 g azadirachtin A/ha initial	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	azadirachtin A	screening test, 0.154 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 0.96 < 2.40 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	azadirachtin B	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 5.45 g/ha total LR_{50} > 0.96 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	14,15-epoxy-azadiradione	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 12 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	Salannin	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 12 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	3-desacetyl-salannin	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 12 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	substance 8 (see Vol. 4)	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 12 g/ha	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea (larvae 2-3d)	Azadiradione	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 12 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	Nimbin	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} < 0.96 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	6-desacetyl-nimbin	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 6.0 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	11-epi azadirachtin D	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 6.0 g/ha	EFSA Journal 2018;16(4):5234
Chrysoperla carnea (larvae 2-3d)	12 decarbo-metoxy-azadirachtin	screening test, 0.960 – 12.0 g/ha	pre-imaginal LR_{50} > 12 g/ha total LR_{50} > 0.96 < 2.40 g/ha	EFSA Journal 2018;16(4):5234
Aphidius rhopalosiphi (adults)	AZA	Extended study on barley plants (3D)	LR_{50} = n.d. **ER_{50} > 5.77 L f.p./ha (equivalent to 60.0 g a.s./ha)**	KCP 10.3.2.2-01 Lemańska, N. 2019 B/54/16
Typhlodromus pyri	AZA	Extended study on leaf discs (2D)	LR_{50} = 3.6 L f.p./ha (eq. 37.1 g a.s./ha) **ER_{50} = 1.3 L f.p./ha (eq. 13.9 g a.s./ha)**	KCP 10.3.2.2-02 Lemańska, N. 2019 B/55/16
Typhlodromus pyri	AZA	Aged residue study	**Residues aged for:** / **% mortality (7d)** — **5.10 L/ha** / **5.70 L/ha** 0 DAA: 7.23 / 27.71 28 DAA: 6.82 / 18.18 56 DAA: -2.01 / -3.45 0 DAA: When compared to the control group, a fecundity reduction ≤ 50 % was observed at both application rates.	KCP 10.3.2.2-03 Varela, S. 2021 S20-07862
Chrysoperla carnea	AZA	Aged residue study	**Residues aged for:** / **% mortality (7d)** — **5.10 L/ha** / **5.70 L/ha** 0 DAA: 62.82 / 88.85 28 DAA: -3.57 / -3.57 56 DAA: 3.33 / 10.00 28 DAA: When compared to the control group, no significant effects were observed at both application rates.	KCP 10.3.2.2-04 Luna, F. 2021 S20-07864
Coccinella septempunctata	AZA	Aged residue study	**Residues aged for:** / **% mortality (7d)** — **5.10 L/ha** / **5.70 L/ha** 0 DAA: 86.11 / 88.89 28 DAA: 0.00 / 0.00 56 DAA: 0.00 / 0.00 28 DAA: When compared to the control group, no significant effects were observed at both application rates.	KCP 10.3.2.2-05 Varela, S. 2021 S20-07865

Species	Substance	Exposure System	Results	Reference
Field or semi-field tests				
Episyrphus balteatus (larvae)	Preparation NeemAzal-T/S initial 1 % (Trifolio M-GmbH)	semi-field test, bean – gauze tent, 42 d, Limit (57.6 a.s. initial)	Mortality corr. : 49 % Reproduction reduction**: 100 % beneficial capacity reduction**: 100 %	EFSA Journal 2018;16(4):5234
Typhlodromus pyri (adults)	Preparation NeemAzal-T/S 1 % (Trifolio M-GmbH)	Field-viniculture test, 42 d, Limit, 2 applications [c] 1. 12.9 g Azadirachtin A/ha initial 2. 18.9 g Azadirachtin A/ha initial	1. application - 58.7 % damage after 7 d 2. application [c]- no damage after 42 d (inclusive time after 1st application)	EFSA Journal 2018;16(4):5234

1 indicate whether initial or aged residues
2 for preparations indicate whether dose is expressed in units of as or preparation
3 indicate if positive percentages relate to adverse effects or not
a: purity: 4 g/kg – no clear indications whether sum of azadirachtins or amount of azadirachtin A
b: no clear concentration response-relationship
*: corresponding to the highest test concentration
**: 100 % mortality during phase of reproduction
c: 2nd application (18.9 g a.s./ha) 14 d after 1st application (12.9 g a.s./ha)

9.7.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to AZA formulation. According to results from laboratory studies of Monograph, both indicator species show a similar mortality toxicity. Therefore, the Applicant considers that extended exposure on *T.Pyri* is covered by the extended study on *Aphidius.*

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for non-target arthropods from all other intended uses (see 9.1.2).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of AZA in All crops

Intended use	All crops
Active substance/product	Azadirachtin / AZA
Application rate (g/ha)	2 x 30 g a.s./ha / 2 × 3.0 L f.p./ha
MAF	1.7

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Azadirachtin			
Typhlodromus pyri	100 g a.s./ha	51 g a.s./ha	0.51
Test species Higher-tier	**Rate with ≤ 50 % effect** (L/ha)**	**$PER_{in\text{-}field}$ (L/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
AZA			
Aphidius rhopalosiphi	>5.77 L f.p./ha	5.1	Yes
Typhlodromus pyri	1.3 L f.p./ha	5.1	**No**
Test species Higher-tier	**Rate with ≤ 50 % effect (L/ha) at 28 DAA**	**$PER_{in\text{-}field}$ (L/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	5.70 L f.p./ha (at 0 DAA)	5.1	Yes
Chrysoperla carnea	5.70 L f.p./ha (at 28 DAA)	5.1	Yes
Coccinella septempunctata	5.70 L f.p./ha (at 28 DAA)	5.1	Yes

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

* Based on a density of 0.9608 g/mL

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

According to results above, no unacceptable in-field risk was obtained for all crops in both species.

zRMS comments:

The PER-in field values for T.Pyri, Chrysoperla carneaa and Coccinella septempunctata for formulation AZA based on Age residue studies and for extended study for Aphidius rhopalosiphi are below the rate with < 50 % effects.

Therefore, this assessment indicates that AZA poses low risk in-field to non-target arthropods following product application according to the proposed use patterns.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of AZA in potato and ornamentals <50cm

Intended use	Potato and ornamentals <50cm
Active substance/product	Azadirachtin / AZA
Application rate (g/ha)	2 x 30 g a.s./ha / 2 × 3.0 L f.p./ha
MAF	1.7
vdf	10 (2D) / 1 (3D), 5 (2D) [1]

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Azadirachtin					

Typhlodromus pyri	100 g a.s./ha	2.38	0.12 g a.s./ha 0.24	10	0.01 0.024
Test species Higher-tier	**Rate with ≤ 50 % effect** (L/ha)**	**Drift rate**	**PER$_{off-field}$ (L/ha)**	**CF**	**corr. PER$_{off-field}$ below rate with ≤ 50 % effect?**
AZA					
Aphidius rhopalosiphi	>5.77 L f.p./ha	2.38	0.12 L f.p./ha	5	Yes
Typhlodromus pyri	1.3 L f.p./ha	2.38	0.012 L f.p./ha	5	Yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* Based on a density of 0.9608 g/mL
** If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.
[1] The vdf value recommended in Central Zone.

Table 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of AZA in tomato and ornamentals >50cm

Intended use	Tomato and ornamentals >50cm
Active substance/product	Azadirachtin / AZA
Application rate (g/ha)	2 x 30 g a.s./ha / 2 × 3.0 L f.p./ha
MAF	1.7
vdf	10 (2D) / 1 (3D), 5 (2D)[1]

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**PER$_{off-field}$ (g/ha)**	**CF**	**HQ$_{off-field}$ criterion: HQ ≤ 2**
Azadirachtin					
Typhlodromus pyri	100 g a.s./ha	7.23	0.37 g a.s./ha 0.72	10	0.04 0.008
Test species Higher-tier	**Rate with ≤ 50 % effect** (g/ha)**	**Drift rate**	**PER$_{off-field}$ (g/ha)**	**CF**	**corr. PER$_{off-field}$ below rate with ≤ 50 % effect?**
AZA					
Aphidius rhopalosiphi	>5.77 L f.p./ha	7.23	0.37 L f.p./ha 0.074	5	Yes Yes
Typhlodromus pyri	1.3 L f.p./ha	7.23	0.037 L f.p./ha 0.074	5	Yes Yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* Based on a density of 0.9608 g/mL
** If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.
[1] The vdf value recommended in Central Zone.

According to results above, no unacceptable off-field risk was obtained for all crops in both species. Therefore, not any risk mitigation measures are needed.

zRMS comments:

The calculations of the risk assessment for off – field were accepted by zRMS-PL. The PER-off field

values for T.Pyri and Aphidius rhopalosiphi are below the rate with < 50 % effects.

Therefore, this assessment indicates that AZA poses low risk off -field to non-target arthropods following application of product according to the proposed use patterns.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

Not relevant.

9.7.3 Overall conclusions

No in-field and off-field risk to non-target arthropods is expected after the application of AZA according to the proposed GAP.

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

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

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Extract: Azadirachtin techn. (Sipcam)	Mixed into substrate 14 d, acute Quartz sand	LC_{50} >1000 mg azadirachtin A/kg d.w.soil $_{nom}$ LC_{50} >8880 mg/kg d.w.soil extract $_{nom}$	EFSA Journal 2018;16(4):5234
Eisenia fetida	Preparation NeemAzal- TS (Trifolio M-GmbH)	Mixed into substrate 14 d, acute	Content of azadirachtin A in the formulation is not derivable. LC_{50} >1000 mg/kg d.w.soil $_{nom}$ product	EFSA Journal 2018;16(4):5234

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	NeemAzal	Mixed into substrate 14 d, chronic	0 % effect mortality 29 – 34.9 % effect reprod.	EFSA Journal 2018;16(4):5234
Eisenia fetida	AZA	Mixed into substrate 56 d, chronic 10% peat	**NOEC ≥ 1000 mg f.p./kg dw soil (equivalent to ≥ 10.6 mg a.s./kg dw soil)**	KCP 10.4.1.1 Wróbel, A. 2020 G/03/17
Field studies				
Earthworm, field, 13 months - no valid data submitted				
Litter bag test				
No data submitted				

* 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 relevant as there is no deviation to the EU agreed endpoints. Additionally, studies were conducted with AZA and the endpoints were considered for the risk assessment.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses (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 AZA in All crops

Intended use	All crops (onion considered as worst-case for PEC_{soil} calculations)		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AZA	≥ 1000	6.918	144.6
AZA*	≥ 10.6	0.066	160.6

TER values shown in bold fall below the relevant trigger.
* Risk assessment based on an endpoint expressed as mg as/kg dw from AZA study.

A chronic study on *Eisenia fetida* was submitted by the Applicant. After the risk assessment, an acceptable chronic risk was obtained for earthworm. In addition, the Applicant wishes to indicate that the value of TER calculated is far above the trigger of 5 (TER = 144.6).

Therefore, the Applicant considers that an acceptable risk to *Folsomia candida* and *Hypoaspis aculeifer* can be concluded on the basis that low risks to other soil macro-organisms was concluded.

zRMS comments:

The TER_{LT} for ppp for earthworm and other soil macro-organism such as Hypoapsis aculifer are above trigger of 5, indicating acceptable risk.

Therefore, it is concluded that the active substance included in ppp AZA does not pose long-term risk to earthworms and other soil macro- and mesofauna when applied AZA according to the proposed uses rates.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The TER_{lt} value for AZA formulation is higher than the Annex VI trigger value of 5, indicating a low long-term risk to earthworms. The TER calculated is far above the trigger and, therefore, the Applicant considers that an acceptable risk to *Folsomia candida* and *Hypoaspis aculeifer* can be concluded on the basis that low risks to other soil macro-organisms was concluded.

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

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

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Azadirachtin technical Purity: 170 g Azadirachtin A+B /kg Approx. 100 g azadirachtin A	28 d, aerobic Loamy sand, ph: 6.03	5.5 % effect at day 28 (< 25 %) at 480 g azadirachtin technical/ha at 82 g azadirachtin A+B /ha	EFSA Journal 2018;16(4):5234
C-mineralisation	Azadirachtin technical Purity: 170 g Azadirachtin A+B /kg Approx. 100 g azadirachtin A	28 d, aerobic Loamy sand, ph: 6.03	5.3 % effect at day 28 (< 25%) at 480 g azadirachtin technical/ha at 82 g azadirachtin A+B /ha	EFSA Journal 2018;16(4):5234
N-mineralisation	Preparation NeemAzal-TS (1 % azadirachthin A)	28 d, aerobic Silty sand, pH: 6	9.0 % effect at day 28 (< 25 %) at 30 L/ha at 333 g as/ha	EFSA Journal 2018;16(4):5234
C-mineralisation	NeemAzal-TS (1% azadirachthin A)	28 d, aerobic Silty sand, pH: 6	13.0 % effect at day 28 (< 25%) at 30 L/ha at 333 g azadirachtin A/ha	EFSA Journal 2018;16(4):5234
N-mineralisation	AZA	28 d, agricultural soil	-1.8 % deviation from control at 7.8 mg f.p./kg dw soil (equivalent to 0.1 mg a.s./kg dw soil) -7.3% deviation from control at 39.2 mg f.p./kg dw soil (equivalent to 0.5 mg a.s./kg dw soil)	KCP 10.5.1 Dec, W. 2018 G/02/17
C-mineralisation	AZA	28 d, agricultural soil	1.7 % deviation from control at 7.8 mg f.p./kg dw soil (equivalent to 0.1 mg a.s./kg dw soil) 1.5% deviation from control at 39.2 mg f.p./kg dw soil (equivalent to 0.5 mg a.s./kg dw soil)	KCP 10.5.2 Dec, W. 2018 G/01/17

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to AZA formulation.

9.9.2 Risk assessment

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group "All crops" also covers the risk for the soil microorganisms from all other intended uses (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of AZA in All crops

Intended use	All crops (onion considered as worst-case for PEC_{soil} calculations)		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Azadirachtin	480 (at 28 d)	0.066	Yes
AZA	39.2 (at 28 d)	6.918	Yes
AZA*	0.5 (at 28 d)	0.066	Yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Azadirachtin	480 (at 28 d)	0.066	Yes
AZA	39.2 (at 28 d)	6.918	Yes
AZA*	0.5 (at 28 d)	0.066	Yes

* Risk assessment based on an endpoint expressed as mg as/kg dw from AZA study.

zRMS comment:

The risk assessment for soil micro-organism after exposure of active substances has been accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PEC_s for the maximum application rate of active substances and the product AZA.

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for the active substance Azadirachtin and AZA formulation indicated a low risk to soil microorganisms.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with Azadirachtin. Full details

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

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

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

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

Species	Substance	Exposure System	Results	Reference
Zea mays	Preparation NeemAzal-TS (Trifolio M-GmbH)	22 d Preliminary screening data limit-test (single application rate)	30.9 g azadirachtin A/ha nom (2940 g/ha product nom) cause 21.1% effect in reduction biomass (fw). No phytotoxicity obtained	EFSA Journal 2018;16(4):5234
Pisum sativum[d], *Brassica oleracea var. capitata*[d], *Daucus carota*[d], *Helianthus annuus*[d], *Allium cepa*[m] and *Avena sativa*[m]	AZA	14 d, seedling emergence	ER_{50} > 6 L f.p./ha (equivalent to 62.4 g a.s./ha)	KCP 10.6.2-01 Wróbel, A. 2020 G/06/17
Pisum sativum[d], *Brassica oleracea var. capitata*[d], *Daucus carota*[d], *Helianthus annuus*[d], *Allium cepa*[m] and *Avena sativa*[m]	AZA	21 d, vegetative vigour	ER_{50} > 6 L f.p./ha (equivalent to 62.4 g a.s./ha)	KCP 10.6.2-02 Wróbel, A. 2020 G/07/17

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to AZA formulation.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Limit tests at rates up to 30.9 g Azadirachtin A/ha were conducted with NeemAzal-TS (Trifolio M-GmbH) and effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). The limit test rates exceed the highest field application rate in all the intended uses and are thus considered as an indicator for an acceptable risk.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of AZA in potato and ornamentals <50cm

Intended use	Potato and ornamentals <50cm			
Product	AZA			
Application rate (L f.p./ha)	2 × 3.0			
MAF	1.7			
Test species	**ER_{50} (L f.p./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L f.p./ha)**	**TER criterion: TER ≥ 5**
Pisum sativum[d], *Brassica oleracea var. capitata*[d], *Daucus carota*[d], *Helianthus annuus*[d], *Allium cepa*[m] and *Avena sativa*[m]	6.0	2.38	0.12	49.4

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

Table 9.10-3: Assessment of the risk for non-target plants due to the use of AZA in tomato

Intended use	Tomato			
Product	AZA			
Application rate (L f.p./ha)	2 × 3.0			
MAF	1.7			
Test species	**ER_{50} (L f.p./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L f.p./ha)**	**TER criterion: TER ≥ 5**
Pisum sativum[d], *Brassica oleracea var. capitata*[d], *Daucus carota*[d], *Helianthus annuus*[d], *Allium cepa*[m] and *Avena sativa*[m]	6.0	7.23	0.37	16.3

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

According to results above, no unacceptable off-field risk on non-target plants was obtained with AZA formulation for all crops. Therefore, not any risk mitigation measures are needed.

zRMS comment:

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. The deterministic risk based on the ER_{50} of 6000 mL product/ha values and PER_{off}- field is considered acceptable.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not relevant.

9.10.3 Overall conclusions

According to the preliminary screening data using a limit-test at a rate of 30.9 g a.s./ha, no phytotoxicity was observed following the use of Azadirachtin. Since the application rates of the intended uses are lower than those used in the preliminary screening data, it can be considered that Azadirachtin poses a low risk to non-target plants according to the intended uses.

In addition, no unacceptable off-field risk on non-target plants was obtained with AZA formulation.

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

Test type / organism	Endpoint
Activated sludge	NOEC = 1000 mg Neem Azal (34% Azadirachtin A)/ L
Pseudomonas sp.	degradation >25% after 14d (100 mg Azadirachtin A /L + 100 mg Na- benzoate /L)

No inhibition of respiration of activated sewage sludge of >10 % was observed up to the highest tested concentration of 1000 mg Neem Azal (34% Azadirachtin A)/L. NOEC is therefore >1000 mg/L for Azadirachtin. It is not expected that Azadirachtin reaches biological sewage treatment plants at higher concentrations. Therefore, the risk to biological methods of sewage treatment is expected to be low from the intended uses.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	AZA
Common Name	AZA
Classification and proposed labelling	
With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: - Code(s) for hazard pictogram(s): - Signal word: - Hazard statement(s): - Precautionary statement: -

AZA is not classified for environment.

zRMS comment:

No classification for aquatic acute hazard is required as the lowest L(E)C_{50}, i.e. mg a.s./L (*O. mykiss*), is above the threshold value of 1 mg/L.

For the chronic classification, the summation method is applied as no adequate testing data of the chronic toxicity of the preparation to aquatic organisms of all trophic levels are available (NOEC only available with the representative products ~~Oikos~~ for *Chironomus riparius*=0.0016 mg a.s./L). Considering the chronic hazard classification (i.e. Aquatic chronic 1) and the M-factor for chronic toxicity (i.e. 10) assigned to the active substance Azadirachtin A the preparation AZA is classified as Aquatic Chronic 2.

Implications for labelling resulting from ecotoxicological assessment according to Regulation (EC) No 1272/2008:

Hazard pictograms

Signal word

~~Warning~~

Hazard statement

H411: Toxic to aquatic life with long lasting effects

Precautionary statements

P391: Collect spillage

EUH phrases

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

Implications for labelling according to Regulation (EC) No 547/2011:

SP 1 Do not contaminate water with the product or its container.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-01	.	2019	Azadirachtin 1% EC Rainbow Trout, Acute Toxicity Test Study No. W/67/17 GLP, Unpublished	Y	SHARDA Crochem Limited
KCP 10.2.1-02	Czarnecka, M.	2019	Azadirachtin 1% EC *Daphnia magna*, Acute immobilisation test Study No. W/69/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.2.1-03	Czarnecka, M.	2019	Azadirachtin 1% EC *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) Growth inhibition test Study No. W/68/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.1.1.1	Parma, P.	2018	Azadirachtin 1% EC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Study No. B/52/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited

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 10.3.1.1.2	Parma, P.	2018	Azadirachtin 1% EC Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test Study No. B/53/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.2.2-01	Lemańska, N.	2019	An extended laboratory test for evaluating the effects of Azadirachtin 1% EC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) Study No. B/54/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.2.2-02	Lemańska, N.	2019	An extended laboratory test for evaluating the effects of Azadirachtin 1% EC on the predatory mite, *Typhlodromus pyri* (Sch.) Study No. B/55/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.2.2-03	Varela, S.	2021	Azadirachtin 1 % EC: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions. Study No. S20-07862 Trialcamp S.L.U. GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.2.2-04	Luna, F.	2021	Azadirachtin 1 % EC. Toxicity to the green lacewing *Chrysoperla carnea* after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions Study No. S20-07864 Trialcamp S.L.U. GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.2.2-05	Varela, S.	2021	Azadirachtin 1 % EC: Toxicity to the ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions Study No. S20-07865 Trialcamp S.L.U. GLP, Unpublished	N	SHARDA Crochem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1	Wróbel, A.	2020	Azadirachtin 1% EC Earthworm Reproduction Test (*Eisenia andrei*) Study No. G/03/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.5.1	Dec, W.	2018	Azadirachtin 1% EC Soil Microorganisms: Nitrogen Transformation Test Study No. G/02/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.5.2	Dec, W.	2018	Azadirachtin 1% EC Soil Microorganisms: Carbon Transformation Test Study No. G/01/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.6.2-01	Wróbel, A.	2020	Azadirachtin 1% EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test Study No. G/06/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.6.2-02	Wróbel, A.	2020	Azadirachtin 1% EC Terrestial Plant Test: Vegetative Vigour Test Study No. G/07/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	SHARDA Crochem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used • dissolved oxygen concentrations were within the range of 80 – 99% of air saturation value (obligatory above 60% of air saturation value). **Agreed endpoint:** **The 96 h LC_{50}> 100 mg product /L correspond to 96 h LC_{50} >1.04 mg a.s./L**

Reference: KCP 10.2.1 - 01

Report "Azadirachtin 1% EC: Rainbow Trout, Acute Toxicity Test". ……. (2019), Report No. W/67/17. …….

Guideline(s):	Yes, OECD Guideline No. 203 (1992)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Materials and methods

Test item:

Description:	Azadirachtin 1% EC
Production batch:	SCL - 281287
A.i. content:	Azadirachtin: 1.04% w/v

Test system:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Strain:	-
Age:	approximately 6.5 months
Average weight:	0.97 g ± 0.08 g
Average length:	4.3 ± 0.13 cm
Source:	The Culture of Salmonidae Fish in Zawoja (Poland)
Acclimation period:	12 days
Diet:	standard granulated fish food in the amount of 2% of their average body weight per day (standard dry food, Aller Aqua, Denmark)

Experimental conditions:

Temperature:	13.4 – 14.1°C
Dissolved O_2:	80 – 99%
Hardness:	-
pH:	7.01 – 7.31
Light and photoperiod:	16h light and 8h dark.
Loading:	-
Test procedure:	Semi-static with renewal of test solution at 24 h interval
Experimental period:	96h

Test design and treatment

Semi-static with renewal of test solution at 24 h interval (96 hours, one replicate of ten fish for each test item concentration and the control).
According to a range finding test, the following nominal test item concentrations was used 100 mg/L plus a negative control. The fish were observed for toxic signs and mortality after for 96 h.
The concentrations of the active substances were chemically determined using a validated liquid chromatographic method with DAD detection. The concentrations of azadirachtin were chemically determined in samples of all fresh test item concentrations and the control collected at exposure initiation and during each renewal, as well as in samples of all spent test item concentrations and the control during each renewal and at exposure termination. The determined concentrations of azadirachtin in fresh samples were in the range of 96.9 – 102.8% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentrations of azadirachtin in spent samples were in the range of 89.9 – 104.1% of the nominal concentration. Therefore, concentration of azadirachtin was stable under the test conditions.

Results

From the original report:

Table 3. Recovery and precision for azadirachtin the fortified samples (n = 5)

Nominal concentration [mg/L]	Determined concentration of azdirachtin in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
Control	0.000	0.000	--	--	--	0.000	--	0.000	--
0.5	0.469	0.461	0.444	0.480	0.497	0.470	94.1	0.020	4.2
5.0	4.88	4.91	4.97	4.93	4.92	4.92	98.4	0.03	0.7

LoQ = 0.5 mg/L
LoD = 0.05 mg/L
SD – standard deviation
RSD – relative standard deviation

Table 15. Concentration and stability of azadirachtin, definitive test

Nominal test item concentration [mg/L]	**control**	**100**	**Day of sampling**
Nominal concentration of azadirachtin [mg/L]	**control**	**1.04**	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	1.055	**exposure initiation fresh**
% of the nominal concentration	---	99.5	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.953	**The first renewal after 24 h - spent**
% of the nominal concentration	---	89.9	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	1.086	**The first renewal after 24 h - fresh**
% of the nominal concentration	---	102.5	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.975	**The second renewal after 48 h - spent**
% of the nominal concentration	---	92.0	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	1.090	**The second renewal after 48 h - fresh**
% of the nominal concentration	---	102.8	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	1.104	**The third renewal after 72 h -spent**
% of the nominal concentration	---	104.1	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	1.028	**The third renewal after 72 h -fresh**
% of the nominal concentration	---	96.9	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.967	**At exposure termination**
% of the nominal concentration	---	91.2	

Mortality is not observed in the test item concentration and the control.

- <u>The endpoint values determined on the basis of the nominal test item concentrations and mortality of fish are given below:</u>
  The LC_{50}/96 h value is higher than to 100 mg/L

- <u>The endpoint values determined on the basis of nominal concentrations of azadirachtin:</u>
  The LC_{50}/96 h value is higher than to 1.04 mg/L

Conclusion
The LC_{50} value of Azadirachtin 1% EC at 96 h was higher than 100 mg/L.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • The percentage of immobilized on of Daphnia magna in the control was 0.0% (criterion: not more than 10%), • The dissolved oxygen concentrations in the test vessels were within the range of 7.4 – 8.4 mg/L (criterion: not less than 3 mg/L) **Agreed endpoint:** **The 48 h EC_{50} >100 mg test item/L 1.06 mg s.a./L.**

Reference:	KCP 10.2.1-02
Report	"Azadirachtin 1% EC: *Daphnia magna*, Acute Immobilization Test", Małgorzata Czarnecka (2019), Study Code: W/69/17. Institute of Industrial Organic Chemistr Branch Pszczyna
Guideline(s):	OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

Immobilization of young *Daphnia magna* (< 24 hours old) exposed to Azadirachtin 1% EC, (batch No. SCL – 281287) was investigated during a 48-hour test in static design. A single test item concentration of 100 mg/L plus the control were used according to a range finding test. Four replicates of each test item concentration and the control with five *Daphnia magna* per replicate were used. The *Daphnia magna* were observed for immobilization after 24 and 48 hours of exposure.

The concentrations of azadirachtin were determined using a validated high performance liquid chromatographic method with DAD detection. Samples of the test item concentration and the control collected at exposure initiation and at exposure termination were chemically determined.
At exposure initiation, the determined concentration of azadirachtin was 98.2% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. At exposure termination, the determined concentration o azadirachtin was 84.6% of the nominal concentration. Therefore, the concentration of azadirachtin was stable under test conditions.

The endpoint values were determined based on the nominal test item concentration and nominal concentration of azadirachtin in the test item.

Results

Preliminary test
In the preliminary test four test item concentrations of 0.1, 1, 10 and 100 mg/L plus the control were used for 48 hours in static system.
In the test, in the control and in all test item concentrations no immobilisation and no abnormal behaviour of Daphnia magna was observed during exposure.

Definitive test
In the definitive test *Daphnia magna* was exposed to the test item concentration of 100 mg/L plus the control for 48 hours in a static system. The results are summarized in the table below.

Table 10.2.1-02-01 Immobilization of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilized *Daphnia magna*								Total of immobilized *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		R1	R2	R3	R4	R1	R2	R3	R4	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
100	20	0	0	0	0	0	0	0	0	0	0

Validity criteria

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

- the percentage of immobilized on of Daphnia magna in the control was 0.0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 7.4 – 8.4 mg/L (criterion: not less than 3 mg/L). s.

From original test

Table 2. Recovery level and precision of azadirachtin in fortified samples (n = 5)

Nominal concentration [mg/L]	Determined concentration of azadirachtin in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
control	0.00000	0.00000	--	--	--	0.00000	--	0.00000	--
0.002	0.00189	0.00204	0.00174	0.00199	0.00183	0.00190	94.9	0.00012	6.4
0.2	0.182	0.191	0.185	0.179	0.184	0.184	92.1	0.004	2.4

LOQ = 0.002 mg/L
LOD = 0.0005 mg/L
SD – standard deviation
RSD – relative standard deviation

Table 7. Concentration and stability of azadirachtin - definitive test

Nominal test item concentration [mg/L]	Control	100	Day of sampling
Nominal concentration of azadirachtin [mg/L]	--	1.06	
Average determined concentration of azadirachtin (n=3) in samples collected [mg/L]	< LoD	1.041	at exposure initiation
% of the nominal concentration	---	98.2	
Average determined concentration of azadirachtin (n=3) in samples collected [mg/L]	< LoD	0.897	at exposure termination
% of the nominal concentration	---	84.6	

LOQ = 0.002 mg/L
LOD = 0.0005 mg/L
--- no value

Conclusion

The EC_{50} value for Azadirachtin 1% EC at 48 hours was higher than 100 mg test item/L or 1.06 mg Azadirachtin/L.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • There was an increase in cell concentration of the negative control culture by a factor of 203.3 which is more than the required factor limit of at least 16 at the end of the test. • The mean coefficient of variation for section by section specific growth rates in the negative control cultures during the course of the test was 14.9 % which is within the required limit of 35%. • The coefficient of variation of average growth rate between replicate cultures of negative control was 3.3% which is within the required limit of 7 %. **Agreed endpoints:** The endpoint values determined on the basis of the nominal test item concentrations: ErC_{50}/72 h >1000.0 mg/L. LOEC/72 h value for growth rate = 62.5 mg/L. NOEC/72 h value for growth rate =15.6 mg/L. EyC_{50}/72 h value =32.34 mg/L. LOEC/72 h value for yield = 62.5 mg/L.

	NOEC/72 h value for yield = 15.6 mg/L. The endpoint values determined on the basis of the active substance concentrations: • Azadirachtin: ErC50/72 h >10.6 mg/L. LOEC/72 h value for growth rate = 0.663 mg/L. NOEC/72 h value for growth rate = 0.165 mg/L. EyC50/72 h value = 0.34 mg/L (95% confidence interval 0.16 – 0.68). LOEC/72 h value for yield =0.663 mg/L. NOEC/72 h value for yield =0.165 mg/L. The endpoint values based on geometric means of determined concentrations of azadirachtin: ErC50/72 h >6.520 mg/L. LOEC/72 h value for growth > 0.250 mg/L. NOEC/72 h value for growth rate =0.057 mg/L. EyC50/72 h value = 0.13 mg/L (95% confidence interval 0.06 – 0.29). LOEC/72 h value for yield = 0.250 mg/L. NOEC/72 h value for yield = 0.057 mg/L.

Reference:	KCP 10.2.1-03
Report	"Azadirachtin 1% EC: Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) Growth Inhibition Test", Małgorzata Czarnecka,. (2019), Study code: W/68/17. Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

The growth of the green algae *Pseudokirchneriella subcapitata* exposed to the test item Azadirachtin 1% EC (batch No. SCL – 281287) was investigated during a 72-hour test. The test was performed in conical flasks of 250 mL capacity covered with cotton plugs. Each of them contained 50 mL of a given test item concentration and the control. The initial density of the algae was 1 x 10^4 cells/mL. A range finding test was carried out using 0.1, 1, 10 and 100 mg/L. According to it, the following test item concentrations were used on the definitive test: 1000, 250, 62.5, 15.6 and 3.9 mg/L plus the control. Three replicates were used for each test item concentration, whereas six replicates were used for control.
The concentrations of the test were determined using a validated high performance liquid chromatographic method with DAD detection. The active ingredient concentration analysis in all test concentrations showed that the percent agreement with the nominal concentration was 98.6 to 108.9 % at the start of the test and 2.0 to 44.7 % at the end of the test (72 hour). The results show that concentrations of azadirachtin were not stable under test conditions.
The endpoint values were determined based on the nominal concentrations of the test item, the nominal concentrations of azadirachtin and geometric means of determined concentrations of azadirachtin.Results

Preliminary test

The preliminary test was performed using the test item concentrations: 0.1, 1, 10 and 100 mg/L plus control.

Table 10.2.1-03.1 Average cell biomass, preliminary test (non-GLP)

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	-5.3*	-26.4*
1.0	-3.7*	-16.6*
10.0	3.6	16.6
100.0	15.4	51.9

Definitive test

In the definitive test, the algae, *Pseudokirchneriella subcapitata*, with an initial cell density of 1 x 10^4 cells/mL were exposed to the test item concentrations: 3.9, 15.6, 62.5, 250.0 and 1000.0 mg/L plus the control. The results are summarized in the table below.

Table 10.2.1-03.2 Growth rate and yield inhibition, definitive 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
3.9	-3.7*	-20.9*
15.6	16.3	58.6
62.5	16.2	58.5
250.0	29.8	80.0
1000.0	31.8	81.8

*** Inhibition is lower than 0.0%, which means that the algal cells density at exposure termination was higher than in the control.**

Validity criteria

In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- There was an increase in cell concentration of the negative control culture by a factor of 203.3 which is more than the required factor limit of at least 16 at the end of the test.
- The mean coefficient of variation for section by section specific growth rates in the negative control cultures during the course of the test was 14.9 % which is within the required limit of 35%.
- The coefficient of variation of average growth rate between replicate cultures of negative control was 3.3% which is within the required limit of 7 %.

Results from original report copied by zRMS to the summary:

Table 2. Recovery level and precision of azadirachtinin fortified samples (n = 5)

Nominal concentration [mg/L]	Determined concentration of azadirachtin in replicates [mg/L]					Average [mg/L]	Recovery [%]	SD [mg/L]	RSD [%]
	1	2	3	4	5				
control	0.00000	0.00000	--	--	--	0.00000	--	0.00000	--
0.002	0.00189	0.00204	0.00174	0.00199	0.00183	0.00190	94.9	0.00012	6.4
0.2	0.182	0.191	0.185	0.179	0.184	0.184	92.1	0.004	2.4

LOQ = 0.002 mg/L
LOD = 0.0005 mg/L
SD – standard deviation
RSD – relative standard deviation

Table 12. Concentration and stability of azadirachtin - definitive test

Nominal test item concentration [mg/L]	control	3.9	15.6	62.5	250.0	1000.0	Day of sampling
Nominal concentration of azadirachtin [mg/L]	control	0.041	0.165	0.663	2.650	10.6	
Geometric mean of determined concentration of azadirachtin* [mg/L]	control	0.012	0.057	0.250	1.602	6.520	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.045	0.163	0.678	2.669	11.291	at exposure initiation fresh
% of the nominal concentration	---	108.9	98.6	102.3	100.7	106.5	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.025	0.142	0.577	2.191	7.731	after 24 h
% of the nominal concentration	---	60.5	85.9	87.1	82.7	72.9	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	0.0145	0.050	0.287	1.160	4.903	after 48 h
% of the nominal concentration	---	35.1	30.2	43.3	43.8	46.3	
Mean concentration (n=3) of azadirachtin [mg/L]	<LoD	<LoD	0.004	0.013	0.981	4.737	at exposure termination
% of the nominal concentration	---	---	2.4	2.0	37.0	44.7	

LOQ = 0.002 mg/L
LOD = 0.0005 mg/L
--- no value
*the geometric mean of the determined azadirachtin concentrations were calculated according to the formula given in the OECD Series on Testing and Assessment No. 23. Annex 2. page 50 [10]

Table 15. Growth rate endpoint values based on the nominal concentrations of azadirachtin - definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>10.6	>10.6	>10.6
E_rC_{20}	>10.6	>10.6	1.07 (0.52 – 1.85)
E_rC_{10}	>10.6	4.35 (1.31 – 10.24)	0.12 (0.02 – 0.28)
LOEC	>10.6	10.6	0.663
NOEC	≥10.6	2.65	0.165

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

Table 16. Yield endpoint values based on the nominal concentrations of azadirachtin - definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>10.6	>10.6 (7.5 – >10.6*)	0.34 (0.16 – 0.68)
E_rC_{20}	>10.6	1.71 (0.2 – 3.8)	0.04 (0.01 – 0.10)
E_rC_{10}	>10.6	0.49 (0.01 – 1.42)	0.02 (0.00 – 0.05)
LOEC	>10.6	10.6	0.663
NOEC	≥10.6	2.65	0.165

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
*value obtained above the tested concentrations range

Table 17. Growth rate endpoint values based on the geometric means of determined concentrations of azadirachtin - definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>6.520	>6.520	>6.520
E_rC_{20}	>6.520	>6.520	0.49 (0.22 – 0.90)
E_rC_{10}	>6.520	2.45 (0.61 – 6.19)	0.04 (0.01 – 0.10)
LOEC	>6.520	6.520	0.250
NOEC	≥6.520	1.602	0.057

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

Table 18. Yield endpoint values based on the geometric means of determined concentrations of azadirachtin - definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>6.520	>6.520 (4.6 – >6.520*)	0.13 (0.1 – 0.3)
E_rC_{20}	>6.520	0.86 (0.06 – 2.08)	0.01 (0.00– 0.03)
E_rC_{10}	>6.520	0.21 (0.00 – 0.70)	0.003 (0.00 – 0.01)
LOEC	>6.520	6.520	0.250
NOEC	≥6.520	1.602	0.057

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
*value obtained above the tested concentrations range

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations:
The concentration causing a 50% inhibition of the growth rate of *Pseudokirchneriella subcapitata*, i.e. the ErC_{50}/72 h value is higher than 1000.0 mg/L.
The LOEC/72 h value for growth rate is 62.5 mg/L.
The NOEC/72 h value for growth rate is 15.6 mg/L.
The concentration causing a 50% inhibition of yield of *Pseudokirchneriella subcapitata*, i.e. the EyC_{50}/72 h value is 32.34 mg/L.
The LOEC/72 h value for yield is 62.5 mg/L.
The NOEC/72 h value for yield is 15.6 mg/L.

The endpoint values determined on the basis of the active substance concentrations:

- Azadirachtin:

The concentration causing a 50% inhibition of the growth rate of Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), i.e. the ErC50/72 h value is higher than 10.6 mg/L.

The LOEC/72 h value for growth rate is 0.663 mg/L.
The NOEC/72 h value for growth rate is 0.165 mg/L.
The concentration causing a 50% inhibition of yield of Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), i.e. the EyC50/72 h value is 0.34 mg/L (95% confidence interval 0.16 – 0.68).
The LOEC/72 h value for yield is 0.663 mg/L.
The NOEC/72 h value for yield is 0.165 mg/L.

The endpoint values based on geometric means of determined concentrations of azadirachtin:
The concentration causing a 50% inhibition of the growth rate of Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), i.e. the ErC50/72 h value is higher than 6.520 mg/L.
The LOEC/72 h value for growth rate is 0.250 mg/L.
The NOEC/72 h value for growth rate is 0.057 mg/L.
The concentration causing a 50% inhibition of yield of Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), i.e. the EyC50/72 h value is 0.13 mg/L (95% confidence interval 0.06 – 0.29).
The LOEC/72 h value for yield is 0.250 mg/L.
The NOEC/72 h value for yield is 0.057 mg/L.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:

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

- The average mortality for the control was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- The 24-hour LD_{50} of the reference item (dimethoate) was 0.12 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee)

Agreed endpoints:

Dose		Nº of tested bees	Mortality after 48 h		LD_{50}	
			Total			
[µg /bee]	[µg a.i./bee]		[no.]	[%]	[µg /bee]	[µg a.i./bee]
0.0 (Control)		30	1	3.3	Above 200	Above 2.65

	12.5	0.17	30	0	0.0		
	25.0	0.33	30	1	3.3		
	50.0	0.66	30	0	0.0		
	100.0	1.33	30	0	0.0		
	200.0	2.65	30	0	0.0		

Reference:	KCP 10.3.1.1.1
Report	"Azadirachtin 1% EC. Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test". Paweł Parma, 2018, Study code B/52/16
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	According to study plan, study B/52/16 should be completed in March 2018, but it was completed in April 2018, which had no impact on the results
GLP:	Yes
Acceptability:	Yes

Materials and methods

The acute oral toxicity study of Azadirachtin 1% EC (batch number: SCL-19345) was conducted to determine the LD_{50} values for honeybees. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee and a control (0.0 µg/bee). The range of doses was selected on the basis of the preliminary test results. Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of a 50% sucrose solution, containing the test item at the doses enumerated above, using a micropipette. During the entire experiment, the insects were caged in groups of 10.
The general condition of the test honeybees and the reliability of the test conducted on them were controlled using the recommended reference item - dimethoate.
After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4 hours after the beginning of the treatment and then every 24 hours after the beginning of the treatment. The acute oral toxicity test ended after the 48-hour exposure.

Results

Table 10.3.1.1.1-01: Acute oral toxicity on honeybees (*Apis mellifera L.*)

Dose		**Nº of tested bees**	**Mortality after 48 h**		**LD_{50}**	
			Total			
[µg /bee]	**[µg a.i./bee]**		**[no.]**	**[%]**	**[µg /bee]**	**[µg a.i./bee]**
0.0 (Control)		30	1	3.3	Above 200	Above 2.65
12.5	0.17	30	0	0.0		
25.0	0.33	30	1	3.3		
50.0	0.66	30	0	0.0		
100.0	1.33	30	0	0.0		
200.0	2.65	30	0	0.0		

Findings

- The mortality in the test item treatments after 48 hours was lower than 50% when compared to the control.
- The median lethal doses of Azadirachtin 1% EC (LD_{50}) after 24 and 48 hours of the exposure are higher than the highest dose used in the study, i.e. 200 µg test item/bee.
- No sublethal toxicity effects (behavioural abnormalities) such as excitement (uncoordinated movement, increased activity, intensive cleaning) or any signs of paralysis with respect to the test item and the control were observed over the 48 hours exposure.
- The reduction in food consumption (sucrose solution) during 48 h ranged from (- 7.63) to 20.79 % when compared to the control. The negative values show that the treated groups consumed more than the control group.

Validity criteria

The following validity criteria were met during the test:

- The average mortality for the control was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- The 24-hour LD_{50} of the reference item (dimethoate) was 0.12 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee)

Conclusion

The median lethal doses (LD_{50}/24 h and LD_{50}/48 h contact) are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee (>2.65 µg a.i./honeybee).

No behavioural abnormalities or any signs of paralysis with respect to the test item and the control were observed over the 48 hours exposure.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:

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

- The average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%).
- The 24 hour LD_{50} of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Agreed endpoints:

Dose		**Nº of tested bees**	**Mortality after 48 h**		**LD_{50}**	
			Total			
[µg /bee]	**[µg a.i./bee]**		**[no.]**	**[%]**	**[µg /bee]**	**[µg a.i./bee]**
0.0 (Control)		30	0	0.0	above 200.0	above 2.65
12.5	0.17	30	0	0.0		
25	0.33	30	0	0.0		
50	0.66	30	0	0.0		
100	1.33	30	0	0.0		

	200	2.65	30	0	0.0		

Reference: KCP 10.3.1.1.2

Report "Azadirachtin 1% EC. Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test", Paweł Parma, 2018, Study code B/53/16

Guideline(s): OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)

Deviations: According to study plan, study B/53/16 should be completed in March 2018, but it was completed in April 2018, which had no impact on the results.

GLP: Yes

Acceptability: Yes

Materials and methods

The acute contact toxicity study of Azadirachtin 1% EC (batch No. SCL-19345) was conducted to determine the effects on honeybees. Five doses of the test item were used. These included: 12.2, 25.0, 52.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary test results. The test item was diluted in distilled water and applied to the dorsal part of thorax using a microapplicator. The volume was 1 µL/bee. During the entire experiment, the insects were caged in groups of 10 under controlled conditions of the temperature and the humidity.
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.

Results

Table 10.3.1.1.2-01: Acute contact toxicity on honeybees (*Apis mellifera* L.)

Dose		**Nº of tested bees**	**Mortality after 48 h**		**LD_{50}**	
			Total			
[µg /bee]	**[µg a.i./bee]**		**[no.]**	**[%]**	**[µg /bee]**	**[µg a.i./bee]**
0.0 (Control)		30	0	0.0	above 200.0	above 2.65
12.5	0.17	30	0	0.0		
25	0.33	30	0	0.0		
50	0.66	30	0	0.0		
100	1.33	30	0	0.0		
200	2.65	30	0	0.0		

Findings

- Mortality of the control group after 48 hours of exposure was 0%.
- Mortality of the treated groups was lower than 50% when compared to the control.
- No sublethal toxicity effects (behavioural abnormalities) such as excitement (uncoordinated movement, increased activity, intensive cleaning) or any signs of paralysis with respect to the test item and the control were observed over the 48 hours exposure.

Validity criteria

The following validity criteria were met during the test:

- The average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%).
- The 24 hour LD_{50} of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Conclusion

The median lethal doses (LD_{50}/24 h and LD_{50}/48 h contact) are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee (>2.65 µg a.i./bee).

With respect to the test results, it can be concluded that the test item, Azadirachtin 1% EC had no adverse effect on mortality of honeybees (*Apis mellifera* L.).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

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

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

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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • After 48 hours, mortality in 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 5.0 mL/ha was 70.0% (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 29.2 (criterion: a minimum of 5.0 mummies/female), • All wasps in the control group gave offspring (criterion: a maxi-

mum of 2 females giving no offspring).

Agreed endpoints:

Study group [application rate] [L/ha]	Tested wasps [no.]	Mortality							
		Dead wasps [no.]						Total	
		Replicates							
		I	II	III	IV	V	VI	[no.]	[%]
Control [0.0]	**30**	0	0	0	0	0	0	**0**	**0.0**
Azadirachtin 1% EC									
0.06	**30**	1	1	0	0	0	0	**2**	**6.7**
0.15	**30**	0	0	0	0	0	3	**3**	**10.0**
0.37	**30**	0	1	5	0	1	0	**7**	**23.3**$^{+}$
0.92	**30**	0	0	0	0	0	0	**0**	**0.0**
2.31	**30**	0	0	0	0	0	0	**0**	**0.0**
5.77	**30**	1	0	1	2	1	0	**5**	**16.7**
LR_{50}	n.d.								
$NOER_{mortality}$	n.d.								

Reference:	KCP 10.3.2.2-01
Report:	"An extended laboratory test for evaluating the effects of Azadirachtin 1% EC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez)". Natalia Lemańska, 2019, B/54/16. Institute of Industrial Organic Chemistry Branch Pszczyna.
Guideline(s):	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., 2000; Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

SUMMARY

The extended laboratory test involved the evaluation of the effects of the test item, Azadirachtin 1% EC on mortality and fecundity of the parasitic wasp, Aphidius rhopalosiphi. Six rates of the test item were used in the definitive test i.e., 0.06, 0.15, 0.37, 0.92, 2.31 and 5.77 L/ha.

Adult female wasps were exposed to the test item applied to barley plants. Observations of settling behaviour were made during the initial 3 hours of exposure. The aims were to determine repellent effects of Azadirachtin 1% EC and to check if the test insects had contact with barley plants sprayed with the test

item. Settling behaviour of females from each replicate was observed five times. Mortality was determined 2, 24, and 48 hours after the introduction of the wasps to the test arenas.
Females which survived the 48-hour exposure to Azadirachtin 1% EC and the ones from the control group were evaluated for fecundity. Fifteen female wasps from the each group treated with Azadirachtin 1% EC and the control were individually introduced into the fecundity units containing barley plants infested with the aphid, Rhopalosiphum padi. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.

Mortality after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.

To verify the sensitivity of the biological test system and the precision of the test procedure, Bi 58 Top 400 EC (400 g dimethoate/L), which is an insecticide, was used as a reference item. The rate of the reference item was 5.0 mL/ha (2.0 g dimethoate/ha). The control group was treated with distilled water.

Materials and methods:

Test item:	name: Azadirachtin 1% EC; content: 1.04% w/v; batch no.: SCL-281287; manufacturing date: January 9th, 2018; expiry date: January 8th, 2020.
Biological test system:	the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae.
– age:	adult females (24 - 48 hours after emerging from mummies)
– source:	a laboratory-bred culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented by a commercial breeder
Experimental design:	8 study groups: - a control group (0.0 L/ha) - Azadirachtin 1% EC at the rate of 0.06 L/ha - Azadirachtin 1% EC at the rate of 0.15 L/ha - Azadirachtin 1% EC at the rate of 0.37 L/ha - Azadirachtin 1% EC at the rate of 0.92 L/ha - Azadirachtin 1% EC at the rate of 2.31 L/ha - Azadirachtin 1% EC at the rate of 5.77 L/ha - Bi 58 Top 400 EC at the rate of 5.0 mL/ha mortality assessment: 6 replicates/group; 5 females/replicate fecundity assessment: 15 replicates/group; 1 females/replicate
Test conditions:	
– temperature:	18-21°C
– relative air humidity:	63-84%
– photoperiod:	16 hours light (mortality assessment and oviposition: 1475 lx; fecundity assessment: 6247 lx): 8 hours dark
Statistical analyses:	Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity, Duncan test, Probit analysis using max. likelihood regression, Chi2 2x2 Table Test with Bonferroni Correction, Williams Multiple Sequential t-test Procedure. – LR_{50} value
Endpoints:	– fecundity reduction (Pr) of the surviving female wasps exposed to Alpha-cypermethrin 10% EC, recorded 12 days after the oviposition period

RESULTS AND DISCUSSION:

The validity criterion concerning mortality was met, because mortality of the control group was 0.0% (criterion: a maximum of 10.0%) after 48 hours of exposure.
Mortality of the wasps exposed to Azadirachtin 1% EC at the rates of 0.06, 0.15, 0.37, 0.92, 2.31 and 5.77 L/ha was 6.7, 10.0, 23.3, 0.0, 0.0, 16.7%, respectively. At the significance level of 0.05, there were statistically significant differences in mortality between the wasps exposed to the test item at rate of 0.37 L/ha and the control group. At the significance level of 0.05, there were statistically significant differences in mortality between the wasps exposed to the test item at the rate of 0.37 and the control group (Chi2 2x2 Table Test with Bonferroni Correction, $p< 0.05$). Mortality of the wasps exposed to Bi 58 Top 400 EC at the rate of 5.0 mL/ha was 70.0% after 48 hours. Therefore, the validity criterion was met. The results showed that the insects were sensitive to dimethoate.
The fecundity assessment showed that the mean number of mummies per female in the control group was 29.2. As for the number of mummies/female in the group treated with Azadirachtin 1% EC at the rates of 0.06, 0.15, 0.37, 0.92, 2.31, 5.77 L/ha was 28.8, 31.1, 25.5, 26.7, 15.4 and 20.4, respectively. Fecundity reduction (Pr) caused by Azadirachtin 1% EC at the rate of 0.06, 0.15, 0.37, 0.92, 2.31, 5.77 L/ha was equal to 1.4, (-6.4), 12.6, 8.4, 47.3 and 30.1%, respectively. The negative value means that in the tested rate there was higher number of mummies than in the control group. At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item and the control group (Williams Multiple Sequential t-test Procedure, $p> 0.05$).

Table 5. Mortality of *A. rhopalosiphi* after 48 hours – definitive test

Study group [application rate] [L/ha]	Tested wasps [no.]	Mortality							
		Dead wasps [no.]						Total	
		Replicates							
		I	II	III	IV	V	VI	[no.]	[%]
Control [0.0]	**30**	0	0	0	0	0	0	**0**	**0.0**
Azadirachtin 1% EC									
0.06	**30**	1	1	0	0	0	0	**2**	**6.7**
0.15	**30**	0	0	0	0	0	3	**3**	**10.0**
0.37	**30**	0	1	5	0	1	0	**7**	**23.3**$^{+}$
0.92	**30**	0	0	0	0	0	0	**0**	**0.0**
2.31	**30**	0	0	0	0	0	0	**0**	**0.0**
5.77	**30**	1	0	1	2	1	0	**5**	**16.7**
LR_{50}	n.d.								
$NOER_{mortality}$	n.d.								
[mL/ha]	**Bi 58 Top 400 EC**								
5.0	**30**	4	2	5	5	2	3	**21**	**70.0**

n.d.: the value could not be determined due to mathematical reasons, it may be assumed that it is higher than the highest rate used in the study (> 5.77 L of Azadirachtin 1% EC/ha)
$^{+}$: statistically significant difference

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:
– after 48 hours, mortality in 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 5.0 mL/ha was 70.0% (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 29.2 (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).

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

- mortality of the control group was 3.3% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 98.3% on day 7 of exposure (criterion: from 50 to 100%

Agreed endpoints:

Study group [application rate]	**Parameter (endpoint)**				
	Mortality		**Reproduction**		
Test item [L/ha]	**Total [%]**	**LR_{50}**	**Mean number of eggs/female (Rr) [no.]**	**Reproduction reduction Pr [%]**	
Control (0.0)	3.3	-	4.2	-	
Azadirachtin 1% EC					
0.06	0.0[a]	3.6 (2.9 – 4.5)* [L t.i./ha]	4.1	2.2	
0.15	0.0[a]		3.3	21.4	
0.37	1.7[a]		3.6	13.7	
0.92	10.3[a+]	37.1 (30.6 – 47.2) [g a.i./ha]	2.3[+]	46.1	
2.31	20.7[a+]		1.6[+]	62.2	
5.77	75.9[a+]		-	-	
$NOER_{mortality}$			**$NOER_{reproduction}$**		

+: statisticaly significant difference
*: 95% confidence limit
a.i.: active ingredient
t.i.: test item
u

Reference: KCP 10.3.2.2-02

Report: "An extended laboratory test for evaluating the effects of Azadirachtin 1% EC on the predatory mite, *Typhlodromus pyri* (Sch.)". Natalia Lemańska, 2019, B/55/16. Institute of Industrial Organic Chemistry Branch Pszczyna.

Guideline(s): ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)

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

SUMMARY

The aim of the laboratory test was to evaluate the effects of the test item, Azadirachtin 1% EC on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).

In consultation with the Sponsor, it was agreed that, a preliminary range - finding test will not be conducted and it was decided to use six rates of the test item in the definitive test. These were 0.06, 0.15, 0.37, 0.92, 2.31 and 5.77 L/ha.

The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (*Pinus* sp.). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and all groups treated with the test item were made after 8, 11, and 14 days of the treatment.

Mortality of *T. pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.

To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Bi 58 Top 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Materials and methods:

Test item:	name: Azadirachtin 1% EC; content: 1.04% w/v; batch no.: SCL-281287; manufacturing date: January 9th, 2018; expiry date: January 8th, 2020.
Biological test system:	the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: Phytoseiidae)
– age:	24-hour-old protonymphs
– source:	a laboratory culture at the ŁUKASIEWICZ RESEARCH NETWORK – INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY BRANCH PSZCZYNA; the culture was augmented by a commercial breeder
Experimental design:	8 study groups: - a control group (0.0 L/ha) - Azadirachtin 1% EC at the rate of 0.06 L/ha - Azadirachtin 1% EC at the rate of 0.15 L/ha - Azadirachtin 1% EC at the rate of 0.37 L/ha - Azadirachtin 1% EC at the rate of 0.92 L/ha - Azadirachtin 1% EC at the rate of 2.31 L/ha - Azadirachtin 1% EC at the rate of 5.77 L/ha - Bi 58 Top 400 EC at the rate of 9.0 mL/ha number of replicates: 3; number of mites in each replicate: 20
Test conditions:	
– temperature:	23 – 27°C
– relative air humidity:	60 – 86%

– photoperiod: 16 hours light (1067 lux): 8 hours dark

Statistical analyses: Probit analysis using max. likelihood regression, Step-down Cochran Armitage test procedure, ShapiroWilk's Test, Levene's Test, Dunnett's Multiple t-test Procedure

Endpoints:
– mite mortality after 7 days of the treatment
– LR_{50} and $NOER_{mortality}$
– reproduction reduction (Pr) after 14 days of the treatment
– ER_{50} and $NOER_{reproduction}$

RESULTS AND DISCUSSION:
In the definitive test, mortality of the control group after 7 days of exposure was 3.3%. After 7 days of exposure to Azadirachtin 1% EC at the rates of 0.06, 0.15, 0.37, 0.92, 2.31 and 5.77 L/ha, the *T. pyri*, corrected mortality percentages were equal to 0.0, 0.0, 1.7, 10.3, 20.7 and 75.9%, respectively.

There were statistically significant differences in mortality between groups treated with the test item at rates of 0.92, 2.31 and 5.77 L/ha and the control group (Step-down Cochran-Armitage test procedure, $p > 0.05$).

On the basis of the obtained results the endpoints regarding mortality could be determined. The LR_{50} value is equal to 3.6 L of Azadirachtin 1% EC/ha (37.4 g a.i./ha). The $NOER_{mortality}$ value is equal to 0.37 L/ha (3.8 g a.i./ha).

After 7 days of exposure to Bi 58 Top 400 EC at the rate of 9.0 mL/ha, corrected mortality was 98.3%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

It should be noticed that Azadirachtin 1% EC has an effect on escape in *Typhlodromus pyri*.

The mean reproduction rate (Rr) in the control group was 4.2 eggs/female. The mean Rr after 14 days of exposure to Azadirachtin 1% EC at rates 0.06, 0.15, 0.37, 0.92 and 2.31 L/ha were 4.1, 3.3, 3.6, 2.3 and 1.6 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by at the rates of 0.06, 0.15, 0.37, 0.92 and 2.31 L/ha were 2.2, 21.4, 13.7, 46.1 and 62.2%, respectively. At the significance level of $\alpha \leq 0.05$, there were statistically significant differences in reproduction between the group treated with the test item at rates of 0.92 and 2.31 L/ha and the control group (Dunnett's Multiple t-test Procedure, $|t| > |t^*|$).

On the basis of the obtained results the endpoints regarding reproduction could be determined. The ER_{50} value is equal to 1.3 L/ha (13.5 g a.i./ha). The $NOER_{reproduction}$ value is equal to 0.37 L/ha (3.8 g a.i./ha). Based on the results it can be stated that Azadirachtin 1% EC has an adverse effect on mortality and fecundity of the tested organisms.

Study group [application rate]	**Parameter (endpoint)**	
	Mortality	**Reproduction**

Test item [L/ha]	Total [%]	LR_{50}	Mean number of eggs/female (Rr) [no.]	Reproduction reduction Pr [%]	ER_{50}
Control (0.0)	3.3	-	4.2	-	-
Azadirachtin 1% EC					
0.06	0.0[a]	3.6 (2.9 – 4.5)* [L t.i./ha]	4.1	2.2	1.3 (0.2 – 9.4)* [L t.i./ha]
0.15	0.0[a]		3.3	21.4	
0.37	1.7[a]		3.6	13.7	
0.92	10.3[a+]	37.1 (30.6 – 47.2) [g a.i./ha]	2.3[+]	46.1	13.9 (1.9 – 97.6) [g a.i./ha]
2.31	20.7[a+]		1.6[+]	62.2	
5.77	75.9[a+]		-	-	
$NOER_{mortality}$			**$NOER_{reproduction}$**		
Bi 58 Top 400 EC					
Reference item [mL/ha]			9.0		
Total mortality			98.3a		

a: the control response of 3.3% was compensated using Abbott's equation
+: statisticaly significant difference
*: 95% confidence limit
a.i.: active ingredient
t.i.: test item

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:
– mortality of the control group was 3.3% on day 7 of exposure (criterion: a maximum of 20%).
– corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 98.3% on day 7 of exposure (criterion: from 50 to 100%)
– the mean number of eggs per female in the control group was 4.2 (required: ≥ 4 eggs per female)

Comments of zRMS:

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

- Maximum mortality in the control group was 17.0 % (exposure of 0 DAA1).
- Minimum mortality (corrected to control) in the toxic reference was 100 % (every exposure of 0, 28 and 56 DAA1).
- Actual minimum cumulative mean number of eggs per female in the control was 5.04 eggs per female (exposure 28 DAA1).

Agreed endpoints:

Azadirachtin 1 % EC **Aging residue periods studied: 0, 28 and 56 DAA1 (days after the test item application)**		
.10 [L of formulated product/ha] (63.70 g Azadirachtin /ha)	5.70 [L of formulated product/ha] (71.20 g Azadirachtin /ha)	
Effects less than 50 % (compared to the control)		
From 0 DAA1 (fresh dried spray residues)	From 0 DAA1 (fresh dried spray residues)	
From 0 DAA1 (fresh dried spray residues)	From 0 DAA1 (fresh dried spray residues)	
No significant effects (compared to the control)		
From 0 DAA1 (fresh dried spray residues)	From 56 DAA1 (aged residue for 56 days)	
From 0 DAA1 (fresh dried spray residues)	From 28 DAA1 (aged residue for 28 days)	

Reference:	KCP 10.3.2.2-03
Report	"Azadirachtin 1 % EC: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions". Sara Varela, 2021. Study code: S20-07862. Trialcamp S.L.U.
Guideline(s):	IOBC (Blümel et al., 2000) modified, Grimm C. et al. (2001), Oomen P.A. (1988) and Pia Ternes et al. (2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The objective of the study was to determine the effects of freshly applied and aged spray deposits of the test item Azadirachtin 1 % EC on the mortality and reproduction on the predatory mite *Typhlodromus pyri* under extended laboratory conditions on apple trees (*Malus domestica* (Borkh), Rosaceae). The effects were evaluated 0, 28 and 56 days after the application of the rates of 5.10 and 5.70 L of formulated product (FP)/ha (equivalent to 63.70 and 71.20 g of azadirachtin /ha).

Material and methods

Test item: Azadirachtin 1 % EC: content: Azadirachtin A [1 % w/v (10 g/L) / 1.3 % w/w (12.4904 g/L)]; Batch No.: SCL-280919; manufacturing date: October 10th, 2020; expiry date: October 09th, 2022.

Reference item: Dimethoate 40% w/v EC: content: dimethoate 400.0 g/L / 409 g/L

Biological test system: *Typhlodromus pyri* Scheuten
– Age: protonymphs (≤ 24 hours old)
– Source: Commercial supplier or stock culture.

Experimental design:

Application code[a]	Treatment group[b]	Application rate [L FP/ha] [c]	Application rate [g a.i./ha] [d]
A1	C	0 [Applied with tap water]	
	T1	5.10	63.70 [g azadirachtin /ha]
	T2	5.70	71.20 [g azadirachtin /ha]
	R	0.45 [e]	184.05 [g dimethoate /ha]
A2	R	0.45 [e]	184.05 [g dimethoate /ha]
A3	R	0.45 [e]	184.05 [g dimethoate /ha]

[a] A1 (day 0), A2 (28 DAA1; days after the application of the test item), A3 (56 DAA1): Semi-field applications
[b] C: control (tap water); T1, T2: test item (Azadirachtin 1 % EC); R: reference item (Dimethoate 40 % w/v EC)
[c] Rate of the test item and the reference item in L of formulated product (FP) per ha
[d] Equivalent active ingredients (a.i.) based on the analysed content: "T1, T2": Azadirachtin 12.4904 g/L and density: 0.9608 g/mL.; "R": Dimethoate 409 g/L
[e] Rate of 0.45 L/ha for the Reference Item (in accordance with Good Agricultural Practices), applied before each exposure.

5 replicates/treatment; 20 individuals/treatment
Four plots with 16 potted apple trees per plot were disposed in one row with approximately 0.5 m distance between pots for the application, simulating a disposal in field. One plot for negative control (tap

water control), one plot for the positive control (toxic reference) and one plot for each rate of the test item were used.

Test dilutions (prepared with tap water) were applied using a compressed air knapsack sprayer simulating commercial application in field at a volume of 800 L/ha with one nozzle "Albuz Hollow Cone Yellow ATR-80" and working at a pressure of 4 bars.

After application, trees were maintained under outdoor conditions in an opened enclosure equipped with a polycarbonate roof closed only when it rains to provide natural ageing conditions and to avoid the washing off by rain. The required reference item rate was obtained in a similar way but pipetting the required formulated.

Test conditions

– Temperature: 24.7 – 25.4 ° C
– Relative air humidity: 80.3 – 88.9 %
– Photoperiod: 16 hours light (1376 - 1991 lux): 8 hours dark

Statistics: Chi² 2x2 Test (0, 28 and 56 DAA1) with mortality (dead + escaped individuals) at 7 d (one sided greater, $\alpha = 0.05$) were used to detect significant differences between mortality data of the test item and the control groups in the exposures (bioassays) of 0, 28 and 56 DAA1.

Reproduction was statistically studied with results in the exposures of 0, 28 and 56 DAA1. At 0, 28 and 56 DAA1, reproduction data met normality (Shapiro-Wilk's Test) and homoscedasticity (Levene's Test). Therefore, STUDENT-t-test for Homogeneous Variances with cumulative offspring/female at 14 d (one-sided smaller, $\alpha = 0.05$) was used to detect significant differences between fecundity data of the test item and the control groups.

Endpoints:

- To study the mortality at 7 days after exposure (lethal effect) to residues on leaves for the following periods: 0 (fresh and dry residue) and 28 and 56 ± 1 days after the test item application (DAA1); actual periods were 0, 28 and 56 DAA1.
- To study the fecundity of the survivor females during 7 days following exposure to residues on leaves for the aforementioned ageing periods.
- The ageing period of the residue at the tested rates with effects below 50%, relative to the control, was determined.

Results

In the control group, mortality below 20 % was achieved at every exposure (actual maximum 17.00 % at 0 DAA1) and an acceptable reproductive capacity ≥ 4.0 eggs/female (minimum: 5.04 eggs/female at 28 DAA1) was assessed over a further 7 days of each exposure. The toxic reference item caused above 50 % mortality (corrected relative to control) in the exposures of 0, 28 and 56 DAA1 (100 % mortality at every exposures).

Based on the results of the present study, residues of the test item "Azadirachtin 1 % EC" applied at the rate of 5.10 L formulated product (FP)/ha caused mortality less than 50 % compared to the control from the exposure with fresh and dried spray residues at 0 DAA1.

At the rate of 5.10 L FP/ha, mortality was not significantly different to the control in any exposure (0, 28 and 56 DAA1). Residues of the test item "Azadirachtin 1 % EC" applied at the rate of 5.70 L FP/ha caused mortality less than 50 % compared to the control from the exposure with fresh and dried spray residues at 0 DAA1. At the rate of 5.70 L FP/ha, mortality was significantly different to the control in the

exposures of 0 and 28 DAA1 and it was not significantly different to the control group in the exposure of 56 DAA1 (Chi^2 2x2 Test, $\alpha = 0.050$; one-sided greater).
Reduction on reproduction of *Typhlodromus pyri* at the rates of 5.10 L FP/ha and 5.70 L FP/ha compared to the control was lower than 50 % from the exposure 0 DAA1. At the rate of 5.10 L FP/ha, reproduction was not significantly different from the control in any exposure (0, 28 and 56 DAA1). At the rate of 5.70 L FP/ha, reproduction was significantly different from the control with fresh and dried spray residues at 0 DAA1 (STUDENT-t test at 0DAA1, one-sided smaller, $\alpha = 0.05$) and it was not significantly different from the exposure 28 DAA1.

Exposure of 0 DAA1 (days after the test item application)					
Treatment group	**Rate [L FP/ha][a]**	**Mean mortality[b] [%]**	**Corrected mortality [%]**	**Reproduction[c] [eggs/female]**	**Reduction in reproduction rate [%]**
Control (tap water)	---	**17.00**	---	**5.70**	---
Azadirachtin 1 % EC	**5.10**	**23.00**	**7.23**	**5.03**	**11.90**
Azadirachtin 1 % EC	**5.70**	**40.00 [sd]**	**27.71**	**3.56 [sd]**	**37.63**
Dimethoate 40 % w/v EC	**0.45**	**100.00**	**100.00**	---	---

Exposure of 28 DAA1 (days after the test item application)					
Treatment group	**Rate [L FP/ha][a]**	**Mean mortality[b] [%]**	**Corrected mortality [%]**	**Reproduction[c] [eggs/female]**	**Reduction in reproduction rate [%]**
Control (tap water)	---	**12.00**	---	**5.04**	---
Azadirachtin 1 % EC	**5.10**	**18.00**	**6.82**	**5.48**	**-8.84**
Azadirachtin 1 % EC	**5.70**	**28.00 [sd]**	**18.18**	**5.30 [sd]**	**-5.10**
Dimethoate 40 % w/v EC	**0.45**	**100.00**	**100.00**	---	---

Exposure of 56 DAA1 (days after the test item application)					
Treatment group	**Rate [L FP/ha][a]**	**Mean mortality[b] [%]**	**Corrected mortality [%]**	**Reproduction[c] [eggs/female]**	**Reduction in reproduction rate [%]**
Control (tap water)	---	**13.00**	---	**6.06**	---
Azadirachtin 1 % EC	**5.10**	**11.25**	**-2.01**	**5.34**	**11.85**
Azadirachtin 1 % EC	**5.70**	**10.00**	**-3.45**	**5.16**	**14.95**
Dimethoate 40 % w/v EC	**0.45**	**100.00**	**100.00**	---	---

[a] Rate in L of formulated product (FP)/ha
[b] "sd": statistically significant increase compared to the control (Chi²-2x2 Test at 0 DAA1 and 28 DAA1, one-sided greater, $\alpha = 0.05$)
[c] "sd": statistically significant decrease compared to the control (Student-t Test at 0 DAA1, one-sided smaller, $\alpha = 0.05$)
[d] Negative value means increase in reproduction rate in comparison to the control group

Test validity criteria

All mortality and reproduction tests were considered to be valid as:

- Maximum mortality in the control group was 17.0 % (exposure of 0 DAA1).
- Minimum mortality (corrected to control) in the toxic reference was 100 % (every exposure of 0, 28 and 56 DAA1).
- Actual minimum cumulative mean number of eggs per female in the control was 5.04 eggs per female (exposure 28 DAA1).

Conclusion

Azadirachtin 1 % EC **Aging residue periods studied: 0, 28 and 56 DAA1 (days after the test item application)**		
Rates	5.10 [L of formulated product/ha] (63.70 g Azadirachtin /ha)	5.70 [L of formulated product/ha] (71.20 g Azadirachtin /ha)
Effects less than 50 % (compared to the control)		

7-d Mortality < 50%	From 0 DAA1 (fresh dried spray residues)	From 0 DAA1 (fresh dried spray residues)
7-14 d Reduction Fecundity < 50%	From 0 DAA1 (fresh dried spray residues)	From 0 DAA1 (fresh dried spray residues)
No significant effects (compared to the control)		
Mortality	From 0 DAA1 (fresh dried spray residues)	From 56 DAA1 (aged residue for 56 days)
Fecundity	From 0 DAA1 (fresh dried spray residues)	From 28 DAA1 (aged residue for 28 days)

Comments of zRMS:

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

- Maximum mortality in the control was 10.34 % (exposure of 0 DAA1).
- Mortality (corrected to control) in the toxic reference was 100% (all exposures).
- The minimum mean number of eggs laid per female per day in the control group was 35.10 (exposure of 56 DAA1) and the mean hatching rate in the control group was 100 % (exposures of 28 and 56 DAA1).

Agreed endpoints:

Azadirachtin 1 % EC Aging residue periods studied: 0, 28 and 56 DAA1 (days after the application of the test item)			
Rates [a]	**Endpoints (DAA1; days after the application of the test item)**		
	Mortality ≤ 50 % [b]	**Fecundity ≥ 15 eggs/female/day**	**Fertility; hatching rate ≥ 70%**
5.10 L FP/ha	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)
5.70 L FP/ha	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)
No significant effects (compared to the control)			
Rates [a]	**Mortality**	**Reproduction [c]**	
5.10 L FP/ha	From 28 DAA1 (28-day-old residues)	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1	
5.70 L FP/ha	From 28 DAA1 (28-day-old residues)	No impact on reproduction in accordance with the validity criteria for the control group 28 DAA1	

[a] Rate in L of formulated product /ha
[b] Corrected mortality to the control
[c] Reproduction was evaluated only qualitatively and no statistical analysis was performed

Reference:	KCP 10.3.2.2-04
Report	"Azadirachtin 1 % EC. Toxicity to the green lacewing *Chrysoperla carnea* after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions". Francisco Luna, 2021. Study code: S20-07864. Trialcamp S.L.U.
Guideline(s):	IOBC (Vogt, H. et al., 2000) modified for the use of natural substrate
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The objective of the study was to evaluate the extent and persistence of effects on the survival and reproduction of the aphid predator *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae) after exposure to fresh and aged residues of the formulation "Azadirachtin 1 % EC" on apple trees (*Malus domestica* (Borkh), Rosaceae), applied at two rates, 5.10 and 5.70 L of formulated product (FP)/ha.

Material and methods

Test item: Azadirachtin 1 % EC: content: azadirachtin: 10 g/L / 12.4904 g/L (1.3 % w/w); Batch No.: SCL-280919; manufacturing date: October 10th, 2020; expiry date: October 09th, 2022.

Reference item: Dimethoate 40% w/v EC: content: dimethoate 400.0 g/L / 409 g/L

Biological test system: *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)
– Age: life stage at start of exposure: larvae (2-3 days old)
– Source: SAUTTER & STEPPER GmbH, Rosenstr. 19, 72119 Ammerbuch - Germany

Experimental design:

Application code[a]	Treatment group	Application rate [a] [L FP/ha]	Application rate [a] [g a.i./ha]
A1	C	0 [Applied with tap water]	
	T1	5.10[a]	63.70 [g azadirachtin /ha]b
	T2	5.70[a]	71.20 [g azadirachtin /ha]b
	R	0.45	184.05 [g dimethoate /ha]c
A2	R	0.45	184.05 [g dimethoate /ha]c
A3	R	0.45	184.05 [g dimethoate /ha]c
An	R	0.45	184.05 [g dimethoate /ha]c

C: Control; T1-T2: Test item; R: Reference item; A1, A2, A3, An: Semi-field application
[a] Rates of the test item proposed by the Sponsor's Representative in L of formulated product (FP) per ha
[b] Equivalent active ingredients (a.i.) based on the analysed content: azadirachtin 1.3 % w/w, and density: 0.9608 g/mL. Values are rounded to two decimals
[c] Active ingredient (a.i.) of the reference item based on the analysed content (dimethoate 409 g/L) and a rate of 0.45 L of formulated product (FP)/ha (in accordance with Good Agricultural Practices). It will be applied before each exposure

Apple trees (*Malus domestica* (Borkh), Rosaceae) were used for trial purposes. Four plots were used with 16 potted plants per plot: one plot for every treatment; water treated control, test item at two rates and toxic reference. The treated plot size was 20 m^2 (10 m x 2 m) for the treatments and the pots were arranged in one crop row (0.5 m between plants).

Application was performed using a compressed air knapsack sprayer and one nozzle "Albuz Hollow Cone Yellow ATR-80" simulating an application in field (volume 800 L/ha), working at pressure of 4 bar and applying the plants outdoors. After application, plants were maintained under outdoor conditions with the use of a translucent roof to cover the crop when it rains to provide natural aging conditions and to avoid the washing-off by rain. The reference item was applied at the same time as the test item. Moreover, the reference item was applied at each aging period using a similar method, a compressed air knapsack sprayer. The same 16 pots with apple plants treated on day 0 were applied with the reference item at 28 and 56 DAA1.

At each aging period, 30 leaves were sampled per treatment group to assemble the test units. Then, thirty larvae, 2-3 days old, were individually confined within test units. The larvae were continuously exposed to the residue on the leaves until at least, 5 days after formation of pupae. Direct treatment effects (mor-

tality) and any change in behaviour, with respect to the control, were assessed until the adults emerged. The number of dead larvae and the number of pupae that failed to develop into adults were combined and the value was used to calculate the total juvenile mortality. Any larvae that escaped from the test units or was killed by mechanical influences during the study were excluded from the mortality calculations.

The sub-lethal effects on the reproductive performance of the emerging adults were evaluated for the treatments of the test item and for the control group in the performed exposures of 28 and 56 DAA1 since the corrected mortality compared to the control was less than 50 % in the test item treatments. Reproduction was evaluated with 2 synchronisations of egg laying (24 h periods) in a week to calculate the eggs per female and day (fecundity rate) and the larvae emerging from eggs to calculate the percentage of viable eggs (fertility rate).

Test conditions

– Temperature: 24.6 – 27.0 °C
– Relative air humidity: 73.2 – 94.6 % *
– Photoperiod: 16 hours light (1120 - 4941 lux): 8 hours dark

*: Humidity above 90 % was not registered for more than 2 hours continuously.

Statistics: Fisher`s Exact Binomial Test (α = 0.050; one-sided greater) was used to detect significant differences between mortality data of the test item groups and the control in the exposures of 0 and 56 DAA1. Mortality in the exposure of 28 DAA1 was not statistically calculated since no mortality was observed in the test item groups.

A quantitative judgement (statistical analysis) of the reproduction data was not conducted. The obtained value with fecundity and fertility were compared to the threshold values for control: fecundity (mean number of eggs per female per day) ≥ 15, and fertility (mean hatching rate) ≥ 70%.

Endpoints:

- Percentage mortality, the mean number of eggs/female/day (fecundity) and the hatching rate (fertility).
- The objective of the study was to evaluate the extent and persistence of effects on survival and reproduction of the formulation Azadirachtin 1 % EC to the green lacewing *Chrysoperla carnea*. The effects were evaluated with the rates of 5.10 and 5.70 L of formulated product (FP)/ha under extended laboratory conditions.

Results

In the control group, cumulative mortality below 20 % was achieved at every exposure (actual maximum 10.34 %), and an acceptable reproductive capacity (minimum: 35.10 eggs/female/day and 100 % hatching rate) was assessed. The toxic reference item caused above 50 % mortality (corrected relative to control) at every exposure (actual 100 %).

Based on the results of the present study performed on *Chrysoperla carnea*, residues of the test item Azadirachtin 1 % EC applied at the rates of 5.10 and 5.70 L of formulated product (FP)/ha caused mortality greater than 50 % (corrected to the control) and significantly different to the control (Fisher's Exact Binomial Test, one-sided greater, α = 0.05) when larvae were exposed the day of the application (exposure 0 DAA1), and caused less than 50 % mortality on aged residues from day 28 (28 and 56 DAA1).

The mean number of eggs laid per female per day in the test item groups was always ≥ 15 (actual between 32.00 and 43.46 eggs/female/day) and the mean hatching rate was always ≥ 70 % (actual 100 %). These values are not considered test item related in accordance with the validity criteria for the control group.

Exposure of 0 DAA1 (days after the test item application)					
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality[b] [%]**	**Corrected mortality [%]**	**Fecundity [eggs/female/day]**	**Fertility mean hatching rate [%]**
Control (tap water)	---	10.34	--	Not studied	
Azadirachtin 1 % EC	5.10	66.67 [sd]	62.82		
Azadirachtin 1 % EC	5.70	90.00 [sd]	88.85		
Dimethoate 40 % w/v EC	0.45	100	100		
Exposure of 28 DAA1 (days after the test item application)					
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality [%]**	**Corrected mortality [%]**	**Fecundity [eggs/female/day]**	**Fertility mean hatching rate [%]**
Control (tap water)	---	3.45	--	39.23	100
Azadirachtin 1 % EC	5.10	0.00	-3.57	33.34	100
Azadirachtin 1 % EC	5.70	0.00	-3.57	32.00	100
Dimethoate 40 % w/v EC	0.45	100	100	Not studied	
Exposure of 56 DAA1 (days after the test item application)					
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality [%]**	**Corrected mortality [%]**	**Fecundity [eggs/female/day]**	**Fertility mean hatching rate [%]**
Control (tap water)	---	0.00	--	35.10	100
Azadirachtin 1 % EC	5.10	3.33	3.33	43.46	100
Azadirachtin 1 % EC	5.70	10.00	10.00	38.63	100
Dimethoate 40 % w/v EC	0.45	100	100	Not studied	

[a] Rate of the test / reference items in L of formulated product (FP) per ha
[b] [sd] =statistically significant increased compared to control (Fisher`s Exact Binomial Test, α=0.050, one-sided greater)
[c] Negative values indicate a decrease in mortality compared to the control

Test validity criteria

All mortality and reproduction tests were considered to be valid as:

- Maximum mortality in the control was 10.34 % (exposure of 0 DAA1).
- Mortality (corrected to control) in the toxic reference was 100% (all exposures).
- The minimum mean number of eggs laid per female per day in the control group was 35.10 (exposure of 56 DAA1) and the mean hatching rate in the control group was 100 % (exposures of 28 and 56 DAA1).

Conclusion

Azadirachtin 1 % EC **Aging residue periods studied: 0, 28 and 56 DAA1** **(days after the application of the test item)**			
Rates [a]	**Endpoints** **(DAA1; days after the application of the test item)**		
	Mortality ≤ 50 % [b]	**Fecundity ≥ 15 eggs/female/day**	**Fertility; hatching rate ≥ 70%**
5.10 L FP/ha	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)
5.70 L FP/ha	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)	From 28 DAA1 (28-day-old residues)
No significant effects (compared to the control)			
Rates [a]	**Mortality**	**Reproduction [c]**	
5.10 L FP/ha	From 28 DAA1 (28-day-old residues)	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1	
5.70 L FP/ha	From 28 DAA1 (28-day-old residues)	No impact on reproduction in accordance with the validity criteria for the control group 28 DAA1	

[a] Rate in L of formulated product /ha
[b] Corrected mortality to the control
[c] Reproduction was evaluated only qualitatively and no statistical analysis was performed

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • The maximum cumulative mortality in the control group was ≤ 30 % (actual maxi-

mum value: 10.00 % in the exposure of 0 DAA1).

- The cumulative mortality in the toxic reference group (corrected mortality) was between 50 % and 100% (actual minimum value: 100.00 % in every exposure).

- The mean number of fertile eggs per female per day in the control group was ≥ 2 (actual minimum: 20.09 in the exposure of 56 DAA).

Agreed endpoints:

Azadirachtin 1 % EC **Aging residue periods studied: 0, 28 and 56 DAA1 (days after the test item application)**		
Rates	5.10 [L of formulated product/ha] (63.70 g Azadirachtin /ha)	5.70 [L of formulated product/ha] (71.20 g Azadirachtin /ha)
Effects less than 50 % (compared to the control)		
Mortality < 50%	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
Fertile eggs per female per day < 2	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
No significant effects (compared to the control)		
Mortality	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
Fertile eggs per female per day < 2	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1

Reference:	KCP 10.3.2.2-05
Report	"Azadirachtin 1 % EC: Toxicity to the ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions". Sara Varela, 2021. Study code: S20-07865. Trialcamp S.L.U.
Guideline(s):	IOBC (Schmuck et al., 2000) modified for the use of natural substrate
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The objective of the study was to evaluate the extent and persistence of effects on the survival and reproduction of the aphid predator *Coccinella septempunctata* L. under extended laboratory conditions on apple trees (*Malus domestica* (Borkh), Rosaceae).

Material and methods

Test item: Azadirachtin 1 % EC: content: Azadirachtin A [1 % w/v (10 g/L) / 1.3 % w/w (12.4904 g/L)]; Batch No.: SCL-280919; manufacturing date: October 10th, 2020; expiry date: October 09th, 2022.

Reference item: Dimethoate 40% w/v EC: content: dimethoate 400.0 g/L / 409 g/L

Biological test system: *Coccinella septempunctata* L. (Coleoptera, Coccinellidae)

– Age: life stage at start of exposures: larvae (3-4 days old). The larvae were fed with aphids of the species *Acyrthosiphon pisum ad libitum*. Adults were provided with aphids (same species as used for the larvae), honey-water solution (1:1 w/w) and a mixture of unspecified pollen types.

– Source: Stock culture from adults supplied by a commercial supplier or from stock culture.

Experimental design:

Application code [a]	Treatment group [b]	Application rate [L FP/ha] [c]	Application rate [g a.i./ha] [d]
A1	C	0 [Applied with tap water]	
	T1	5.10	63.70 [g azadirachtin /ha]b
	T2	5.70	71.20 [g azadirachtin /ha]b
	R	0.45 [e]	184.05 [g dimethoate /ha]c
A2	R	0.45 [e]	184.05 [g dimethoate /ha]c
A3	R	0.45 [e]	184.05 [g dimethoate /ha]c

[a] A1 (day 0), A2 (28 DAA1; days after the application of the test item), A3 (56 DAA1): Semi-field applications
[b] C: control (tap water); T1, T2: test item (Azadirachtin 1 % EC); R: reference item (Dimethoate 40 % w/v EC)
[c] Rate of the test item and the reference item in L of formulated product (FP) per ha
[d] Equivalent active ingredients (a.i.) based on the analysed content: "T1, T2": Azadirachtin 12.4904 g/L and density: 0.9608 g/mL.; "R": Dimethoate 409 g/L
[e] Rate of 0.45 L/ha for the Reference Item (in accordance with Good Agricultural Practices), applied before each exposure

Apple trees (*Malus domestica* (Borkh), Rosaceae) of the variety GOLDEN were used for the trial exposures. Four plots were used with 16 potted trees per plot: one plot for water treated control, one plot for each rate of the test item and one plot for the toxic reference. The treated plot size was 20 m^2 (10 m x 2 m) for the treatments and the pots were arranged in one crop row (0.5 m between plants).

Application was performed using a compressed air knapsack sprayer and one nozzle "Albuz Hollow Cone Yellow ATR-80" simulating an application in field (volume 800 L/ha), working at pressure of 4 bars and applying the trees outdoors. After application, plants were maintained under outdoor conditions with the use of a translucent roof to cover the crop when it rains to provide natural ageing conditions and to avoid the washing-off by rain. The reference item was applied at the same time as the test item. Moreover, the reference item was applied at each ageing period using a similar method, a compressed air knapsack sprayer; the same 16 pots with apple trees on day 0 were applied with the reference item in 28 and 56 DAA1.

At each aging period, at least 40 leaflets from different plants per treatment group were sampled in order to assemble the test units. Then, forty larvae, 3-4 days old, were individually confined within test units. The larvae were continuously exposed to the residue on the leaves until they moult to adults. Direct treatment effects (mortality) and any change in behaviour, with respect to the control, were assessed until the adults emerged. Mortality assessments were carried out daily, at least every working day, and the number of dead larvae/pupae was recorded together. Pupation and hatching of the adults were recorded. The number of dead larvae and the number of pupae that fail to develop into adults were combined in order to calculate the total juvenile mortality.

Reproduction was not possible to be studied with fresh and dried residue at 0 DAA1 since the observed mortality (corrected to control) was above the 50 %. However, reproduction was studied at further exposures after the 28 and 56 DAA1.

Test conditions

– Temperature: 24.6 – 27.0 °C
– Relative air humidity: 73.2 – 94.6 % *
– Photoperiod: 16 hours light (1002 - 2261 lux): 8 hours dark

*: Humidity above 90 % was not registered for more than 2 hours continuously, not considered deviation.

Statistics: Fisher`s Exact Binomial Test (0 DAA1) with mortality at 21 d (one-sided greater, α = 0.05) was used to detect significant differences between mortality data of the test item and the control groups in the exposure (bioassays) of 0 DAA1. No statistical analyses were needed to be performed at the exposures 28 and 56 DAA1 since 0 % of mortality were detected at the two rates of the test item.

A quantitative judgment (statistical analysis) of the reproduction data was not conducted.

Endpoints:

- Percentage mortality, the mean number of eggs/female/day (fecundity), the percentage of fertile eggs (hatching rate) and the mean number of fertile eggs/female/day.
- The objective of the study was to evaluate the extent and persistence of effects on survival and reproduction of the formulation Azadirachtin 1 % EC to the ladybird *Coccinella septempunctata* L. The effects were evaluated with the rates of 5.10 and 5.70 L of formulated product (FP)/ha under extended laboratory conditions.

Results

Azadirachtin 1 % EC applied to apple trees caused mortality above 50 % and statistically significant lethal effects on *Coccinella septempunctata* at the test item rates of 5.10 and 5.70 L of formulated product/ha with fresh and dry residues (exposure of 0 DAA) when compared to the control group. Mortality with 28 and 56-d old residue was less than 50 % and without statistically significant differences compared to the control.

Reproduction with the tested rates of 5.10 and 5.70 L FP/ha was possible to be studied in the performed exposures of 28 and 56 DAA.

The mean fecundity in the test item groups were 15.34 (T1) and 21.53 (T2) eggs per female per day compared to 28.15 eggs per female per day in the control group at 28 DAA1. The mean fecundity in the test item groups was 24.06 (T1) and 14.88 (T2) eggs per female per day compared to 20.92 eggs per female per day in the control group at 56 DAA1.

The mean hatching rates were 98.09 (T1) and 99.62 (T2) % in the test item treatment groups compared to 99.95 % in the control group in the exposure of 28 DAA. The mean hatching rates were 99.80 (T1) and 99.91 (T2) % in the test item treatment groups compared to 100.00 % in the control group in the exposure of 56 DAA.

The mean of fertile eggs per female per day were 15.21 (T1) and 21.46 (T2) % in the test item treatment groups compared to 28.14 % in the control group in the exposure of 28 DAA. The mean of fertile eggs per female per day were 24.10 (T1) and 14.88 (T2) % in the test item treatment groups compared to 20.92 % in the control group in the exposure of 56 DAA.

More than 2 fertile eggs per female per day is considered a normal reproductive output for the control treatment, so the test item is considered as harmless in reproduction when larvae are exposed to the residue after 28 days of the application.

Exposure of 0 DAA1 (days after the test item application)				
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality [b] [%]**	**Corrected mortality [%]**	**Fertility [Fertile eggs per female per day]**
Control (tap water)	--	10.00	--	n.d.
Azadirachtin 1 % EC	5.10	87.50 [sd]	86.11	n.d.
Azadirachtin 1 % EC	5.70	90.00 [sd]	88.89	n.d.

The reference item caused a mortality of 100 %

Exposure of 28 DAA1 (days after the test item application)				
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality [b] [%]**	**Corrected mortality [%]**	**Fertility [Fertile eggs per female per day]**
Control (tap water)	--	0.00	--	28.15
Azadirachtin 1 % EC	5.10	0.00	0.00	15.34
Azadirachtin 1 % EC	5.70	0.00	0.00	21.53

The reference item caused a mortality of 100 %

Exposure of 56 DAA1 (days after the test item application)				
Treatment group	**Rate [a] [L FP/ha]**	**Mean mortality [b] [%]**	**Corrected mortality [%]**	**Fertility [Fertile eggs per female per day]**
Control (tap water)	--	0.00	--	20.92
Azadirachtin 1 % EC	5.10	0.00	0.00	24.06
Azadirachtin 1 % EC	5.70	0.00	0.00	14.88

The reference item caused a mortality of 100 %
a Rate in L of formulated product (FP)/ha
b sd = Statistically significantly increased compared to control (Fisher`s Exact Binomial Test with mortality, one-sided greater, $\alpha = 0.05$).
c n.d.: not determined, since the corrected mortality was above 50 %

Test validity criteria

All mortality and reproduction tests were considered to be valid as:

- The maximum cumulative mortality in the control group was ≤ 30 % (actual maximum value: 10.00 % in the exposure of 0 DAA1).
- The cumulative mortality in the toxic reference group (corrected mortality) was between 50 % and 100% (actual minimum value: 100.00 % in every exposure).
- The mean number of fertile eggs per female per day in the control group was ≥ 2 (actual minimum: 20.09 in the exposure of 56 DAA).

Conclusion

Azadirachtin 1 % EC **Aging residue periods studied: 0, 28 and 56 DAA1 (days after the test item application)**		
Rates	5.10 [L of formulated product/ha] (63.70 g Azadirachtin /ha)	5.70 [L of formulated product/ha] (71.20 g Azadirachtin /ha)
Effects less than 50 % (compared to the control)		
Mortality < 50%	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
Fertile eggs per female per day < 2	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
No significant effects (compared to the control)		
Mortality	From 28 DAA1 (aged residue for 28 days)	From 28 DAA1 (aged residue for 28 days)
Fertile eggs per female per day < 2	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1	No impact on reproduction in accordance with the validity criteria for the control group from 28 DAA1

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

Not required.

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

Not required.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

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

- each replicate produced 110 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 13.8% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 0.0% (criterion: ≤ 10%).

Agreed endpoints:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of azadirachtin/kg dry weight of artificial soil]
EC_{10}	> 1000.0	>10.6
EC_{20}	> 1000.0	>10.6
EC_{50}	> 1000.0	>10.6
NOEC (reproduction)	≥ 1000.0	≥10.6
LOEC (reproduction)	> 1000.0	>10.6
LC_{50}	> 1000.0	>10.6
NOEC (survival)	≥ 1000.0	≥10.6
LOEC (survival)	> 1000.0	>10.6

Reference KCP 10.4.1.1

Report "Azadirachtin 1% EC. Earthworms reproduction test (*Eisenia andrei*)" Anna Wróbel, (2020) Study code: G/03/17. Institute of Industrial Organic Chemistry Branch Pszczyna

Guideline(s) OECD Guideline No. 222 (2016)

Deviations Deviations from OECD Guideline No. 222 (2016), SOP/G/36 and the Study Plan:
The test should be performed at a temperature range between 18 and 22°C. The range of temperature registered in the definitive test was 20.0 – 23.2°C. Short-term deviation from the recommended maximum range of temperature did not have impact on the generated results.
Deviation from the Study Plan:
The study finished in July 2020, not in September 2019 as it had been planned.
These deviations did not affect the study results.

GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item	Azadirachtin 1% EC: SCL – 281287, active substance: azadirachtin 1.04% w/v
Artificial soil	10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
Test organism	the earthworm, Eisenia andrei obtained from a standard laboratory culture cultivated at the Łukasiewicz research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Toxicology
Test design	Test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicates
Concentration of the test item	control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg/kg dry weight of the artificial soil
Test conditions	temperature: 20.0 – 23.2°C; pH at the beginning of the experiment: 5.58 – 5.83; pH at the end of the experiment: 5.51 – 5.64; soil moisture content at the beginning of the experiment: 21.5– 26.5% (40.3 – 49.7% of the maximum water holding capacity); soil moisture content at the end of the experiment: 23.1 – 28.7% (43.3 – 53.7% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 680.4 – 782.5 lux light intensity at the end of the experiment: 753.6 – 790.4 lux
Statistical analysis	EC_{10}, EC_{20}, EC_{50}, LC_{50} – 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 Sequential t-test Procedure NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a value suggested by the ToxRat Professional 2.10 statistical computer software.
Endpoints	EC_{10}, EC_{20}, EC_{50}, NOEC, LOEC LC_{50}, NOEC, LOEC

Results and discussions

On the basis of the results, it was concluded that after 4 weeks, at the control group no mortality of adult earthworms was observed. Earthworms mortality was observed at the concentrations 5.6, 100, 180, and 320 mg/kg dry artificial soil. It was equal to 5.0, 10.0, 10.0, and 2.5 %, respectively. No mortality of earthworms at the concentrations 10, 18, 32, 56, 560, and 1000 mg/kg dry artificial soil was observed.

The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 1000.0 mg of the test item/kg dry weight of artificial soil (10.6 mg of azadirachtin/kg dry weight of artificial soil).

After 4 weeks of the experiment, the treated living earthworms did not exhibit any changes in appearance and behaviour.

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of artificial soil, the body weight decrease was between 9.1 to 21.3%. As for the control group, the body weight decrease was equal to 16.1%.

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 83 - 168 per replicate. The mean number of juveniles in the control group was equal to 110 per replicate.

After 8 weeks of the experiment, it was concluded that Azadirachtin 1% EC had no statistically significant impact on reproduction of the earthworms at concentrations between 5.6 – 1000.0 mg/kg dry weight of artificial soil.
The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC_{10}) is higher than 1000 mg of the test item/kg dry weight of artificial soil (>10.6 mg azadirachtin/kg dry weight of artificial soil).
The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC_{20}) is is higher than 1000 mg of the test item/kg dry weight of artificial soil (>10.6 mg azadirachtin/kg dry weight of artificial soil).
The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) is is higher than 1000 mg of the test item/kg dry weight of artificial soil (>10.6 mg azadirachtin/kg dry weight of artificial soil).
The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal or higher than 1000 mg of the test item/kg dry weight of artificial soil (≥ 10.6 mg azadirachtin/kg dry weight of artificial soil).
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is higher than 1000.0 mg of the test item/kg dry weight of artificial soil (>10.6 mg azadirachtin/kg dry weight of artificial soil).
After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Validity criteria
The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced 110 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 13.8% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 0.0% (criterion: ≤ 10%).

Conclusion
The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of azadirachtin/kg dry weight of artificial soil]
EC_{10}	> 1000.0	>10.6
EC_{20}	> 1000.0	>10.6
EC_{50}	> 1000.0	>10.6
NOEC (reproduction)	≥ 1000.0	≥10.6
LOEC (reproduction)	> 1000.0	>10.6
LC_{50}	> 1000.0	>10.6
NOEC (survival)	≥ 1000.0	≥10.6
LOEC (survival)	> 1000.0	>10.6

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 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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • The coefficients of variation (CV) in the control group were 5.9, 2.9, 3.1 and 2.5%, 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% **Agreed endpoints:** On the basis of the results, it was concluded that at the concentration corresponding to the PEC: 7.8 mg/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg/kg of soil (0.5 mg a.s./kg of soil), Azadirachtin 1% EC did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference: 10.5.1

Report "Azadirachtin 1% EC_Soil Microorganisms: Nitrogen Transformation Test" Weronika Dec, 2018, G/02/17

Guideline(s): OECD Guideline No. 216 (2000) / EU Method C.21.

Deviations: Deviations from the OECD Guideline No. 216 (2000), the EU Method C.21.:
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 for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer.
The deviations did not affect the results of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) -

Materials and methods

Test item:

Description:	Azadirachtin 1% EC
Production batch:	SCL- 19345
Active ingredients content:	azadirachtin – 1.3% (w/v)

Vehicle and control: Distilled water

Test system:

Species: Microorganisms

Source:	Agricultural soil collected from a place belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna.

Experimental conditions:

Temperature:	19 – 21°C
Humidity:	52.5% – 57.3% MWHC incubation in darkness.

Study design and methods

Test design and treatment: Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days.
Concentrations of the test item:
Control; PEC: 7.8 mg of the test item/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg of the test item/kg of soil (0.5 mg a.s./kg of soil)

Results The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentration corresponding to the PEC: 7.8 mg/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg/kg of soil (0.5 mg a.s./kg of soil) did not exceed 25% on 28 day of analysis.

Deviations from the control based on nitrates formation rate for selected time interval [%]:

Time interval [d]	**PEC 7.8 mg of the test item/kg of soil (0.1 mg a.s./kg of soil)**	**5 x PEC 39.2 mg of the test item/kg of soil (0.5 mg a.s./kg of soil)**
0 - 7	2.9	0.3
0 - 14	-2.4	3.0
0 - 28	-1.8	-7.3

"-" values of nitrate formation rate higher than the one obtained for the control group

Validity

The coefficients of variation (CV) in the control group were 5.9, 2.9, 3.1 and 2.5%, 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%.

Conclusion

On the basis of the results, it was concluded that at the concentration corresponding to the PEC: 7.8 mg/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg/kg of soil (0.5 mg a.s./kg of soil), Azadirachtin 1% EC did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Comments of zRMS:

Reference: KCP 10.5.2

Report "Azadirachtin 1% EC. Soil Microorganisms: Carbon Transformation Test. Weronika Dec, March, 2018, G/01/17.

Guideline(s): OECD Guideline No. 217 (2000) / EU Method C.22

Deviations: Deviations from the Study Plan:

	The completion of the study should be January 2018 instead of January 2017. It was an editorial error. The study was finished in March 2018 and not in January 2018 as it was planned. The deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Materials

Test item:

Description:	Azadirachtin 1% EC
Production batch:	SCL- 19345
Active ingredients content:	Azadirachtin – 1.3% (w/v)

Test system:

Species:	Microorganisms
Source:	Agricultural soil taken from the area belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna.

Experimental conditions:

Temperature:	19 – 21°C
Humidity:	52.5 – 55.0% of MWHC
Air changes:	-
Light and photoperiod:	Dark (24/24h)

Study design and methods

Test design and treatment:	3 portions of soil: one control group and two groups containing the test item weighing 1500 g each. Every portion was divided into three replicates weighing 500 g each. Test duration: 28 days. Concentrations of the test material**:** Control, PEC: 7.8 mg of the test item/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg of the test item/kg of soil (0.5 mg a.s./kg of soil).
Statistics:	In order to determine significance of differences between the control and the treated groups, the Shapiro-Wilk's Test on Normal Distribution, the Levene's Test on Variance Homogeneity and Williams Multiple Sequential t-test Procedure were used.

Results

The difference in the soil respiration rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 7.8 mg/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg/kg of soil (0.5 mg a.s./kg of soil) did not exceed 25% on any day of analysis.

Oxygen (O_2) consumption - deviations from the control [%]:

Day	**PEC 7.8 mg of the test item/kg of soil (0.1 mg a.s./kg of soil)**	**5 x PEC 39.2 mg of the test item/kg of soil (0.5 mg a.s./kg of soil)**
0	3.2	3.3
7	3.3	3.3
14	-0.3	1.0
28	1.7	1.5

"-" the value of the oxygen consumption higher than the one obtained for the control group

Conclusion

On the basis of the results, it was concluded that Azadirachtin 1% EC at the concentrations corresponding to the PEC: 7.8 mg/kg of soil (0.1 mg a.s./kg of soil) and 5 x PEC: 39.2 mg/kg of soil (0.5 mg a.s./kg of soil), did not have any long-term adverse effects on the process of carbon transformation in aerobic surface soils.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. The seedling emergence in the control (validity criterion: at least 70%) was as follows: 95.2% – sunflower, 95.2% – pea, 100.0% – cabbage, 90.0% – carrot, 95.0% – onion, 95.0% – oats,, - the mean survival of the emerged control seedlings was 100% for cabbage, pea, carrot, sunflower and onion and 94.7% for oats (validity criterion: at least 90%);

- the control seedlings did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Agreed endpoints:

Azadirachtin 1% EC: ER_{50}, and NOER values.

Endpoint value		**Sunflower** *Helianthus annus*	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Shoot length (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	2000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	20.8	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4

Reference: KCP 10.6.2-01

Report: "Azadirachtin 1% EC. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test". Anna Wróbel., G/06/17, 2020. Institute of Industrial Organic Chemistry, Branch Pszcyna

Guideline(s): OECD No. 208 (2006)

Deviations: Deviations from OECD Guideline No. 208:
According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 67.60 and 121.5 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
Deviation from the study plan:
The study was finished in July 2020 and not in January/February 2020 as it had been planned.
The carbon dioxide concentration was determined at the beginning experiment and during experiment second time. Not at the end experiment as it had been planned.
All deviations did not affect results of the experiment..

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Summary

The study, aimed at evaluating the effect of Azadirachtin 1% EC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. The test item was sprayed onto the soil surface. For each species, five application rates were used. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots 3 (sunflower, pea, cabbage) or 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 and then every 2 – 3 days) and visual phytotoxicity (after 7 and 14 days). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed.

The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER_{10}, ER_{25}, ER_{50}, and NOER.

Material and methods

Test item:	Azadirachtin 1% EC Batch number: SCL-281287 Production date: January 09, 2018 Expiry date: January 08, 2020
Test species::	sunflower (*Helianthus annuus*) pea (*Pisum sativum*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*).
Test design:	Number of rates: 5 application rates + control Number of replicates: 4 (carrot, onion, oats) or 7 (sunflower, cabbage, pea) Number of seeds: 20 (carrot, onion, oats) or 21 (sunflower, cabbage, pea) The total number of plants per application rate: 20 (carrot, onion, oats) or 21 (sunflower, cabbage, pea) Test termination: 14 days after the emergence of 50% of the control seedlings
Test duration:	14 days after 50 % emergence of the control seedlings.
Application rates:	a control, 74.1, 222.2 666.7, 2000, 6000 mL test item/ha (i.e. 0.8, 2.3, 6.9, 20.8, 62.4 g of azadirachtin/ha) volume of deionized water used to prepare the highest rate corresponded 1000 L water/ha
Soil:	sandy loam
Endpoints:	ER_{10}, ER_{25}, ER_{50}, NOER
Test conditions:	Temperature: 18.6 – 26.9°C Humidity: 45.4 – 82.5% Photoperiod – 16h day:8h night Light intensity: 67.60 – 121.5 $\mu E/m^2/s$ Carbon dioxide concentration: 323– 342 ppm
Statistical analysis:	ER_{10}, ER_{25}, ER_{50} – probit analysis, NOER:

In order to determine the NOER values for the emergence the following statistical tests were used:
Fisher's Exact Binomial Test with Bonferroni Correction.
In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:
Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Validity criteria:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
  95.2% – sunflower,
  95.2% – pea,
  100.0% – cabbage,
  90.0% – carrot,
  95.0% – onion,
  95.0% – oats,,
- the mean survival of the emerged control seedlings was 100% for cabbage, pea, carrot, sunflower and onion and 94.7% for oats (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Findings

Azadirachtin 1% EC: ER_{50}, and NOER values.

Endpoint value		**Sunflower** *Helianthus annus*	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Shoot length (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	2000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	20.8	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4

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

The following validity criteria were met:
- the seedling emergence (validity criterion: at least 70%) was as follows:
81.0 – 88.1% – sunflower,
85.7 – 95.2% – cabbage,
83.3 – 88.1% – pea,
92.5 – 100% – carrot,
92.5 – 97.5% – onion,
87.5 – 92.5% – oats,
- the mean survival of the emerged control seedlings was 100% for all tested species (validity criterion: at least 90%),
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Agreed endpoints:

Endpoint value		**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota* **)**	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	> 6000*	> 6000*	> 6000*	> 6000*	> 6000*	> 6000*
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Shoot length (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	2000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	20.8
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4

*- the value could not be determined; it can be probaby higher than the highest rate of the test item used in the experiment, i.e. 6000 mL test item/ha.

Reference: KCP 10.6.2-02

Report "Azadirachtin 1% EC: Terrestial Plant Test: Vegetative Vigour Test". Anna Wróbel , 2020, Study code: G/07/17 Institute of Industrial Organic Chemistry, Branch Pszczyna

Guideline(s): OECD Guideline No. 227 (2006)

Deviations: Deviations from OECD Guideline No. 227:
According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 93.3 – 200.9 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
Deviations from SOP/G/70 and the Study Plan:
According to SOP/G/70 and the Study Plan, the light intensity should be 50 – 150 µE/m2/s. During the experiment the light intensity was between 93.3 – 200.9 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
The study finished in August 2020 not in January/February 2020 as it had been planned.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Azadirachtin 1% EC; Batch Number SCL-281287; active substance: azadirachtin 1.04% (w/v)

Test species: sunflower (Helianthus annuus), cabbage (Brassica oleracea var. capitata), pea (Pisum sativum), carrot (Daucus carrota), onion (Allium cepa), oats (Avena sativa)

Soil: Sandy loam soil containing 1.2% organic carbon

Study design: number of rates: 5 application rates + control; number of replicates: 4 or 7 pots/application
the total number of plants per application rate – 20 or 21;
test termination: 21 days after the spraying.

Application rates: 6000, 2000, 666.7, 222.2 and 74.1 mL test item/ha (i.e. 62.4 g of azadirachtin/ha, 20.8 g of azadirachtin/ha + 6.9 g of azadirachtin/ha, 2.3 g of azadirachtin/ha, 0.8 g of azadirachtin/ha)
Volume of deionised water used to prepare the highest rate: 1000 L water/ha

Test conditions: temperature: 18.1 – 24.4°C, humidity: 48.5 – 89.9%, light – dark cycles (16h:8h), light intensity: 93.3– 200.9 µE/m^2/s, carbon dioxide concentration: 354- 371 ppm.

Statistical analysis: The ER_{10}, ER_{25}, ER_{50} and NOEC values were determined by using a Probit analysis in the NCSS (Number Cruncher Statistical System) and one-way ANOVA using GraphPad Prism 8.0.

Endpoints: ER_{10}, ER_{25}, ER_{50} and NOEC

Results and Conclusions

The test item, i.e. Azadirachtin 1% EC applied at rates ranging from 6000 to 74.1 mL test item/ha had a varied impact on vegetative vigour of all the plant species tested. The impact depended on the rate of the test item and species used.
There was mortality observed for all the plant species tested at rates ranging from 6000 to 74.1 mL test item/ha. The phytotoxic symptoms for all plant species tested were observed at all the rates of the test item used. The following phytotoxic symptoms were not observed on 21 days after the test item application: chlorosis, necrosis, wilting, leaf deformation, stem deformation or death.

The endpoint values showing the impact of the test item on vegetative vigour of the plant species tested are presented in table given below:

Endpoint value		**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota* **)**	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	> 6000*	> 6000*	> 6000*	> 6000*	> 6000*	> 6000*
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4
Shoot length (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	2000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	20.8

Plant dry weight (plants without roots)							
ER_{50}	mL/ha	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
	g/ha	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4	> 62.4
NOER	mL/ha	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
	g/ha	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4	≥ 62.4

*- the value could not be determined; it can be probaby higher than the highest rate of the test item used in the experiment, i.e. 6000 mL test item/ha.

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

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

A 2.8 KCP 10.8 Monitoring data