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

NATIONAL ADDENDUM

Poland

Applicant: Sharda Cropchem España S.L.

Submission date: June 2022

MS Finalisation date: July 2022

Version history

When	What
July 2022	Evaluation of the ppp by zRMS-PL

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-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.

It should be noted that National Addendum for Poland for AZA submitted for the applicant is concerned on risk assessment for aquatic organism with new PEC_{sw} values calculated by VSmod.

In addition, the chronic studies for bees were submitted and zRMS-PL evaluated them in Appendix 2.

For these reason only risk assessment for aquatic organism was updated in the NA for Poland.

No additional risk assessment calculations are required for product AZA until GD for Bees, 2013 will be implemented at EU level.

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)

Please, refer to the core dossier.

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.

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

Fruiting Vegetables (Tomato): ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies.*

Potato: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies.*

Ornamentals >50cm: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed buffer zone of 5m to surface water bodies with 50% of nozzles reduction OR an unsprayed buffer zone of 10m to surface water bodies.*

Ornamentals <50cm: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies*.

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. The applicant submitted the new chronic studies for adult bees and larvae for product AZA according to recommendation given in EU Reg.284/2009.

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)

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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

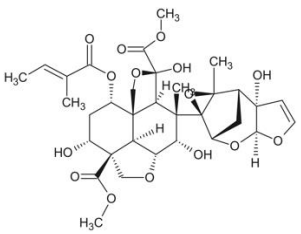
Grouping according to application rate			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
All crops	Fruiting vegetables (including Tomato, Strawberry and Melon uses), Citrus and Potato	Same application rate (3 L prod./ha), same number of applications (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

Grouping according to application rate			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
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

Please, refer to the core dossier.

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)

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.2.2.4 Effects of secondary poisoning

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

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

Please, refer to the core dossier.

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

9.3.1 Toxicity data

Please, refer to the core dossier.

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). No additional calculations are required for PL registration of the product AZA. Please, refer to the core dossier.

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

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.3.2.2 Higher-tier risk assessment

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.3.2.3 Drinking water exposure

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.3.2.4 Effects of secondary poisoning

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

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

Please, refer to the core dossier.
The risk assessment is considered acceptable for all uses proposed in the GAP. No additional calculations are required for PL registration of the product AZA.

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
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

Species	Substance	Exposure System	Results	Reference
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
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

Species	Substance	Exposure System	Results	Reference
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 (µg/L)				LC_{50} >100000	EC_{50} >100000	E_rC_{50} >1000000
AF				100	100	10
RAC (µg/L)				>1000	>1000	>100000
Distance	**% Drift**	**Nozzles reduction (%)**	**PEC $_{gl-max}$ (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.

NOTE: The Steps 1, 2 and 3 calculations were already done in the core dossier and for consistency have been reported here but only Polish relevant scenarios.

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
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**

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
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**

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 $_{gl\text{-}max}$ (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							
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**
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**

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							
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
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**

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.
NOTE: The Steps 4 calculations were already done in the core dossier and for consistency have been reported here but only polish relevant scenarios. Only VFSMOD calculations are new in the Addenda.

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	R1 stream	0.244/0.53	0.157/0.349	-/0.244	-/0.187	-/0.128
RAC 0.47		**PEC/RAC ratio**				
None	R1 stream	0.519/1.14	0.334/0.743	-/0.519	-/0.398	-/0.272
RAC 0.16		**PEC/RAC ratio**				
None	R1 stream	1.**525/3.35**	0.981/**2.181**	**-/1.525**	**-/1.169**	-/0.800

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

Table 9.5-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on surface water VFSMOD 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)	5
	No spray buffer (m)	5
None	R1 stream	-/0.056
RAC 0.47		**PEC/RAC ratio**
None	R1 stream	-/0.119
RAC 0.16		**PEC/RAC ratio**
None	R1 stream	-/0.350

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

zRMS comment:

The Steps 4 PEC_{sw} calculations were already done in the Core dossier for AZA. The applicant for consistency presented results for Fruiting vegetables (*Tomato)* only for scenarios R1 (stream) for which the max buffer zone was needed. Based on the PEC_{sw} FOCUS step 4 calculations and RAC of 0.16 µg/ L the following risk mitigation measures was needed to surface water bodies:

Fruiting Vegetables (Tomato)

- 20 m no-spray buffer and 20 m vegetative filter strip

Further, the applicant provided the refinement of PEC_{sw} values using PEC_{sw} VFSMOD Step 4 calculations.

Based on PEC_{sw} VFSMOD Step 4 calculations and RAC = 0.16 µg/L, the following risk mitigation measures to surface water bodies is now proposed:

Fruiting Vegetables (Tomato)

- 5 m no-spray buffer and 5m vegetative filter strip

Finally for use in *Fruiting Vegetables (Tomato):* **5 m no-spray buffer and 5m vegetative filter strip to surface water bodies** was agreed by zRMS for use in Poland**.**

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 lowest endpoint for fish and sediment dwelling organisms with mitigation of spray drift and run-off for the use of AZA in Potato (single/multiple application)

Intended use **Active substance** **Application rate (g/ha)**		Potato Azadirachtin 2 × 25			
Nozzle reduction	Vegetative strip (m)	None	5	10	15
	No spray buffer (m)	5	5	10	15
None	R1 stream	0.202/0.444	0.131/0.290	-/0.202	-/0.155
RAC 0.47		**PEC/RAC ratio**			
None	R1 stream	0.430/0.945	0.279/0.617	-/0.430	-/0.330
RAC 0.16		**PEC/RAC ratio**			
None	R1 stream	**1.263/2.775**	0.819/**1.813**	-/**1.263**	-/0.969

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

Table 9.5-12: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Azadirachtin based on surface water VFSMOD 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 Potato (single/multiple application)

Intended use **Active substance** **Application rate (g/ha)**		Potato Azadirachtin 2 × 25
Nozzle reduction	Vegetative strip (m)	5
	No spray buffer (m)	5
None	R1 stream	-/0.046
RAC 0.47		**PEC/RAC ratio**
None	R1 stream	-/0.098
RAC 0.16		**PEC/RAC ratio**
None	R1 stream	-/0.288

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

zRMS comment:

The Steps 4 PEC_{sw} calculations were already done in the Core dossier for AZA. The applicant for consistency presented results for Potato only for scenarios R1 (stream) for which the max buffer zone was needed.

Based on the PEC_{sw} FOCUS step 4 calculations and RAC of 0.16 µg/L the following risk mitigation measures was needed to surface water bodies:

Potato

- 15 m no-spray buffer and 15 m vegetative filter strip

Due to that fact 15 m no-spray buffer and 15 m vegetative filter strip calculated by modelling approach according to the Austrian Environmental Agency AGES which is not recommended for Poland, the following risk mitigation measures was proposed:

Potato

- 20 m no-spray buffer and 20 m vegetative filter strip is recommended for Poland

Further, the applicant provided the refinement of PEC_{sw} values using PEC_{sw} VFSMOD Step 4 calculations.

Based on PEC_{sw} VFSMOD Step 4 calculations and RAC = 0.16 µg/L value, the following risk mitigation measures to surface water bodies now is proposed.

Potato

- 5 m no-spray buffer and 5m vegetative filter strip

Finally for use in Potato: **5 m no-spray buffer and 5m vegetative filter** to surface water bodies was agreed by zRMS for use in Poland.

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 lowest endpoint for fish and 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 **(>50 cm)**	
Active substance		Azadirachtin	
Application rate (g/ha)		2 × 30	
Nozzle reduction	Vegetative strip (m)	None	
	No spray buffer (m)	5	10
None	D3 ditch	0.309/0.272	0.112/0.098
50 %		0.154/0.136	-/-
None	D4 stream	0.271/0.253	0.098/0.091
50 %		0.136/0.126	-/-
None	R1 stream	0.272/0.240	0.099/0.086
50 %		0.136/0.120	-/-

Intended use **Active substance** **Application rate (g/ha)**		Bush ornamentals **(>50 cm)** Azadirachtin 2 × 30	
Nozzle reduction	Vegetative strip (m)	None	
	No spray buffer (m)	5	10
RAC 0.47		**PEC/RAC ratio**	
None	D3 ditch	0.657/0.579	0.238/0.209
50 %		0.328/0.289	-/-
None	D4 stream	0.577/0.538	0.209/0.194
50 %		0.289/0.268	-/-
None	R1 stream	0.579/0.511	0.211/0.183
50 %		0.289/0.255	-/-
RAC 0.16		**PEC/RAC ratio**	
None	D3 ditch	**1.931/1.700**	0.700/0.613
50 %		0.963/0.850	-/-
None	D4 stream	**1.694/1.581**	0.613/0.569
50 %		0.850/0.788	-/-
None	R1 stream	**1.700/1.500**	0.619/0.538
50 %		0.850/0.750	-/-

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

zRMS comment:

The Steps 4 calculations were already done in the Core dossier for AZA. The applicant for consistency provided in this NA the results for scenarios D3, D4 and R1 for Poland for which FOCUS STEP 3 PEC_{sw}/RAC was above trigger of 1, indicating unacceptable risk.

After the PEC_{sw} FOCUS step 4 calculations for D3, D4 and R1 scenarios and RAC of 0.16 µg/L value, the following risk mitigation measures was proposed to surface water bodies:

- D3 ditch, D4 stream, R1 stream: 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.

Finally for use in Ornamentals >50 cm: 5 **m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer surface water bodies** was agreed by zRMS for Poland.

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 lowest endpoint for fish and 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 **Active substance** **Application rate (g/ha)**		Herbaceous ornamentals (**Ornamentals <50 cm)** Azadirachtin 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	-/-	-/-	-/-	-/-
	R1 stream	0.282/0.920	0.173/0.599	0.116/0.417	-/0.320	-/0.218
RAC 0.47		**PEC/RAC ratio**				
None	D3 ditch	0.111/0.091	-/-	-/-	-/-	-/-
	R1 stream	0.600/1.957	0.368/1.274	0.247/0.887	-/0.681	-/0.464
RAC 0.16		**PEC/RAC ratio**				
None	D3 ditch	0.325/0.269	-/-	-/-	-/-	-/-
	R1 stream	**1.763/5.750**	**1.081/3.744**	0.725/**2.606**	**-/2.000**	**-/1.363**

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 surface water VFSMOD 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 Bulb Vegetables (herbaceous ornamentals) (single/multiple application)

Intended use **Active substance** **Application rate (g/ha)**		Herbaceous ornamentals (Ornamentals **<50 cm)** Azadirachtin 2 × 30
Nozzle reduction	Vegetative strip (m)	5
	No spray buffer (m)	5
None	R1 stream	0.045/0.051
RAC 0.47		**PEC/RAC ratio**
None	R1 stream	0.096/0.109
RAC 0.16		**PEC/RAC ratio**
None	R1 stream	0.281/0.319

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

zRMS comment:

The Steps 4 calculations were already done in the Core dossier for AZA and for consistency was provided by the applicant in NA but only for relevant scenarios R1 (stream) and D3 for Poland for which FOCUS STEP 3 PEC_{sw}/RAC was above trigger of 1, indicating unacceptable risk.

The calculations with PECsw FOCUS STEP 4 and RAC of 0.16 µg/L did not indicate acceptable risk with 20 m no-spray buffer and 20 m vegetative filter strip for R1 scenario.

The applicant provided the refinement of PEC_{sw} values using PEC_{sw} VFSMOD Step 4 calculations for R1 scenario.

Based on PECsw VFSMOD Step 4 calculations and RAC = 0.16 µg/L value, the following risk mitigation measures to surface water now is proposed:

- 5 m no-spray buffer and 5m vegetative filter strip

Finally for use in ornamentals <50 cm: **5 m no-spray buffer and 5m vegetative filter strip to surface water bodies** was agreed by zRMS for Poland.

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

Fruiting Vegetables (Tomato)

- R1 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

Potato

- R1 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

Ornamentals >50 cm

- D3 ditch, D4 stream, R1 stream: 5 m no-spray buffer with 50% of nozzles reduction or 10 m no-spray buffer.

Ornamentals <50 cm

- D3 ditch: 5 m no-spray buffer.
- R1 stream: 5 m no-spray buffer and 5 m vegetative filter strip.

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.

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

Fruiting Vegetables (Tomato): ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies.*

Potato: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to sur-*

face water bodies.

Ornamentals >50cm: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed buffer zone of 5m to surface water bodies with 50% of nozzles reduction OR an unsprayed buffer zone of 10m to surface water bodies.*

Ornamentals <50cm: ***Spe3*** *– To protect aquatic organisms, respect an unsprayed vegetated buffer zone of 5m to surface water bodies*.

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 A/bee**	EFSA Journal 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
Apis mellifera	AZA	Chronic, 10 d	LDD_{50} = 47.48 µg/bee/day (0.63 µg a.s./bee/day) NOEDD = 11.08 µg/bee/day (0.15 µg a.s./bee/day)	KCP 10.3.1.2 Prabha, K.L., 2022, 9036/2021*
Apis mellifera	AZA	Larval, repeated exposure	NOED = 39.5 µg/larva (0.52 µg a.s./larva) ED_{50} = 102.42 µg/larva (1.36 µg a.s./larva)	KCP 10.3.1.3 Prabha, K.L., 2022, 9035/2021*

Species	Substance	Exposure System	Results	Reference
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.				

*Study summaries are included in this dossier (see appendix 2). However, they have not been considered further as they are not relevant to the currently approved risk assessment scheme (SANCO/10329/2002).

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 comment:

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 and the chronic test for larvae should be provided for authorization of plant protection product.

The applicant submitted these studies which were summarised in Appendix 2.

No additional risk assessment calculations are required for product AZA until GD for Bees, 2013 will be implemented at EU 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

Please, refer to the core dossier.

9.7.1.1 Justification for new endpoints

Please, refer to the core dossier.

9.7.2 Risk assessment

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.7.2.1 Risk assessment for in-field exposure

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.7.2.2 Risk assessment for off-field exposure

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

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

Please, refer to the core dossier.

The risk assessment indicates that AZA poses low risk to in- and -off -field non-target arthropods following application of product according to the proposed use patterns. Therefore, any risk mitigation measures are needed.

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

9.8.1 Toxicity data

Please, refer to the core dossier.

9.8.1.1 Justification for new endpoints

Please, refer to the core dossier.

9.8.2 Risk assessment

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.8.2.1 First-tier risk assessment

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

Please, refer to the core dossier.

The risk assessment indicates acceptable long-term risk to earthworms and other soil macro- and mesofauna when applied AZA according to the proposed uses rates.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Please, refer to the core dossier.

9.9.1.1 Justification for new endpoints

Please, refer to the core dossier.

9.9.2 Risk assessment

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.9.3 Overall conclusions

Please, refer to the core dossier.

According to results presented in the Core dossier, no unacceptable risk for microorganism was obtained with AZA formulation for all crops.

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

9.10.1 Toxicity data

Please, refer to the core dossier.

9.10.1.1 Justification for new endpoints

Please, refer to the core dossier.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

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

Please, refer to the core dossier. No additional calculations are required for PL registration of the product AZA.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not relevant.

9.10.3 Overall conclusions

Please, refer to the core dossier.
According to results presented in the Core dossier . No unacceptable off-field risk on non-target plants was obtained with AZA formulation for all crops. Therefore, any risk mitigation measures are needed.

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

Please, refer to the core dossier.

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: Aquatic Chronic 13~~ ~~Code(s) for hazard pictogram(s):~~ ~~Signal word:~~ ~~Hazard statement(s): H412: Harmful to aquatic life with long lasting effects~~ ~~EU specific statements: EUH401~~ ~~Precautionary statement: P391, P501~~

zRMS comment:

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

Hazard pictograms

Signal word

-

-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 10.3.1.2	Prabha, K.L.	2022	Chronic Oral Toxicity Study of Azadirachtin 1% EC on adult honey bee (*Apis mellifera*). Study No. 9036/2021 Bioscience Research Foundation GLP, Unpublished	N	SHARDA Crochem Limited
KCP 10.3.1.3	Prabha, K.L.	2022	Effect of Azadirachtin 1% EC on larvae of honey bee, *Apis mellifera* (L.) following repeated expoure. Study No. 9035/2021 Bioscience Research Foundation 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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2-04	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
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

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 10.6.2-02	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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please, refer to the core dossier.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

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

- There was no mortality in control group.
- The average mortality in the reference substance treated group is ≥ 50 % at the end of the test (10 days following start of exposure)

Agreed endpoints:

Endpoint	Value	Value Based on Azadirachtin[a] content
LDD_{50}	47.48 ± 5.85 µg/bee	0.63 ± 0.08 µg as/bee
LC_{50}	2286.14 ± 307.76mg/kg food	30.33± 4.0 mg as/kg food.
NOEDD	11.08 µg /bee	0.15 µg as/bee
NOEC	468.8 mg /kg	6.22 mg as/kg

a - based on the content of **Azadirachtin** in the test the item, i.e., 1.3% w/v, Density: 0.98 g/cm^3

Reference:	KCP 10.3.1.2
Report	"Chronic Oral Toxicity Study of Azadirachtin 1% EC on adult honey bee (*Apis mellifera*)". Ms. K. Lakshmi Prabha. 2022. Bioscience Research Foundation. Report No. 9036/2021
Guideline(s):	OECD test No. 245 Guideline for the Testing of Chemicals: Honey bee (*Apis mellifera* L.), Chronic Oral Toxicity Test – 10 Day Feeding (9 October 2017).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Azadirachtin 1% EC Batch No.: SCL-31046
Test system:	Species: *Apis mellifera* (L.) strain carnica

	Source: Bee hive maintained at BRF test facility Stage: 2 days old young adult bee
Dose response test:	Control, 9.4, 18.8, 37.5, 75.0 and 150.0 µg/bee/day.
Number of adult bees:	10 bees/ replicate 3 replicates 5 concentrations
Duration:	10 days

Results and discussions

Based on the results of range finding study, the Dose Response Test was conducted and no deviation was observed in range finding study.

Honeybees were acclimatized for 24 hours. The bees were orally treated with control, 9.4, 18.8, 37.5, 75.0 and 150 µg/bee concentrations in 50% w/v sucrose solution. A concurrent control group with 50% w/v sucrose solution was also maintained.

At the end of every 24 (10 days exposure) hour observation bees treated with control group were appeared normal and no toxic sign was observed.

- From day 1 to day 3exposure, 0% mortality and no clinical signs were observed in bees treated with control, 9.4, 18.8, 37.5, 75.0 and 150 µg /bee.
- On day 4, mortality and toxicity signs (affected) was observed in bees exposed to 150 µg /bee whereas bees exposed to control, 9.4, 18.8, 37.5 and 75.0 µg/bee appeared normal.
- On day 5, mortality and toxicity signs (affected) was observed in bees exposed to 37.5, 75.0 and 150 µg/bee whereas bees exposed to control, 39.51 and 59.3 µg /bee appeared normal.
- On day 6, mortality and toxicity signs (affected) was observed in bees exposed to 37.5, 75.0 and 150 µg/bee whereas bees exposed to control, 9.4 and 18.8 µg/bee appeared normal.
- On day 7, mortality and toxicity signs (affected) was observed in bees exposed to18.8, 37.5, 75.0 and 150 µg/bee whereas bees exposed to control and 9.4 µg/bee appeared normal.
- On day 8, mortality and toxicity signs (affected) was observed in bees exposed 18.8, 37.5, 75.0 and 150 µg /bee whereas bees exposed to control and 9.4 µg /bee appeared normal.
- On day 9, mortality and toxicity signs (affected) was observed in bees exposed to18.8, 37.5, 75.0 and 150 µg /bee whereas bees exposed to control and 9.4 µg/bee appeared normal.

Table 1: Dose response test – summary of mortality and test item intake

Initial		Consumed		No. of tested bees	Mortality		LC_{50}	LDD_{50}
Concentration [mg /kg of food]	Dose [µg/20mg/ bee/ day]	Concentration [mg /kg of food]	Dose [µg/bee/ day]		total No. of bees [%]		[mg/kg]	[µg/bee/ day]
Azadirachtin 1% EC								
0.0 Control				30	0	0.00	2286.14 ±307.76	47.48 ±5.85
468.8	9.40	470.00	11.08	30	3	10.00		
937.5	18.80	940.00	21.16	30	8	26.67		
1875	37.50	1875.00	39.85	30	11	36.67		
3750	75.00	3750.00	72.07	30	17	56.67		
7500	150.00	7500.00	147.00	30	28	93.33		
Dimethoate								
0.8	0.016	0.8	0.009	30	23	76.67	Not determined	
MCPA 750 g/L SL	**NOEC [mg/kg]**			468.8				
	NOEDD [µg /bee/ day]			11.08				

Table 2: Dose response test – summary of feed consumption data

Initial		Consumed		Consumption of A 50% Sucrose Solution* [mg/bee/day]			Average con-sumption [mg/bee/day]
Concentration [mg/kg of food]	Dose [µg/20mg/bee/ day]	Concentration [mg/kg of food]	Dose [µg/bee/ day]	replicates			
				I	II	III	
Azadirachtin 1% EC							
0.0 Control				24.98	24.73	25.76	25.16
468.8	9.4	470	11.08	23.33	23.76	23.81	23.63
937.5	18.8	940	21.16	22.50	22.18	23.03	22.57
1875	37.5	1875	39.85	19.58	21.73	22.45	21.25
3750	75.0	3750	72.07	17.45	21.45	18.76	19.22
7500	150.0	7500	147.00	25.24	19.03	14.54	19.60
0.8 (Reference)	0.02	0.8	0.009	11.54	10.86	10.17	10.86

24.17

Table 3: Endpoints

Endpoint	Value	Value Based on Azadirachtin[a] content
LDD_{50}	47.48 ± 5.85 µg/bee	0.63 ± 0.08 µg as/bee
LC_{50}	2286.14 ± 307.76mg/kg food	30.33± 4.0 mg as/kg food.

NOEDD	11.08 µg /bee	0.15 µg as/bee
NOEC	468.8 mg /kg	6.22 mg as/kg

a - based on the content of **Azadirachtin** in the test the item, i.e., 1.3% w/v, Density: 0.98 g/cm^3

Validity criteria

- There was no mortality in control group.
- The average mortality in the reference substance treated group is ≥ 50 % at the end of the test (10 days following start of exposure.

Conclusion

Based on the experimental results and feed consumption (test item intake), the LDD_{50} of **Azadirachtin 1% EC** was 47.48 ± 5.85µg/bee which is equivalent to 0.63±0.08 µg azadirachtin/bee.

LC_{50} was determined as 2286.14 ± 307.76 mg/kg food which is equivalent to 30.33± 4.0 mg azadirachtin/kg food.

The NOEC was determined as 468.8 mg /kg which is equivalent to 6.22 mg azadirachtin/kg.

NOEDD was determined as 11.08 µg/bee which is equivalent to 0.15 µg azadirachtin/bee.

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

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

- Larval mortality in the controls: In control (A1), the cumulative larval mortality from D3 to D8 was 8.33% (Criterion: should be ≤15% across all control replicates).
- Adult emergence rate: In control (A1), the adult emergence rate on D22 was 83.33% respectively. (Criterion: should be ≥ 70% across all control replicates).
- Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 51.52% (Criterion: should be > 50% across all reference replicates).

Agreed endpoints:

		Up to D22	
Test item doses	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on Azadirachtin[a] content (µg a.s./larva)**
	ED_{10}	52.12	0.69
	ED_{20}	65.73	0.87
	ED_{50}	102.42	1.36
	NOED	39.50	0.52
Test item concentrations	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on Azadirachtin[a] content (µg a.s./larva)**
	ED_{10}	338.82	4.495
	ED_{20}	427.25	5.668
	ED_{50}	665.81	8.832
	NOED	256.80	3.407

[a]: based on the content of Azadirachtin in the test the item, ie., 1.3% w/w; density; 0.98 g/cm given by the sponsor

Reference: KCP 10.3.1.3

Report "Effect of Azadirachtin 1% EC on larvae of honey bee, *Apis mellifera* (L.)

	following repeated exposure". Ms. K. Lakshmi Prabha. 2022. Bioscience Research Foundation. Report No. 9035/2021
Guideline(s):	OECD Guideline for the testing of chemicals, No. 239 (2016): Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Azadirachtin 1% EC Batch No.: SCL-280919
Test system:	Species: *Apis mellifera* (L). strain carnica Source: Bee hive maintained at BRF test facility Stage: First instalar larvae (one day old) during grafting of queen-right colonies in good health conditions.
Dose response test:	Control, 39.5, 59.3, 88.9, 133.3 and 200 µg a.s./ larvae.
Number of adult bees:	12 bees/ replicate 3 replicates 5 concentrations
Duration:	22 days

Results and discussions

On D8, larval mortalities of 8.33% was observed in control group. Pupal mortality (between D8 and D15) was 0% in the control group. The control group showed a total mortality of 16.67% (A1) on D22.

In the test item group 39.5, 59.3, 88.9, 133.3 and 200 200 µg/ larvae, larval mortalities on D8 was 0.00, 3.03, 12.12, 33.33 and 42.42%. Pupal mortalities 2.78, 5.56, 11.11, 25.00 and 30.56% in the test item treatment groups. Total mortalities at D22 was 3.33, 16.67, 36.67, 70.00 and 90.00%.

Mortality in the reference (R1) was 51.52% across all replicates on D8, Pupal mortality was D(15) 16.67%. Total mortalities at D22 was 86.67% respectively.

Table 1: Toxicity of Azadirachtin 1% EC to larvae of *Apis mellifera L.*

Treatment group	Test solution (ID)	Dose (µg/ larva)	Conc. (mg/kg food)	On D8			On D15		On D 22		
				Larval mortality D3 to D8		Mean (OO)	Pupal stage D8 to D15		Total mortality D3-D22		Adult emergence rate %
				mor (%)	corr (%)	(%)	mor (%)	corr (%)	mor (%)	corr (%)	(%)
Control	A1	-	-	8.33	-	0	0.00	-	16.67	-	83.33
Test item	T1	39.5	256.8	8.33	0.00	0	2.78	2.78	19.44	3.33	80.56
	T2	59.3	385.2	11.11	3.03	0	5.56	5.56	30.56	16.67	69.44
	T3	88.9	578	19.44	12.12	0	11.11	11.11	47.22	36.67	52.78
	T4	133.3	867	38.89	33.33	0	25.00	25.00	75.00	70.00	25.00
	T5	200	1300	47.22	42.42	0	30.56	30.56	91.67	90.00	8.33
Ref. item	R1	7.39	48	55.56	55.56	0	16.67	16.67	88.89	86.67	11.11

Note: D-Day, Mor- Mortality, corT.-Corrected Mortality, O0-Other observation. Results are averages based on 3 replicates, containing 12 larvae each;
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): reference item was corrected by Al and test item was corrected by A2; negative values are set to "O"; calculations are performed with non-rounded values; CL. confidence limit
00: Other observations (e. g. remaining food)
1 Average% of pupal mortality was calculated according to the following formula:
Sum of dead between D8 and D22 / Sum of living larvae on D8 x 100%
2 Adult emergence [%] = 100 [%] – Mortality of D22 [%]

		Up to D22	
Test item doses	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on Azadirachtin[a] content (µg a.s./larva)**
	ED_{10}	52.12	0.69
	ED_{20}	65.73	0.87
	ED_{50}	102.42	1.36
	NOED	39.50	0.52
Test item concentrations	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on Azadirachtin[a] content (µg a.s./larva)**
	ED_{10}	338.82	4.495
	ED_{20}	427.25	5.668
	ED_{50}	665.81	8.832
	NOED	256.80	3.407

[a]: based on the content of Azadirachtin in the test the item, ie., 1.3% w/w; density; 0.98 g/cm given by the sponsor

Validity criteria

- Larval mortality in the controls: In control (A1), the cumulative larval mortality from D3 to D8 was 8.33% (Criterion: should be ≤15% across all control replicates).
- Adult emergence rate: In control (A1), the adult emergence rate on D22 was 83.33% respectively. (Criterion: should be ≥ 70% across all control replicates).
- Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 51.52% (Criterion: should be > 50% across all reference replicates).

Conclusion

In a repeated exposure larval toxicity study with Azadirachtin 1% EC, the ED_{50} (successful adult emergence up to D22) was calculated to be 102.42 ± 3.70 µg/larva (i.e., 1.36 µg Azadirachtin/larva), which is equivalent to an EC_{50} of 665.81 ± 24.05 mg/kg (i.e., 5.4 mg Azadirachtin/kg food)

The ED_{10} (successful adult emergence up to D22) was calculated to be 52.12 µg/larva (i.e., 0.69 µg Azadirachtin/larva) which is equivalent to an EC_{10} of 338.82 mg/kg (i.e., 4.495 mg Azadirachtin/kg food).

The ED_{20} (successful adult emergence up to D22) was calculated to be 65.73 µg/larva (i.e., 0.87 µg Azadirachtin/larva) which is equivalent to an EC_{20} of 427.25 mg/kg (i.e, 5.668 mg Azadirachtin/kg food).

The NOED was 39.5 ug/larva (i.e., 0.52 mg Azadirachtin/larva) and the corresponding NOEC was 256.8 mg/kg food (i.e., 3.407 mg Azadirachtin/kg food).

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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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

Please, refer to the core dossier.

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