REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: ASA-01

Product name(s): **VIARES**

Chemical active substance:

Acetamiprid, 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: XXXX

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When	What
2025-03-24	Update on evaluator request
May 2025	Version evaluated 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: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter rape (BRSNW)	F	pollen beetle *Brassicogethes aeneus* (MEIAE)	spray	Spring BBCH 50-60	a) 1 b) 1	-	a) 0.08-0.1 L/ha b) 0.08-0.1 L/ha	a) 24-30 g a.s./ha b) 24-30 g a.s./ha	200-400 L/ha	NR	-							
2	PL	Apple (MABSD)	F	Aphids *Aphdidae* (APXXSP)	spray	Spring BBCH 56-75	a) 1 b) 1	-	a) 0.03-0.05 L/10000 m^2 LWA b) 0.03-0.05 L/10000 m^2 LWA	a) 9-15 g a.s./10000 m^2 LWA b) 9-15 g a.s. /10000 m^2 LWA	500-900 L/ha	14 days	max. 0.075 L/ha max. 22.5 g as/ha							
3	PL	Apple (MABSD)	F	codling moth *Cydia pomonella* (CARPPO)	spray	Spring BBCH 57-75	a) 1 b) 2	7-10 days	a) 0.07-0.09 L/10000 m^2 LWA b) 0.14-0.18 L/ 10000 m^2 LWA	a) 21-27 g a.s./10000 m^2 LWA b) 42-54 g a.s. /10000 m^2 LWA	500-750 L/ha	14 days	max. 0.09 L/ha max. 27 g as/ha							
Minor uses art. 51																				
4	PL	Wild apple (MABSY) Pear (PYUCO) Chinese Pear (PYULI) Quince (CYDOB) Medlar (MSPGE)	F	Aphids *Aphdidae* (APXXSP)	spray	Spring BBCH 56-75	a) 1 b) 1	-	a) 0.03-0.05 L/10000 m^2 LWA b) 0.03-0.05 L/10000 m^2 LWA	a) 9-15 g a.s./10000 m^2 LWA b) 9-15 g a.s. /10000 m^2 LWA	500-900 L/ha	14 days	max. 0.075 L/ha max. 22.5 g as/ha							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
5	PL	Wild apple (MABSY) Pear (PYUCO) Chinese Pear (PYULI) Quince (CYDOB) Medlar (MSPGE)	F	codling moth *Cydia pomonella* (CARPPO)	spray	Spring BBCH 57-75	a) 1 b) 2	7-10 days	a) 0.07-0.09 L/10000 m^2 LWA b) 0.14-0.18 L/ 10000 m^2 LWA	a) 21-27 g a.s./10000 m^2 LWA b) 42-54 g a.s. /10000 m^2 LWA	500-750 L/ha	14 days	max. 0.09 L/ha max. 27 g as/ha							

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

9.1.1 Overall conclusions

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

Birds

Effects on birds for ASA-01 were not evaluated as part of the EU review of acetamiprid. However further data on ASA-01 is not relevant as data for the active substance on toxicity to birds are considered essential. It is possible to extrapolate from data for the active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for ASA-01 with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of ASA-01 to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance, as well as SV_{90} and SV_m.

Drinking water exposure leaf scenario has not been performed since ASA-01 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. Drinking water exposure puddle scenario has not been performed since the ratios of effective application rates to relevant endpoints do not exceed 50 (Koc < 500 L/kg).

Exposure for earthworm-eating birds and fish-eating birds via secondary poisoning was not required since log P_{ow} of acetamiprid are blow the trigger value of 3.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to birds from the proposed use of ASA-01. No risk management measures are required.

Terrestrial vertebrates (other than birds)

Effects on mammals for ASA-01 were not evaluated as part of the EU review of acetamiprid. However further data on ASA-01 is not relevant as data for the active substance on toxicity to mammals are considered essential. It is possible to extrapolate from data for the active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for ASA-01 with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of ASA-01 to mammals were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance, as well as SV_{90} and SV_m.

Drinking water exposure puddle scenario has not been performed since the ratios of effective application rates to relevant endpoints do not exceed 50 (Koc < 500 L/kg).

Exposure for earthworm-eating mammals and fish-eating mammals via secondary poisoning was not required since log P_{ow} of acetamiprid are blow the trigger value of 3.

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

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Effects on aquatic organisms for ASA-01 were not evaluated as part of the EU review of acetamiprid. Acute toxicity studies of ASA-01 to invertebrates and algae were submitted in this dossier.

Risk assessments for ASA-01 with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290.

PECsw/RAC values were calculated with PECsw values obtained for active substance and its metabolites calculated in Steps 1, 2, 3 and 4 were below the 1 for acute and long-term risk indicating no unacceptable risk to aquatic organisms for most scenarios. For scenarios with PEC/RAC above 1 safe use has not been confirmed so further risk mitigations from and risk refinement is required at national level.

<table>
<tr><td colspan="3">rape 1 x 30 g as/ha (use no. 1)</td></tr>
<tr><td>D2 ditch
D2 stream
D3 ditch
D4 stream
D5 stream
R1 stream
R3 stream</td><td colspan="2">10mVFS + 10 m NSS + 50%DRN or
20mVFS + 20 m NSS</td></tr>
<tr><td>D4 pond
D5 pond
R1 pond</td><td colspan="2">NR</td></tr>
<tr><td colspan="3">orchards 1 x 22.5 g as/ha (use no. 2)</td></tr>
<tr><td>D3 ditch
R1 stream
R4 stream</td><td rowspan="3">20mVFS + 100 m NSS or
20mVFS+50m NSS +50% DRN</td><td>~~20mVFS +90%DRN or~~
~~20mVFS+50mFS+50%DRN~~</td></tr>
<tr><td>D4 stream
D5 stream
R3 stream</td><td>~~20mVFS +90%DRN or~~
~~20mVFS+50mFS+50%DRN or~~
~~20mVFS+100mFS~~</td></tr>
<tr><td>D4 pond
D5 pond
R1 pond</td><td>~~5mFS+90%DRN or~~
~~10mFS+75%DRN or~~
~~15mFS+50%DRN or~~
~~20mFS~~</td></tr>
<tr><td colspan="3">orchards 2 x 27 g as/ha (use no. 3)</td></tr>
<tr><td>D4 stream
D5 stream</td><td rowspan="2">20mVFS + 100 m NSS or
20mVFS+50m NSS +90% DRN</td><td>~~20mVFS+50mFS+90%DRN or~~
~~20mVFS+100mFS~~</td></tr>
<tr><td>D3 ditch
D4 pond
D5 pond
R1 pond
R1 stream
R3 stream
R4 stream</td><td>~~20mVFS+90%DRN or~~
~~20mVFS+50mFS~~</td></tr>
</table>

~~FS – filter strip~~ VFS – vegetated filter strip DRN – drift reducing nozzles
NSS – non-sprayed strip

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that ASA-01 in accordance with GAP does not pose unacceptable risk to aquatic organisms under condition that appropriate risk mitigations are ~~20m vegetated buffer zone is~~ applied in rape (use no. 1) and orchards (use no. 2, 3, 4, 5). ~~In case of rape (use no. 1) no risk mitigations measures are required.~~

For Poland D3, D4 and R1 scenarios are characteristic so proposed risk mitigations measures are:

rape 1 x 30 g as/ha (use no. 1)	10mVFS +10 m NSS + 50%DRN or 20mVFS +20 m NSS
orchards 1 x 22.5 g as/ha (use no. 2, 4)	20mVFS + +20 m NSS +90%DRN or 20mVFS+50m NSS FS+50%DRN or 20mVFS+100m NSS FS
orchards 2 x 27 g as/ha (use no. 3, 5)	20mVFS+50m NSS FS +90%DRN or 20mVFS+100m NSS FS

FS – filter strip VFS – vegetated filter strip DRN – drift reducing nozzles
NSS – non-sprayed strip

Classification of ASA-01 was done on the basis of formulation test results as well as active substance properties. The proposed classification of the product ASA-01 is:

Aquatic Chronic 1, H410

9.1.1.3 Effects on bees (KCP 10.3.1)

Effects on bees for ASA-01 were not evaluated as part of the EU review of acetamiprid. Toxicity studies of ASA-01 to bees and bumblebees were submitted in this dossier.

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

The risks of ASA-01 to honeybees was assessed from Hazard Quotients (HQ) and Exposure Toxicity Ratio (ETR) between toxicity endpoints, estimated from acute oral and contact studies with formulated product as well as the maximum single application rate.

All the hazard quotients were considerably less than the respective triggers, indicating that ASA-01 in accordance with GAP poses a low risk to bees. No risk management measures are required.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Effects on non-target arthropods for ASA-01 were not evaluated as part of the EU review of acetamiprid. Toxicity studies of ASA-01 to non-target arthropods were submitted in this dossier.

Risk assessments for ASA-01 with the proposed use pattern was carried out according to the guidance for risk assessment for arthropods "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and in consideration of the recommendations of the guidance document ESCORT 2.

The in-field risk of ASA-01 to non-target arthropods was evaluated by comparison of % effects rate with derived from laboratory tests and in-field predicted rate. The off-field risk of ASA-01 to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from laboratory tests with the formulated product ASA-01 as well as off-field predicted environmental rate. No risk was determined in-field and off-field after application of ASA-01 in accordance with GAP. No risk management measures are required.

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

Effects on earthworms and other soil micro-organisms for ASA-01 were not evaluated as part of the EU review of acetamiprid. The toxicity studies to earthworm and *Hypoaspis aculeifer* as well as nitrogen transformation test for ASA-01 were submitted in this dossier.

Risk assessments for ASA-01 with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Earthworms, *Folsomia candida* and *Hypoaspis aculeifer*
The risk of ASA-01 to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* was assessed from toxicity exposure ratio (TER) between the selected toxicity endpoints for metabolite IM-1-5 s and the formulated product ASA-01 as well as the maximum soil PECs.

The chronic TER values were greater than the trigger of 5, indicating an acceptable risk to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* following application of ASA-01 in accordance with GAP. No risk management measures are required.

Micro-organisms
The risk of ASA-01 to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for metabolite IM-1-5 and the formulated product ASA-01 with the maximum soil PECs.

According to the performed risk assessment it was assessed that the application of ASA-01 in accordance with GAP does not pose unacceptable risk to soil micro-organisms. No risk management measures are required.

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

Effects on non-target terrestrial plants for ASA-01 were not evaluated as part of the EU review of acetamiprid. The studies on seedling emergence and vegetative vigour for ASA-01 were submitted in this dossier.

The risk of ASA-01 to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation ASA-01 and off-field predicted environmental rate. The TER values were greater than the trigger of 5, indicating an acceptable risk to non-target terrestrial plants following application of ASA-01 in accordance with GAP. No risk management measures are required.

The risk of ASA-01 to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation ASA-01 and off-field predicted environmental rate. The TER val-ues were greater than the trigger of 5, indicating an acceptable risk to non-target terrestrial plants following application of ASA-01 in accordance with GAP. No risk management measures are required.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

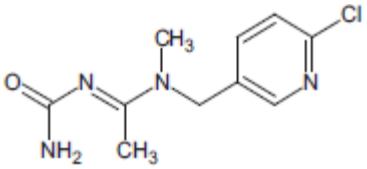
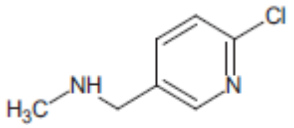
Table 9.1-2: Critical use pattern of ASA-01 grouped according to crop group

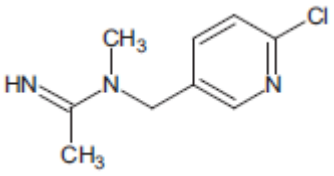
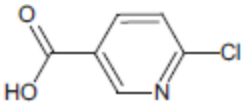
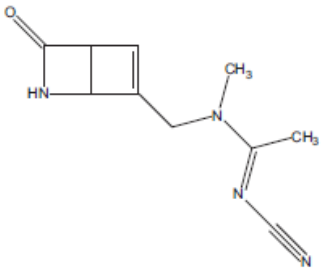
Grouping according to criterion			
Uses no	**Covered Uses no**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
1	1	Crop: rape Growth stage: BBCH 50-60 Application rate: max. 0.1 L/ha (30 g acetamiprid/ha) Number of applications: 1 Application interval: N/A	Relevant parameter for: all areas of the risk assessment
2	2, 4	Crop: apple tree Growth stage: BBCH 56-75 Application rate: max. 0.075 L/ha (22.5 g acetamiprid/ha) Number of applications: 1 Application interval: N/A	Relevant parameter for: all areas of the risk assessment
3	3, 5	Crop: apple tree Growth stage: BBCH 57-75 Application rate: max. 0.09 L/ha (27 g acetamiprid/ha) Number of applications: 2 Application interval: 7	Relevant parameter for: all areas of the risk assessment

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 ASA-01 is indicated in the table.

Table 9.1-3 Metabolites of acetamiprid

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IM-1-2		240.69	Maximum in soil: 55% Maximum in water/sediment: 13.4%	Soil: yes Water/sediment: yes
IM-1-4		156.61	Maximum in soil: 72% Maximum in water/sediment: 81.5% (aerobic mineralisation study)	Soil: yes Water/sediment: yes

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IM-1-5		197.66	Maximum in soil: 20% (calcareous soils only)	Soil: yes Water/sediment: yes
IC-0 6-chloronicotinic acid (IV-0)		157.55	Maximum in soil: 11.3% Maximum in water/sediment: 29.5%	Soil: yes Water/sediment: yes
IB-1-1		204.23	Maximum in water/sediment: 35% (aqueous photochemical degradation)	Soil: no Water/sediment: yes

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The submitted screening step and first tier assessment of the acute and long-term risk for birds were accepted. The endpoints used in risk assessment were agreed at the EU level (EFSA Journal 2016;14(11):4610). **Acetamiprid**. The TER_A values for birds are above the trigger value of 10 at screening step (orchard) and at first tier assessment (oilseed rape) indicating an acceptable acute risk for birds. The TER_{LT} values for long-term risk are above the trigger value of 5 at screening step (orchard) and at first tier assessment (oilseed rape) indicating an acceptable acute risk for birds. No further refinement is required. The risk to birds following application of ASA-01 in accordance with the proposed pattern use is acceptable.

9.2.1 Toxicity data

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

Effects on birds of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. However, the provision of further data on the ASA-01 is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Anas plaryrhynchos (mallard duck)	acetamiprid	Acute	LD_{50} = 98 mg/kg bw	EFSA Journal 2016;14(11):4610
Colinus virginianus (bobwhite quail)	acetamiprid	Acute	LD_{50} > 100 mg/kg bw	EFSA Journal 2016;14(11):4610
Poephila guttata (zebra finch)	acetamiprid	Acute	LD_{50} = 5.7 mg/kg bw	EFSA Journal 2016;14(11):4610
Geometric mean	acetamiprid	Acute	**LD_{50} = 38.2 mg/kg bw**	EFSA Journal 2016;14(11):4610
	acetamiprid	Long-term	**LD_{50} /10 = 3.8 mg/kg bw**	EFSA Journal 2016;14(11):4610
Anas plaryrhynchos (mallard duck)	acetamiprid	Long-term	NOAEL = 9.5 mg/kg bw/d	EFSA Journal 2016;14(11):4610

9.2.1.1 Justification for new endpoints

Not relevant. No new endpoints were used.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use no. 3 also covers the risk for birds from uses no. 2, 4 and 5 (see 9.1.2).

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ASA-01 in rape (use no. 1)

Intended use	rape (use no. 1)				
Active substance/product	acetamiprid				
Application rate (g/ha)	1 × 30				
Acute toxicity (mg/kg bw)	38.2				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

Screening assessment					
winter rape appl. rate: 30 g as/ha	Small omnivorous bird	158.8	1	4.76	**8.03**
First-tier assessment					
winter rape BBCH 50-60 appl. rate: 30 g as/ha	Small insectivorous bird "dunnock" BBCH 30-99	7.4	1	0.22	173.64
	Small omnivorous bird "lark" BBCH ≥ 40	6.0	1	0.18	212.22
	Medium herbivorous/ granivorous bird "pigeon" BBCH ≥ 40	2.0	1	0.06	636.67
Reprod. toxicity (mg/kg bw/d)	3.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
winter rape appl. rate: 30 g as/ha	Small omnivorous bird	64.8	1 x 0.53	1.03	**3.69**
First-tier assessment					
winter rape BBCH 50-60 appl. rate: 30 g as/ha	Small insectivorous bird "dunnock" BBCH 30-99	2.7	1 x 0.53	0.04	95
	Small omnivorous bird "lark" BBCH ≥ 40	2.7	1 x 0.53	0.04	95
	Medium herbivorous/granivorous bird "pigeon" BBCH ≥ 40	0.9	1 x 0.53	0.01	380

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.

The acute and reproductive screening TER values for small omnivorous bird was below the relevant trigger values in oilseed rape (use no. 1) and further evaluation was necessary. The acute and reproductive first-tier TER values for small insectivorous bird, small omnivorous bird and medium herbivorous/granivorous bird exceed the relevant trigger value of 10 and 5, respectively, indicating no unacceptable acute and reproductive risk following applications of ASA-01 in oilseed rape (use no. 1).

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)				
Active substance/product	acetamiprid				
Application rate (g/ha)	2 × 27				
Acute toxicity (mg/kg bw)	38.2				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					

orchards appl. rate: 2 x 27 g as/ha, interval 7	small insectivorous bird	46.8	1.4	1.77	21.58
Reprod. toxicity (mg/kg bw/d)	3.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
orchards appl. rate: 2 x 27 g as/ha, interval 7	small insectivorous bird	18.2	1.6 x 0.53	0.42	9.05

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.

The acute and reproductive screening TER values for small insectivorous bird exceed the relevant trigger value of 10 and 5, respectively, indicating no unacceptable acute and reproductive risk following applications of ASA-01 in orchards (use no. 2, 3, 4, 5).

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 ASA-01 is not a product for spray applications / 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 106.5 (arithmetic mean), acetamiprid 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 no. 3 also covers the risk for birds from all other intended uses (see 9.1.2).

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 since 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).

Effective application rate (g/ha) =	2 x 27 (worst case)			
Acute toxicity (mg/kg bw) =	38.2	quotient	=	1.41
Reprod. toxicity (mg/kg bw/d) =	3.8	quotient	=	14.2

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of acetamiprid amounts to 0.8 at 25 °C (neutral pH) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. ASA-01 does not pose unacceptable risk to birds following application in accordance with GAP. No risk management measures are required.

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

zRMS Comments:	The submitted screening step and first tier assessment of the acute and long-term risk for mammals were accepted. A higher tier reproductive risk assessment for frugivorous mammal “dormouse” in orchards was also accpetd. The endpoints used in risk assessment were agreed at the EU level (EFSA Journal 2016;14(11):4610). **Acetamiprid**. Oilseed rape. The TER_A and TER_{LT} values for mammals are above the trigger value of 10 and 5, respectively at screening step (acute) and at first tier assessment (long-term/reproductive) indicating an acceptable risk for mammals. Orchards. The TER_A values for long-term risk are above the trigger value of 10 at screening step. In first tier assessment the long-term risk was identified for frugivorous mammal, dormouse, at BBCH 71-79. Further risk refinement was required. The refinement based on deposition factor was accepted. TER_{LT} value is above the trigger value of 5 indicating an acceptable reproductive risk for mammals. No further refinement is required.

	The risk to mammals following application of ASA-01 in accordance with the proposed pattern use is acceptable.

9.3.1 Toxicity data

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

Effects on mammals of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. However, the provision of further data on the ASA-01 is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	acetamiprid	Acute	**LD_{50} = 146 mg/kg bw**	EFSA Journal 2016;14(11):4610
Rat	acetamiprid	Long-term 90-d study	NOAEL = 12.4 mg/kg bw	EFSA Journal 2016;14(11):4610
Rat	acetamiprid	Long-term Developmental neurotoxicity study	**NOAEL = 2.5 mg/kg bw**	EFSA Journal 2016;14(11):4610

9.3.1.1 Justification for new endpoints

Not relevant. No new endpoints were used.

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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use no. 3 also covers the risk for mammals from use no. 2, 4, 5 (see 9.1.2).

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ASA-01 in rape (use no. 1)

Intended use	rape (use no. 1)				
Active substance/product	acetamiprid				
Application rate (g/ha)	1 × 30				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
winter rape appl. rate: 30 g as/ha	Small herbivorous mammal	118.4	1	3.55	41.13
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
winter rape appl. rate: 30 g as/ha	Small herbivorous mammal	48.3	1 x 0.53	0.77	**3.25**
First-tier assessment					
winter rape BBCH 50-60 appl. rate: 30 g as/ha	small insectivorous mammal "shrew" BBCH ≥ 20	1.9	1 x 0.53	0.03	83.33
	small herbivorous mammal "vole" BBCH ≥ 40	18.1	1 x 0.53	0.29	8.62
	Large herbivorous mammal "lagomorph" all season	14.3	1 x 0.53	0.23	10.87
	Small omnivorous mammal "mouse" BBCH ≥ 40	1.9	1 x 0.53	0.03	83.33

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.

The acute screening TER value for small herbivorous mammals exceed the relevant trigger value of 10, indicating no unacceptable acute risk following applications of ASA-01 in oilseed rape (use no. 1). However, in case of reproductive exposure, screening risk assessment failed, and further evaluation was necessary. The reproductive first-tier TER values for small insectivorous mammals, small herbivorous mammals, large herbivorous mammals and small omnivorous mammal exceed the relevant trigger value of 5, indicating no unacceptable reproductive risk following applications of ASA-01 in oilseed rape (use no. 1).

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)
Active substance/product	acetamiprid
Application rate (g/ha)	2 × 27
Acute toxicity (mg/kg bw)	146
TER criterion	10

Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** **(mg/kg bw/d)**	**TER_a**
Screening assessment					
orchards appl. rate: 2 x 27 g as/ha, interval 7	Small herbivorous mammal	136.4	1.4	5.16	28.29
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m** **(mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
orchards appl. rate: 2 x 27 g as/ha, interval 7	Small herbivorous mammal	72.3	1.6 x 0.53	1.66	**1.51**
First-tier assessment					
Orchards BBCH 56-75 appl. rate: 22.5 g as/ha (use no. 2, 4)	Small herbivorous mammal "vole" BBCH ≥ 40	21.7	1 x 0.53	0.26	9.62
	Frugivorous mammal "dormouse" BBCH 71-79	22.7	1 x 0.53	0.27	9.26
	Large herbivorous mammal "lagomorph" BBCH ≥ 40	4.3	1 x 0.53	0.05	50
	Small omnivorous mammal "mouse" BBCH ≥ 40	2.3	1 x 0.53	0.03	83.33
Orchards BBCH 56-75 appl. rate: 2 x 27 g as/ha, interval 7 (use no. 3, 5)	Small herbivorous mammal "vole" BBCH ≥ 40	21.7	1.6 x 0.53	0.50	5.0
	Frugivorous mammal "dormouse" BBCH 71-79	22.7	1.6 x 0.53	0.52	**4.81**
	Large herbivorous mammal "lagomorph" BBCH ≥ 40	4.3	1.6 x 0.53	0.1	25
	Small omnivorous mammal "mouse" BBCH ≥ 40	2.3	1.6 x 0.53	0.05	50

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.

The acute screening TER value for small herbivorous mammals exceed the relevant trigger value of 10, indicating no unacceptable acute risk following applications of ASA-01 in orchards (use no. 2, 3, 4, 5). However, in case of reproductive exposure, screening risk assessment failed, and further evaluation was necessary. The reproductive first-tier assessment was performed for use no. 2, 4 and use no. 3, 5 separately.

The reproductive TER values for small herbivorous mammals, frugivorous mammal, large herbivorous mammals and small omnivorous mammal exceed the relevant trigger value of 5, indicating no unacceptable reproductive risk following applications of ASA-01 in orchards (use no. 2, 4).

In case of orchards (use no. 3, 5), the reproductive TER values for herbivorous mammals and omnivorous mammals exceed the relevant trigger value of 5. However, in case of frugivorous mammals, the reproductive TER was below of trigger of 5, indicating that risk following applications of ASA-01 in orchards (use no. 3, 5) cannot be excluded and further higher-tier risk assessment is necessary.

9.3.2.2 Higher-tier risk assessment

The higher-tier reproductive risk assessment for frugivorous mammal "dormouse" are conducted according to recommendations of EFSA/2009/1438.

Frugivorous mammal "dormouse"

The food intake rate per body weight for garden dormouse (*Eliomys quercinus*) consuming 100% of currants equals 1.16 g fw/g bw/d and mean RUD in currants equals 19.5 mg/kg food in accordance with Appendix A of EFSA Journal 2009; 7(12): 1438 was used for risk refinement. Additionally, a deposition factor (DF) of 0.4 (BBCH 56) in accordance with EFSA Journal 2014;12(5):3662 was applied since at this stage it can be assumed that fruits are covered by leaves and frugivorous mammal feeds mostly on ground under tree.

Table 9.3-4: Hier-tier assessment of the long-term/reproductive risk for frugivorous mammals due to the use of ASA-01 in orchards (use no. 3)

Intended use		orchards (use no. 3)					
Active substance/product		acetamiprid					
Application rate (g/ha)		2 × 27, interval 7d					
Chronic toxicity (mg/kg bw)		2.5					
TER criterion		5					
Focal species	**Food category, % in diet**	**FIR/bw**	**RUDm × DF (mg/kg food)**	**MAFm × TWA**	**PT**	**DDDm (mg/kg bw/d)**	**TERlt**
garden dormouse BBCH 56-75 appl. rate: 2 x 27 g as/ha, interval 7 (use no: 3, 5)	Orchard fruits, 100%	1.16	19.5 × 0.4	1.6 x 0.53	1	0.21	11.9

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.

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 106.5 (arithmetic mean), acetamiprid 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 no. 3 also covers the risk for mammals from all other intended uses (see 9.1.2).

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 since 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).

Effective application rate (g/ha) =	2 x 27 (worst case)			
Acute toxicity (mg/kg bw) =	146	quotient	=	0.37
Reprod. toxicity (mg/kg bw/d) =	2.5	quotient	=	21.6

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of acetamiprid amounts to 0.8 at 25 °C (neutral pH) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. ASA-01 does not pose unacceptable risk to mammals following application in accordance with GAP. No risk management measures are required.

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

9.5 Effects on aquatic organisms (KCP 10.2)

Evaluation Comments: IIIA 10.5	The endpoints used for substance risk assessment were agreed at the EU level. New studies were submitted and evaluated in Appendix 2. The study with additional aquatic plant species *Myriophyllum spicatum* was submitted and accepted. The RAC = 0.0235 µg/L for sediment dwelling organisms represents the worst case and was used in risk assessment.

The following application pattern was taken into consideration:

- Winter OSR 1 x 30 g a.s./ha;
- Pome fruits 1 x 22.5 g a.s./ha;
- Pome fruits 2 x 27 g a.s./ha.

For risk assessment the PECsw and PECsed values evaluated in Section 8 (Step 4) were taken into consideration.
The mitigation measures were proposed for every application rate.

Crop	**Application rate g a.s./ha**	**Mitigation measure**
Winter OSR	1 x 30	20 m NSS + 20 VFS m or 10 m NSS + 10 VFS m + 50%
Pome fruits	1 x 22.5	100 m NSS + 20 VFS m or 50 m NSS + 20 VFS m + 50% DRT or 20mVFS +20 m NSS +90%DRT
Pome fruits	2 x 27	100 m NSS + 20 VFS m or 50 m NSS + 20 VFS m + 90% DRT

The proper mitigation measures should be considered at MS level in accordance with the national requirements.

For **Poland** relevant scenarios (D3, D4 and R1) were considered and following mitigation measures are required:

- Winter OSR 20 m NSS + 20 VFS m or 10 m NSS + 10 VFS m + 50%;
- Pome fruits 100 m NSS + 20 VFS m or 50 m NSS + 20 VFS m + 50% DRT;
- Pome fruits 100 m NSS + 20 VFS m or 50 m NSS + 20 VFS m + 50% DRT or 20 m NSS + 20 VFS m + 59% DRT

Metabolites of acetamipirid. All relevant metabolites were considered. The risk is acceptable for all relevant metabolites based on PECsw values obtained in Step 1.

Formulation ASA-01. The proposed mitigation measures were added by evaluator (using the Drift Calculator in SWASH model):

- Winter OSR 1 m NSS;
- Pome fruits 20 m NSS;
- Pome fruits 20 m NSS

Based on above mentioned formulation risk assessment caused by drift – no additional mitigation measure is required.

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with acetamiprid and its relevant

metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on aquatic organisms of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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 – acetamiprid and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish - acute				
Oncorhynchus mykiss	acetamiprid	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Journal 2016;14(11):4610
Lepomis macrochirus	acetamiprid	96 h, f	LC_{50} > 119.3 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Cyprinodon variegatus	acetamiprid	96 h, f	LC_{50} = 100 mg a.s./L $_{nom}$	EFSA Journal 2016;14(11):4610
Oncorhynchus mykiss	metabolite IM-1-4	96 h, ss	**LC_{50} > 98.1 mg a.s./L**	EFSA Journal 2016;14(11):4610
Fish - chronic				
Pimephales promelas	acetamiprid	35 d, f	**NOEC = 9.4 mg a.s./L $_{mm}$** EC_{10} > 150 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Amphibians - chronic				
Xenopus laevis	acetamiprid	21 d, f	**$NOEC_{growth}$ = 2.6 mg a.s./L $_{mm}$**	EFSA Journal 2016;14(11):4610
Aquatic invertebrates - acute				
Daphnia magna	acetamiprid	48 h, s	EC_{50} = 49.8 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Chironomus riparius	acetamiprid	48 h, s	EC_{50} = 0.0207 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Gammarus fasciatus	acetamiprid	48 h, s	EC_{50} = 0.10 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Mysidopsis bahia	acetamiprid	48 h, f	EC_{50} = 0.066 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Gammarus pulex	acetamiprid	48 h, s	EC_{50} = 0.050 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Simulium latigonium	acetamiprid	48 h, s	EC_{50} = 0.0037 mg a.s./L $_{mm}$	EFSA Journal 2016;14(11):4610
Geometric mean aquatic insects	acetamiprid	-	**EC_{50} = 0.0085 mg a.s./L $_{mm}$**	EFSA Journal 2016;14(11):4610
Daphnia magna	Metabolite IM-1-2	48 h, ss	EC_{50} > 99.8 mg/L	EFSA Journal 2016;14(11):4610
Chironomus riparius	Metabolite IM-1-2	48 h, s	**EC_{50} = 15.0 mg/L**	EFSA Journal 2016;14(11):4610

Daphnia magna	Metabolite IM-1-4	48 h, ss	EC_{50} = 43.9 mg/L	EFSA Journal 2016;14(11):4610
Mysidopsis bahia	Metabolite IM-1-4	48 h, s	**EC_{50} = 19 mg/L**	EFSA Journal 2016;14(11):4610
Chironomus riparius	Metabolite IM-1-4	48 h, s	EC_{50} = 76.0 mg/L	EFSA Journal 2016;14(11):4610
Daphnia magna	Metabolite IM-1-5	48 h, s	**EC_{50} = 25 mg/L**	EFSA Journal 2016;14(11):4610
Chironomus riparius	Metabolite IM-1-5	48 h, s	EC_{50} = 68 mg/L	EFSA Journal 2016;14(11):4610
Daphnia magna	Metabolite IC-0	48 h, ss	**EC_{50} > 95.1 mg/L**	EFSA Journal 2016;14(11):4610
Chironomus riparius	Metabolite IC-0	48 h, s	EC_{50} > 100 mg/L	EFSA Journal 2016;14(11):4610
Daphnia magna	Metabolite IB-1-1	48 h, ss	EC_{50} > 100.8 mg/L	EFSA Journal 2016;14(11):4610
Chironomus riparius	Metabolite IB-1-1	48 h, s	**EC_{50} > 100 mg/L**	EFSA Journal 2016;14(11):4610
Aquatic invertebrates – chronic				
Daphnia magna	acetamiprid	21 d, ss	NOEC = 5 mg a.s./Lmm **EC_{10} = 2.96 mg a.s./L mm**	EFSA Journal 2016;14(11):4610
Daphnia magna	Metabolite IM-1-5	21 d, ss	**$NOEC_{rep}$ = 26 mg/L**	EFSA Journal 2016;14(11):4610
Sediment dwelling organisms				
Chironomus riparius	acetamiprid	28 d, s	$NOEC_{emerg}$ = 0.00096 mg a.s./L mm **$EC_{10emerg}$ = 0.000235 mg a.s./L mm**	EFSA Journal 2016;14(11):4610
Algae				
Scenedesmus subspicatus	acetamiprid	72 h, s	E_bC_{50} / E_rC_{50} > 98.3 mg a.s./L mm	EFSA Journal 2016;14(11):4610
Anabaena flos-aquae	acetamiprid	120 h, s	**EC_{50} >1.3 mg a.s./L mm**	EFSA Journal 2016;14(11):4610
Higher plant				
Lemna gibba	acetamiprid	14 d, s	Fronds number **EC_{50} > 1.0 mg a.s./L mm**	EFSA Journal 2016;14(11):4610
Higher-tier studies (micro- or mesocosm studies)				
Outdoor mesocosm study: Effect assessment on macroinvertebrates, zooplankton, phytoplankton, periphyton and macrophytes in outdoor mesocosms. Test substance: Acetamiprid 20 SG (Mospilan 20 SG). 2 applications with a 14 day interval. Study duration: 82 days. Treatment rates: 0.5, 1.1, 2.6 and 6.0 µg a.s./L. Endpoints: NOEC and NOEAEC <0.5 µg/L based on class 5B effects on Naididae at 0.5-6.0 µg/L . Considering however the uncertainty associated with the findings for Naididae (not expected to be more sensitive than insects based on mode of action; relatively low numbers in control, although MDD was low) the reported conclusion by the study author NOEC based on class 2 effects to derive the ETO-RAC 1.1 µg/L; NOEAEC to derive ERO-RAC 1.1 µg/L based on class 5B effects on Cloeon dipterum at 2.6 µg/L) could be acceptable in case the findings for Naididae in the present study are negated by prolonged toxicity laboratory studies (e.g. at least 28 days duration) with representative taxa of Naididae.				

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ASA-01

Species	Substance	Exposure System	Results	Reference
Daphnia magna	ASA-01	48 h, s	EC_{50} = 9.313 mg/L_{nom} (2.563 mg as/ L_{nom}) LOEC = 6.250 mg/ L_{nom} (1.720 mg as/ L_{nom}) NOEC = 3.130 mg/L_{nom} (0.861 mg as/ L_{nom})	Kacperek-Karetta Z/ 2023/ Study code: W-44-22 KCP 10.2.1.2/01
Pseudokirchneriella subcapitata	ASA-01	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ (27.54 mg a.s./L) E_yC_{50} > 100 mg/L $_{nom}$ (27.54 mg a.s./L)	Jasnota D/2020/Study code: W/50/19 KCP 10.2.1.3/01
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

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

9.5.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.5-2. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2 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 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ASA-01 in rape 1 x 30 g as/ha (use no. 1)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Sed. dwell. prolonged**	**Amphibians**	**Mesocosm**
Test species		***Cyprinodon variegatus***	***Pimephales promelas***	Geomean.	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***	***Chironomus riparius***	***Xenopis laevis***	-
Endpoint (µg/L)		LC_{50} 100 000	NOEC 9 400	EC_{50} 8.5	NOEC 2 960	EC_{50} 1 300	EC_{50} 1 000	NOEC 0.235	NOEC 2 600	NOEC/NOEAEC 1.1
AF		100	10	100	10	10	10	10	10	3
RAC (µg/L)		1000	940	0.085	296	130	100	0.0235	260	0.37
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**									
Step 1										
-	9.0787	0.01	0.01	**106.81**	0.03	0.07	0.09	**386.33**	0.03	**24.74**
Step 2										
N-Europe	0.2947	0.00	0.00	**3.47**	0.00	0.00	0.00	**12.54**	0.00	0.80
S-Europe	0.3598	0.00	0.00	**4.23**	0.00	0.00	0.00	**15.31**	0.00	0.98
Step 3										
D2/ditch	0.1926	0.00	0.00	**2.27**	0.00	0.00	0.00	**8.20**	0.00	0.52
D2/stream	0.1714	0.00	0.00	**2.02**	0.00	0.00	0.00	**7.29**	0.00	0.47
D3/ditch	0.1901	0.00	0.00	**2.24**	0.00	0.00	0.00	**8.09**	0.00	0.52
D4/pond	0.006561	0.00	0.00	**2.24**	0.00	0.00	0.00	**8.09**	0.00	0.52
D4/stream	0.1460	0.00	0.00	**1.72**	0.00	0.00	0.00	**6.21**	0.00	0.40
D5/pond	0.006561	0.00	0.00	0.08	0.00	0.00	0.00	0.28	0.00	0.02
D5/stream	0.1541	0.00	0.00	**1.81**	0.00	0.00	0.00	**6.56**	0.00	0.42
R1/pond	0.01415	0.00	0.00	0.17	0.00	0.00	0.00	0.60	0.00	0.04

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants	Sed. dwell. prolonged	Amphibians	Mesocosm
R1/stream	0.2837	0.00	0.00	**3.34**	0.00	0.00	0.00	**12.07**	0.00	0.77
R3/stream	0.1757	0.00	0.00	**2.07**	0.00	0.00	0.00	**7.48**	0.00	0.48

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 use in rape (use no. 1), calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive groups of aquatic organisms (*Daphnia magna* and Chironomus *riparius*) in most of FOCUS Steps 1-3 scenarios. Therefore, additional risk with RAC based on mesocosm studies has been performed where all PEC/RAC values were below 1 indicating no unacceptable risk.

Table 9.5-4: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for mesocosm with mitigation of spray drift and run-off for the use of ASA-01 in in rape 1 x 30 g as/ha (use no. 1)

Intended use		rape						
Active substance		acetamiprid						
Application rate (g as/ha)		1 × 30						
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	None	None	10 VFSmod	20 VFSmod
	No-spray buffer (m)	1/3	5	10	15	20	10	20
None	D2/ditch	0.1926	0.05221	0.02769	-	-	0.02769	0.01439
50 %		-	-	-	-	-	0.01385	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D2/stream	0.1714	0.06261	0.03320	-	-	0.03320	0.01725
50 %		-	-	-	-	-	0.01660	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D3/ditch	0.1901	0.05154	0.02734	-	-	0.02734	0.01421
50 %		-	-	-	-	-	0.01367	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D4/pond	0.006561	0.005676	0.004080	-	-	0.004080	0.002725
50 %		-	-	-	-	-	0.002040	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D4/stream	0.1460	0.05335	0.02829	-	-	0.02829	0.01470
50 %		-	-	-	-	-	0.01414	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D5/pond	0.006561	0.005677	0.004081	-	-	0.004081	0.002725
50 %		-	-	-	-	-	0.002040	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D5/stream	0.1541	0.05630	0.02986	-	-	0.02986	0.01551
50 %		-	-	-	-	-	0.01493	-
75 %		-	-	-	-	-	-	-

90 %		-	-	-	-	-	-	-
None	R1/pond	0.01415	0.01348	0.01227	-	-	0.004080	0.002725
50 %		-	-	-	-	-	0.002040	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	R1/stream	0.2837	0.2837	0.2837	-	-	0.02407	0.01251
50 %		-	-	-	-	-	0.01204	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	R3/stream	0.1757	0.06421	0.04989	-	-	0.03405	0.01769
50 %		-	-	-	-	-	0.01702	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
RAC (µg/L) 0.0235		**PEC/RAC ratio**						
None	D2/ditch	**8.2**	**2.22**	**1.18**	-	-	**1.18**	0.61
50 %		-	-	-	-	-	0.59	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D2/stream	**7.29**	**2.66**	**1.41**	-	-	**1.41**	0.73
50 %		-	-	-	-	-	0.71	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D3/ditch	**8.09**	**2.19**	**1.16**	-	-	**1.16**	0.60
50 %		-	-	-	-	-	0.58	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D4/pond	0.28	0.24	0.17	-	-	0.17	0.12
50 %		-	-	-	-	-	0.09	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D4/stream	**6.21**	**2.27**	**1.2**	-	-	**1.20**	0.63
50 %		-	-	-	-	-	0.60	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	D5/pond	0.28	0.24	0.17	-	-	0.17	0.12
50 %		-	-	-	-	-	0.09	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-

None	D5/stream	**6.56**	**2.4**	**1.27**	-	-	**1.27**	0.66
50 %		-	-	-	-	-	0.64	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	R1/pond	0.6	0.57	0.52	-	-	0.17	0.12
50 %		-	-	-	-	-	0.09	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	R1/stream	**12.07**	**12.07**	**12.07**	-	-	**1.02**	0.53
50 %		-	-	-	-	-	0.51	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-
None	R3/stream	**7.48**	**2.73**	**2.12**	-	-	**1.45**	0.75
50 %		-	-	-	-	-	0.72	-
75 %		-	-	-	-	-	-	-
90 %		-	-	-	-	-	-	-

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~~5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ASA-01 in orchards 1 x 22.5 g as/ha (use no. 2)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Sed. dwell. prolonged**	**Amphibians**	**Mesocosm**
Test species		***Cyprinodon variegatus***	***Pimephales promelas***	Geomean.	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***	***Chironomus riparius***	***Xenopis laevis***	-
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	EC_{50}	NOEC	NOEC	NOEC/NOEAEC
(µg/L)		100 000	9 400	8.5	2 960	1 300	1 000	0.235	2 600	1.1
AF		100	10	100	10	10	10	10	10	3
RAC (µg/L)		1000	940	0.085	296	130	100	0.0235	260	0.37
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**									
Step 1										
-	8.7919	0.01	0.01	**103.43**	0.03	0.07	0.09	**374.12**	0.03	**23.96**
Step 2										
N-Europe	2.1898	0.00	0.00	**25.76**	0.01	0.02	0.02	**93.18**	0.01	**5.97**
S-Europe	2.1898	0.00	0.00	**25.76**	0.01	0.02	0.02	**93.18**	0.01	**5.97**
Step 3										
D3/ditch	1.753	0.00	0.00	**20.62**	0.01	0.01	0.02	**74.60**	0.01	**4.78**
D4/pond	0.1063	0.00	0.00	**1.25**	0.00	0.00	0.00	**4.52**	0.00	0.29
D4/stream	1.858	0.00	0.00	**21.86**	0.01	0.01	0.02	**79.06**	0.01	**5.06**
D5/pond	0.1063	0.00	0.00	**21.86**	0.01	0.01	0.02	**79.06**	0.01	**5.06**
D5/stream	1.899	0.00	0.00	**22.34**	0.01	0.01	0.02	**80.81**	0.01	**5.17**
R1/pond	0.1062	0.00	0.00	**1.25**	0.00	0.00	0.00	**4.52**	0.00	0.29
R1/stream	1.424	0.00	0.00	**16.75**	0.00	0.01	0.01	**60.60**	0.01	**3.88**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants	Sed. dwell. prolonged	Amphibians	Mesocosm
R2/stream	1.909	0.00	0.00	**22.46**	0.01	0.01	0.02	**81.23**	0.01	**5.20**
R3/stream	1.993	0.00	0.00	**23.45**	0.01	0.02	0.02	**84.81**	0.01	**5.43**
R4/stream	1.392	0.00	0.00	**16.38**	0.00	0.01	0.01	**59.23**	0.01	**3.79**

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 use orchards (use no. 2, 4), calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (*Daphnia magna*, *Chironomus riparius* and mesocosm RAC) 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 and mesocosm studies.

Table 9.5-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for mesocosm with mitigation of spray drift and run-off for the use of ASA-01 in orchards x 22.5 g as/ha (use no. 2)

Intended use		orchards						
Active substance		acetamiprid						
Application rate (g as/ha)		1 × 22.5						
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	None	None	10 VFS	20 VFS
	No spray buffer (m)	1/3	5	10	15	20	10	20
None	D3/ditch	1.753	1.378	0.8459	0.3807	0.1935	0.8459	0.1935
50 %		-	0.6888	0.4230	0.1903	-	-	-
75 %		-	0.3444	0.2115	0.09516	-	-	-
90 %		-	0.1378	0.08459	0.03807	-	-	-
None	D4/pond	0.1063	0.1197	0.06561	0.03464	0.02122	0.06561	0.02122
50 %		-	0.05983	0.03281	0.01732	-	-	-
75 %		-	0.02991	0.01640	0.008660	-	-	-
90 %		-	0.01197	0.006561	0.003464	-	-	-
None	D4/stream	1.858	1.597	0.9806	0.4411	0.2242	0.9806	0.2242
50 %		-	0.7982	0.4902	0.2206	-	-	-
75 %		-	0.3993	0.2451	0.1103	-	-	-
90 %		-	0.1597	0.09806	0.04411	-	-	-
None	D5/pond	0.1063	0.1197	0.06561	0.03464	0.02122	0.06561	0.02122
50 %		-	0.05983	0.03281	0.01732	-	-	-
75 %		-	0.02991	0.01640	0.008660	-	-	-
90 %		-	0.01197	0.006561	0.003464	-	-	-
None	D5/stream	1.899	1.632	1.002	0.4507	0.2291	1.002	0.2291
50 %		-	0.8157	0.5009	0.2254	-	-	-
75 %		-	0.4080	0.2505	0.1127	-	-	-
90 %		-	0.1632	0.1002	0.04507	-	-	-
None	R1/pond	0.1062	0.1196	0.06557	0.03462	0.02121	0.06557	0.02121
50 %		-	0.05979	0.03279	0.01731	-	-	-
75 %		-	0.02990	0.01639	0.008963	-	-	-
90 %		-	0.01196	0.007332	0.004929	-	-	-
None	R1/stream	1.424	1.224	0.7515	0.3380	0.1718	0.7515	0.1718
50 %		-	0.6117	0.3756	0.1690	-	-	-
75 %		-	0.3060	0.1878	0.1453	-	-	-

90 %		-	0.1453	0.1453	0.1453	-	-	-
None	R2 stream	1.909	1.640	1.007	0.4530	0.2303	1.007	0.2303
50 %		-	0.8199	0.5035	0.2265	-	-	-
75 %		-	0.4101	0.2518	0.1133	-	-	-
90 %		-	0.1640	0.1007	0.04530	-	-	-
None	R3 stream	1.993	1.712	1.052	0.4730	0.2404	1.052	0.2404
50 %		-	0.8560	0.5257	0.2365	-	-	-
75 %		-	0.4282	0.2629	0.1183	-	-	-
90 %		-	0.1712	0.1052	0.04730	-	-	-
None	R4 stream	1.392	1.196	0.7345	0.3303	0.1679	0.7345	0.1679
50 %		-	0.5979	0.3671	0.2081	-	-	-
75 %		-	0.2991	0.2081	0.2081	-	-	-
90 %		-	0.2081	0.2081	0.2081	-	-	-
RAC (µg/L) 0.37		**PEC/RAC ratio**						
None	D3/ditch	**4.74**	**3.72**	**2.29**	**1.03**	0.52	**2.29**	0.52
50 %		-	**1.86**	**1.14**	0.51	-	-	-
75 %		-	0.93	0.57	0.26	-	-	-
90 %		-	0.37	0.23	0.1	-	-	-
None	D4/pond	0.29	0.32	0.18	0.09	0.06	0.18	0.06
50 %		-	0.16	0.09	0.05	-	-	-
75 %		-	0.08	0.04	0.02	-	-	-
90 %		-	0.03	0.02	0.01	-	-	-
None	D4/stream	**5.02**	**4.32**	**2.65**	**1.19**	0.61	**2.65**	0.61
50 %		-	**2.16**	**1.32**	0.6	-	-	-
75 %		-	**1.08**	0.66	0.3	-	-	-
90 %		-	0.43	0.27	0.12	-	-	-
None	D5/pond	0.29	0.32	0.18	0.09	0.06	0.18	0.06
50 %		-	0.16	0.09	0.05	-	-	-
75 %		-	0.08	0.04	0.02	-	-	-
90 %		-	0.03	0.02	0.01	-	-	-
None	D5/stream	**5.13**	**4.41**	**2.71**	**1.22**	0.62	**2.71**	0.62
50 %		-	**2.2**	**1.35**	0.61	-	-	-
75 %		-	**1.1**	0.68	0.3	-	-	-
90 %		-	0.44	0.27	0.12	-	-	-
None	R1/pond	0.29	0.32	0.18	0.09	0.06	0.18	0.06
50 %		-	0.16	0.09	0.05	-	-	-
75 %		-	0.08	0.04	0.02	-	-	-
90 %		-	0.03	0.02	0.01	-	-	-

None	R1/stream	**3.85**	**3.31**	**2.03**	0.91	0.46	**2.03**	0.46
50 %		-	**1.65**	**1.02**	0.46	-	-	-
75 %		-	0.83	0.51	0.39	-	-	-
90 %		-	0.39	0.39	0.39	-	-	-
None	R2 stream	**5.16**	**4.43**	**2.72**	**1.22**	0.62	**2.72**	0.62
50 %		-	**2.22**	**1.36**	0.61	-	-	-
75 %		-	**1.11**	0.68	0.31	-	-	-
90 %		-	0.44	0.27	0.12	-	-	-
None	R3 stream	**5.39**	**4.63**	**2.84**	**1.28**	0.65	**2.84**	0.65
50 %		-	**2.31**	**1.42**	0.64	-	-	-
75 %		-	**1.16**	0.71	0.32	-	-	-
90 %		-	0.46	0.28	0.13	-	-	-
None	R4 stream	**3.76**	**3.23**	**1.99**	0.89	0.45	**1.99**	0.45
50 %		-	**1.62**	0.99	0.56	-	-	-
75 %		-	0.81	0.56	0.56	-	-	-
90 %		-	0.56	0.56	0.56	-	-	-

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: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for mesocosm with mitigation of spray drift and run-off for the use of ASA-01 in orchards 1 x 22.5 g as/ha (use no. 2)

Intended use		orchards								
Active substance		acetamiprid								
Application rate (g as/ha)		1 × 22.5								
Nozzle reduction	Vegetative strip (m)	None	None	None	None	None	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
	No spray buffer (m)	1/3	5	10	15	20	10	20	50	100
None	D3 ditch	1.753	1.378	0.8459	0.3807	0.1935	0.8459	0.1935	0.02185	0.004137
50 %		-	0.6888	0.4230	0.1903	-	-	0.09671	0.01092	-
75 %		-	0.3444	0.2115	0.09516	-	-	0.04837	-	-
90 %		-	0.1378	0.08459	0.03807	-	-	0.01935	-	-
None	D4 pond	0.1063	0.1197	0.06561	0.03464	0.02122	0.06561	0.02122	0.003870	0.000925
50 %		-	0.05983	0.03281	0.01732	-	-	0.01061	0.001935	-
75 %		-	0.02991	0.01640	0.008660	-	-	0.005305	-	-
90 %		-	0.01197	0.006561	0.003464	-	-	0.002122	-	-
None	D4 stream	1.858	1.597	0.9806	0.4411	0.2242	0.9806	0.2242	0.02532	0.004793
50 %		-	0.7982	0.4902	0.2206	-	-	0.1121	0.01266	-
75 %		-	0.3993	0.2451	0.1103	-	-	0.05606	-	-
90 %		-	0.1597	0.09806	0.04411	-	-	0.02242	-	-
None	D5 pond	0.1063	0.1197	0.06561	0.03464	0.02122	0.06561	0.02122	0.003870	0.000925
50 %		-	0.05983	0.03281	0.01732	-	-	0.01061	0.001935	-
75 %		-	0.02991	0.01640	0.008660	-	-	0.005305	-	-
90 %		-	0.01197	0.006561	0.003464	-	-	0.002122	-	-
None	D5 stream	1.899	1.632	1.002	0.4507	0.2291	1.002	0.2291	0.02588	0.004898
50 %		-	0.8157	0.5009	0.2254	-	-	0.1146	0.01294	-
75 %		-	0.4080	0.2505	0.1127	-	-	0.05729	-	-
90 %		-	0.1632	0.1002	0.04507	-	-	0.02291	-	-
None	R1 pond	0.1062	0.1196	0.06557	0.03462	0.02121	0.06557	0.02121	0.003868	0.000924
50 %		-	0.05979	0.03279	0.01731	-	-	0.01060	0.001934	-
75 %		-	0.02990	0.01639	0.008963	-	-	0.005301	-	-
90 %		-	0.01196	0.007332	0.004929	-	-	0.002121	-	-
None	R1 stream	1.424	1.224	0.7515	0.3380	0.1718	0.7515	0.1718	0.01940	0.003673
50 %		-	0.6117	0.3756	0.1690	-	-	0.08590	0.009702	-
75 %		-	0.3060	0.1878	0.1453	-	-	0.04296	-	-
90 %		-	0.1453	0.1453	0.1453	-	-	0.01718	-	-

Intended use		orchards								
Active substance		acetamiprid								
Application rate (g as/ha)		1 × 22.5								
Nozzle reduction	Vegetative strip (m)	None	None	None	None	None	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
	No spray buffer (m)	1/3	5	10	15	20	10	20	50	100
None	R2 stream	1.909	1.640	1.007	0.4530	0.2303	1.007	0.2303	0.02601	0.004923
50 %		-	0.8199	0.5035	0.2265	-	-	0.1151	0.01300	-
75 %		-	0.4101	0.2518	0.1133	-	-	0.05758	-	-
90 %		-	0.1640	0.1007	0.04530	-	-	0.02303	-	-
None	R3 stream	1.993	1.712	1.052	0.4730	0.2404	1.052	0.2404	0.02715	0.005140
50 %		-	0.8560	0.5257	0.2365	-	-	0.1202	0.01358	-
75 %		-	0.4282	0.2629	0.1183	-	-	0.06012	-	-
90 %		-	0.1712	0.1052	0.04730	-	-	0.02404	-	-
None	R4 stream	1.392	1.196	0.7345	0.3303	0.1679	0.7345	0.1679	0.01897	0.003590
50 %		-	0.5979	0.3671	0.2081	-	-	0.08396	0.009483	-
75 %		-	0.2991	0.2081	0.2081	-	-	0.04199	-	-
90 %		-	0.2081	0.2081	0.2081	-	-	0.01679	-	-
RAC (µg/L) 0.0235		**PEC/RAC ratio**								
None	D3 ditch	**74.60**	**58.64**	**36.00**	**16.20**	**8.23**	**36.00**	**8.23**	0.93	0.18
50 %		-	**29.31**	**18.00**	**8.10**	-	-	**4.12**	0.46	-
75 %		-	**14.66**	**9.00**	**4.05**	-	-	**2.06**	-	-
90 %		-	**5.86**	**3.60**	**1.62**	-	-	0.82	-	-
None	D4 pond	**4.52**	**5.09**	**2.79**	**1.47**	**0.90**	**2.79**	0.90	0.16	0.04
50 %		-	**2.55**	**1.40**	0.74	-	-	0.45	0.08	-
75 %		-	**1.27**	0.70	0.37	-	-	0.23	-	-
90 %		-	0.51	0.28	0.15	-	-	0.09	-	-
None	D4 stream	**79.06**	**67.96**	**41.73**	**18.77**	**9.54**	**41.73**	**9.54**	**1.08**	0.20
50 %		-	**33.97**	**20.86**	**9.39**	-	-	**4.77**	0.54	-
75 %		-	**16.99**	**10.43**	**4.69**	-	-	**2.39**	-	-
90 %		-	**6.80**	**4.17**	**1.88**	-	-	0.95	-	-
None	D5 pond	**4.52**	**5.09**	**2.79**	**1.47**	0.90	**2.79**	0.90	0.16	0.04
50 %		-	**2.55**	**1.40**	0.74	-	-	0.45	0.08	-
75 %		-	**1.27**	0.70	0.37	-	-	0.23	-	-
90 %		-	0.51	0.28	0.15	-	-	0.09	-	-
None	D5 stream	**80.81**	**69.45**	**42.64**	**19.18**	**9.75**	**42.64**	**9.75**	**1.10**	0.21
50 %		-	**34.71**	**21.31**	**9.59**	-	-	**4.88**	0.55	-

Intended use		orchards								
Active substance		acetamiprid								
Application rate (g as/ha)		1 × 22.5								
Nozzle reduction	Vegetative strip (m)	None	None	None	None	None	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
	No spray buffer (m)	1/3	5	10	15	20	10	20	50	100
75 %		-	**17.36**	**10.66**	**4.80**	-	-	**2.44**	-	-
90 %		-	**6.94**	**4.26**	**1.92**	-	-	0.97	-	-
None	R1 pond	**4.52**	**5.09**	**2.79**	**1.47**	0.90	**2.79**	0.90	0.16	0.04
50 %		-	**2.54**	**1.40**	0.74	-	-	0.45	0.08	-
75 %		-	**1.27**	0.70	0.38	-	-	0.23	-	-
90 %		-	0.51	0.31	0.21	-	-	0.09	-	-
None	R1 stream	**60.60**	**52.09**	**31.98**	**14.38**	**7.31**	**31.98**	**7.31**	0.83	0.16
50 %		-	**26.03**	**15.98**	**7.19**	-	-	**3.66**	0.41	-
75 %		-	**13.02**	**7.99**	**6.18**	-	-	**1.83**	-	-
90 %		-	**6.18**	**6.18**	**6.18**	-	-	0.73	-	-
None	R2 stream	**81.23**	**69.79**	**42.85**	**19.28**	**9.80**	**42.85**	**9.80**	**1.11**	0.21
50 %		-	**34.89**	**21.43**	**9.64**	-	-	**4.90**	0.55	-
75 %		-	**17.45**	**10.71**	**4.82**	-	-	**2.45**	-	-
90 %		-	**6.98**	**4.29**	**1.93**	-	-	0.98	-	-
None	R3 stream	**84.81**	**72.85**	**44.77**	**20.13**	**10.23**	**44.77**	**10.23**	**1.16**	0.22
50 %		-	**36.43**	**22.37**	**10.06**	-	-	**5.11**	0.58	-
75 %		-	**18.22**	**11.19**	**5.03**	-	-	**2.56**	-	-
90 %		-	**7.29**	**4.48**	**2.01**	-	-	1.02	-	-
None	R4 stream	**59.23**	**50.89**	**31.26**	**14.06**	**7.14**	**31.26**	**7.14**	0.81	0.15
50 %		-	**25.44**	**15.62**	**8.86**	-	-	**3.57**	0.40	-
75 %		-	**12.73**	**8.86**	**8.86**	-	-	**1.79**	-	-
90 %		-	**8.86**	**8.86**	**8.86**	-	-	0.71	-	-

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

Table 9.5-~~67~~: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ASA-01 in orchards 2 x 27 g as/ha (use no. 3)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Sed. dwell. prolonged**	**Amphibians**	**Mesocosm**
Test species		***Cyprinodon variegatus***	***Pimephales promelas***	Geomean.	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***	***Chironomus riparius***	***Xenopis laevis***	**-**
Endpoint (µg/L)		LC_{50} 100 000	NOEC 9 400	EC_{50} 8.5	NOEC 2 960	EC_{50} 1 300	EC_{50} 1 000	NOEC 0.235	NOEC 2 600	NOEC/NOEAEC 1.1
AF		100	10	100	10	10	10	10	10	3
RAC (µg/L)		1000	940	0.085	296	130	100	0.0235	260	0.37
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**									
Step 1										
-	21.1005	0.02	0.02	**248.24**	0.07	0.16	0.21	**897.89**	0.08	**57.49**
Step 2										
N-Europe	4.0801	0.00	0.00	**48.00**	0.01	0.03	0.04	**173.62**	0.02	**11.12**
S-Europe	4.0801	0.00	0.00	**48.00**	0.01	0.03	0.04	**173.62**	0.02	**11.12**
Step 3										
D3/ditch	1.812	0.00	0.00	**21.32**	0.01	0.01	0.02	**77.11**	0.01	**4.94**
D4/pond	0.1427	0.00	0.00	**1.68**	0.00	0.00	0.00	**6.07**	0.00	0.39
D4/stream	1.904	0.00	0.00	**22.40**	0.01	0.01	0.02	**81.02**	0.01	**5.19**
D5/pond	0.1940	0.00	0.00	**22.40**	0.01	0.01	0.02	**81.02**	0.01	**5.19**
D5/stream	2.058	0.00	0.00	**24.21**	0.01	0.02	0.02	**87.57**	0.01	**5.61**
R1/pond	0.1784	0.00	0.00	**2.10**	0.00	0.00	0.00	**7.59**	0.00	0.49
R1/stream	1.459	0.00	0.00	**17.16**	0.00	0.01	0.01	**62.09**	0.01	**3.98**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants	Sed. dwell. prolonged	Amphibians	Mesocosm
R2/stream	1.955	0.00	0.00	**23.00**	0.01	0.02	0.02	**83.19**	0.01	**5.33**
R3/stream	2.056	0.00	0.00	**24.19**	0.01	0.02	0.02	**87.49**	0.01	**5.60**
R4/stream	1.426	0.00	0.00	**16.78**	0.00	0.01	0.01	**60.68**	0.01	**3.89**

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 use orchards (use no. 3, 5), calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (*Daphnia magna*, *Chironomus riparius* and mesocosm RAC) 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 and mesocosm studies.

Table 9.5-7: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for mesocosm with mitigation of spray drift and run-off for the use of ASA-01 in orchards x 27 g as/ha (use no. 3)

Intended use		orchards						
Active substance		acetamiprid						
Application rate (g as/ha)		2 × 27						
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	None	None	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	15	20	10	20
None	D3/ditch	1.812	1.397	0.8254	0.4533	0.2138	0.8254	0.2138
50 %		-	0.6987	0.4129	0.2266	-	-	-
75 %		-	0.3494	0.2064	0.1133	-	-	-
90 %		-	0.1397	0.08257	0.04534	-	-	-
None	D4/pond	0.1427	0.1601	0.09089	0.04769	0.02738	0.09089	0.02738
50 %		-	0.07998	0.04540	0.02383	-	-	-
75 %		-	0.03996	0.02269	0.01190	-	-	-
90 %		-	0.01597	0.009063	0.004755	-	-	-
None	D4/stream	1.904	1.616	0.9548	0.5243	0.2472	0.9548	0.2472
50 %		-	0.8081	0.4774	0.2621	-	-	-
75 %		-	0.4041	0.2387	0.1310	-	-	-
90 %		-	0.1616	0.09548	0.05243	-	-	-
None	D5/pond	0.1940	0.2177	0.1235	0.06480	0.03719	0.1235	0.03719
50 %		-	0.1087	0.06169	0.03236	-	-	-
75 %		-	0.05428	0.03081	0.01616	-	-	-
90 %		-	0.02168	0.01230	0.006451	-	-	-
None	D5/stream	2.058	1.747	1.032	0.5666	0.2672	1.032	0.2672
50 %		-	0.8734	0.5160	0.2833	-	-	-
75 %		-	0.4367	0.2580	0.1416	-	-	-
90 %		-	0.1747	0.1032	0.05666	-	-	-
None	R1/pond	0.1784	0.1999	0.1124	0.06090	0.03384	0.1124	0.03384
50 %		-	0.1008	0.05807	0.03140	-	-	-
75 %		-	0.05134	0.03000	0.01837	-	-	-
90 %		-	0.02203	0.01581	0.01194	-	-	-
None	R1/stream	1.459	1.239	0.7317	0.4018	0.1894	0.7317	0.1894
50 %		-	0.6193	0.3658	0.2008	-	-	-
75 %		-	0.3096	0.1830	0.1767	-	-	-
90 %		-	0.1767	0.1767	0.1767	-	-	-

None	R2 stream	1.955	1.660	0.9807	0.5385	0.2539	0.9807	0.2539
50 %		-	0.8300	0.4904	0.2692	-	-	-
75 %		-	0.4150	0.2452	0.1346	-	-	-
90 %		-	0.1660	0.09807	0.05385	-	-	-
None	R3 stream	2.056	1.746	1.031	0.5663	0.2670	1.031	0.2670
50 %		-	0.8729	0.5157	0.2831	-	-	-
75 %		-	0.4364	0.2579	0.1415	-	-	-
90 %		-	0.1746	0.1031	0.05663	-	-	-
None	R4 stream	1.426	1.211	0.7152	0.3927	0.1852	0.7152	0.1852
50 %		-	0.6053	0.3576	0.1963	-	-	-
75 %		-	0.3027	0.1788	0.1561	-	-	-
90 %		-	0.1561	0.1561	0.1561	-	-	-
RAC (µg/L) 0.37		**PEC/RAC ratio**						
None	D3/ditch	**4.9**	**3.78**	**2.23**	**1.23**	0.58	**2.23**	0.58
50 %		-	**1.89**	**1.12**	0.61	-	-	-
75 %		-	0.94	0.56	0.31	-	-	-
90 %		-	0.38	0.22	0.12	-	-	-
None	D4/pond	0.39	0.43	0.25	0.13	0.07	0.25	0.07
50 %		-	0.22	0.12	0.06	-	-	-
75 %		-	0.11	0.06	0.03	-	-	-
90 %		-	0.04	0.02	0.01	-	-	-
None	D4/stream	**5.15**	**4.37**	**2.58**	**1.42**	0.67	**2.58**	0.67
50 %		-	**2.18**	**1.29**	0.71	-	-	-
75 %		-	**1.09**	0.65	0.35	-	-	-
90 %		-	0.44	0.26	0.14	-	-	-
None	D5/pond	0.52	0.59	0.33	0.18	0.1	0.33	0.1
50 %		-	0.29	0.17	0.09	-	-	-
75 %		-	0.15	0.08	0.04	-	-	-
90 %		-	0.06	0.03	0.02	-	-	-
None	D5/stream	**5.56**	**4.72**	**2.79**	**1.53**	0.72	**2.79**	0.72
50 %		-	**2.36**	**1.39**	0.77	-	-	-
75 %		-	**1.18**	0.7	0.38	-	-	-
90 %		-	0.47	0.28	0.15	-	-	-
None	R1/pond	0.48	0.54	0.3	0.16	0.09	0.3	0.09
50 %		-	0.27	0.16	0.08	-	-	-
75 %		-	0.14	0.08	0.05	-	-	-
90 %		-	0.06	0.04	0.03	-	-	-
None	R1/stream	**3.94**	**3.35**	**1.98**	**1.09**	0.51	**1.98**	0.51

50 %		-	**1.67**	0.99	0.54	-	-	-
75 %		-	0.84	0.49	0.48	-	-	-
90 %		-	0.48	0.48	0.48	-	-	-
None	R2 stream	**5.28**	**4.49**	**2.65**	**1.46**	0.69	**2.65**	0.69
50 %		-	**2.24**	**1.33**	0.73	-	-	-
75 %		-	**1.12**	0.66	0.36	-	-	-
90 %		-	0.45	0.27	0.15	-	-	-
None	R3 stream	**5.56**	**4.72**	**2.79**	**1.53**	0.72	**2.79**	0.72
50 %		-	**2.36**	**1.39**	0.77	-	-	-
75 %		-	**1.18**	0.7	0.38	-	-	-
90 %		-	0.47	0.28	0.15	-	-	-
None	R4 stream	**3.85**	**3.27**	**1.93**	**1.06**	0.5	**1.93**	0.5
50 %		-	**1.64**	0.97	0.53	-	-	-
75 %		-	0.82	0.48	0.42	-	-	-
90 %		-	0.42	0.42	0.42	-	-	-

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: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for mesocosm with mitigation of spray drift and run-off for the single/double use of ASA-01 in orchards 2 x 27 g as/ha (use no. 3)

Intended use	orchards										
Active substance	acetamiprid										
Application rate (g as/ha)	2 × 27										
Vegetative strip (m)	-	-	-	-	-	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
No spray buffer (m)	1/3	5	10	15	20	10	20	20	50	50	100
Nozzle Reduction (%)	-	-	-	-	-	-	-	90	-	90	-
D3 ditch	2.104/ 1.812	1.653/ 1.397	1.015/ 0.8254	0.4566/ 0.4533	0.2321/ 0.2138	1.015/ 0.8254	0.2321/ 0.2138	0.02321/ 0.02138	0.02622/ 0.01898	0.01311/ 0.009491	0.004963/ 0.002991
D4 pond	0.1276/ 0.1427	0.1436/ 0.1601	0.07874/ 0.09089	0.04156/ 0.04769	0.02546/ 0.02738	0.07874/ 0.09089	0.02546/ 0.02738	0.002546/ 0.002729	0.004644/ 0.004056	0.002322/ 0.002026	0.001110/ 0.000822
D4 stream	2.230/ 1.904	1.916 / 1.616	1.176/ 0.9548	0.5294/ 0.5243	0.2690/ 0.2472	1.176/ 0.9548	2.230/ 1.904	0.02690/ 0.02472	0.03039/ 0.02194	0.01519/ 0.01097	0.005753/ 0.003457
D5 pond	0.1276/ 0.1940	0.1437/ 0.2177	0.07875/ 0.1235	0.04157/ 0.06480	0.02546/ 0.03719	0.07875/ 0.1235	0.1276/ 0.1940	0.002546/ 0.003701	0.004645/ 0.005502	0.002323/ 0.002747	0.001110/ 0.001113
D5 stream	2.409/ 2.058	2.069/ 1.747	1.271/ 1.032	0.5717/ 0.5666	0.2906/ 0.2672	1.271/ 1.032	0.2906/ 0.2672	0.02906/ 0.02672	0.03282/ 0.02371	0.01641/ 0.01186	0.006213/ 0.003736
R1 pond	0.1275/ 0.1784	0.1435/ 0.1999	0.07869/ 0.1124	0.04154/ 0.06090	0.02544/ 0.03384	0.07869/ 0.1124	0.02544/ 0.03384	0.002544/ 0.003368	0.004642/ 0.005007	0.002321/ 0.002500	0.001109/ 0.001013

Intended use	orchards										
Active substance	acetamiprid										
Application rate (g as/ha)	2 × 27										
Vegetative strip (m)	-	-	-	-	-	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
No spray buffer (m)	1/3	5	10	15	20	10	20	20	50	50	100
Nozzle Reduction (%)	-	-	-	-	-	-	-	90	-	90	-
R1 stream	1.709/ 1.459	1.468/ 1.239	0.9015/ 0.7317	0.4057/ 0.4018	0.2062/ 0.1894	0.9015/ 0.7317	0.2062/ 0.1894	0.02062/ 0.01894	0.02328/ 0.01681	0.01164/ 0.008407	0.004409/ 0.002649
R2 stream	2.291/ 1.955	1.968 / 1.660	1.208/ 0.9807	0.5437/ 0.5385	0.2763/ 0.2539	1.208/ 0.9807	0.2763/ 0.2539	0.02763/ 0.02539	0.03121/ 0.02254	0.01560/ 0.01127	0.005909/ 0.003551
R3 stream	2.392/ 2.056	2.055/ 1.746	1.262/ 1.031	0.5677/ 0.5663	0.2885 / 0.2670	1.262/ 1.031	0.2885/ 0.2670	0.02885/ 0.02670	0.03259/ 0.02370	0.01629/ 0.01185	0.006170/ 0.003734
R4 stream	1.670/ 1.426	1.435/ 1.211	0.8812/ 0.7152	0.3965/ 0.3927	0.2015/ 0.1852	0.8812/ 0.7152	0.2015/ 0.1852	0.02015/ 0.01852	0.02276/ 0.01643	0.01138/ 0.008217	0.004309/ 0.002589
RAC (µg/L) 0.0235	**PEC/RAC ratio**										
D3 ditch	**89.53/77.11**	**70.34/59.45**	**43.19/35.12**	**19.43/19.29**	**9.88/9.10**	**43.19/35.12**	**9.88/9.10**	0.99/0.91	1.12/0.81	0.56/0.40	0.21/0.13
D4 pond	**5.43/6.07**	**6.11/6.81**	**3.35/3.87**	**1.77/2.03**	**1.08/1.17**	**3.35/3.87**	**1.08/1.17**	0.11/0.12	0.20/0.17	0.10/0.09	0.05/0.03
D4 stream	**94.89/81.02**	**81.53/68.77**	**50.04/40.63**	**22.53/22.31**	**11.45/10.52**	**50.04/40.63**	**94.89/81.02**	**1.14/1.05**	**1.29**/0.93	0.65/0.47	0.24/0.15
D5 pond	**5.43/8.26**	**6.11/9.26**	**3.35/5.26**	**1.77/2.76**	**1.08/1.58**	**3.35/5.26**	**5.43/8.26**	0.11/0.16	0.20/0.23	0.10/0.12	0.05/0.05
D5 stream	**102.51/87.57**	**88.04/74.34**	**54.09/43.91**	**24.33/24.11**	**12.37/11.37**	**54.09/43.91**	**12.37/11.37**	**1.24/1.14**	**1.40/1.01**	0.70/0.50	0.26/0.16

Intended use	orchards										
Active substance	acetamiprid										
Application rate (g as/ha)	2 × 27										
Vegetative strip (m)	-	-	-	-	-	10 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod	20 VFSmod
No spray buffer (m)	1/3	5	10	15	20	10	20	20	50	50	100
Nozzle Reduction (%)	-	-	-	-	-	-	-	90	-	90	-
R1 pond	**5.43/7.59**	**6.11/8.51**	**3.35/4.78**	**1.77/2.59**	**1.08/1.44**	**3.35/4.78**	**1.08/1.44**	0.11/0.14	0.20/0.21	0.10/0.11	0.05/0.04
R1 stream	**72.72/62.09**	**62.47/52.72**	**38.36/31.14**	**17.26/17.10**	**8.77/8.06**	**38.36/31.14**	**8.77/8.06**	0.88/0.81	0.99/0.72	0.50/0.36	0.19/0.11
R2 stream	**97.49/83.19**	**83.74/70.64**	**51.40/41.73**	**23.14/22.91**	**11.76/10.80**	**51.40/41.73**	**11.76/10.80**	**1.18/1.08**	**1.33**/0.96	0.66/0.48	0.25/0.15
R3 stream	**101.79/87.49**	**87.45/74.30**	**53.70/43.87**	**24.16/24.10**	**12.28/11.36**	**53.70/43.87**	**12.28/11.36**	**1.23/1.14**	**1.39/1.01**	0.69/0.50	0.26/0.16
R4 stream	**71.06/60.68**	**61.06/51.53**	**37.50/30.43**	**16.87/16.71**	**8.57/7.88**	**37.50/30.43**	**8.57/7.88**	0.86/0.79	0.97/0.70	0.48/0.35	0.18/0.11

Table 9.5-89: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ASA-01 in rape (use no. 1)

Group		**Fish acute**	**Inverteb. acute**		**Inverteb. acute**		**Inverteb. acute**	**Inverteb. prolonged**		**Inverteb. acute**	**Inverteb. acute**
Metabolite		**IM-1-4**	**IM-1-4**		**IM-1-2**		**IM-1-5**	**IM-1-5**			**IC-0**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***		***Chironomus riparius***		***Daphnia magna***	***Daphnia magna***		***Daphnia magna***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} 98 100	EC_{50} 19 000		EC_{50} 15 000		EC_{50} 25 000	NOEC 26 000		EC_{50} 95 100	EC_{50} 100 000
AF		100	100		100		100	10		100	100
RAC (µg/L)		981	190		150		250	2600		951	1000
FOCUS Scenario	**IM-1-4 PEC $_{gl\text{-}max}$ (µg/L)**			**IM-1-2 PEC $_{gl\text{-}max}$ (µg/L)**		**IM-1-5 PEC $_{gl\text{-}max}$ (µg/L)**			**IC-0 PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1											
-	8.9877	0.01	0.05	7.0411	0.05	1.2585	0.01	0.00	2.5868	0.00	0.00

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

For the intended use rape (use no. 1), calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

Table 9.5-910: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ASA-01 in orchards (use no. 2)

Group		**Fish acute**	**Inverteb. acute**		**Inverteb. acute**		**Inverteb. acute**	**Inverteb. prolonged**		**Inverteb. acute**	**Inverteb. acute**
Metabolite		**IM-1-4**	**IM-1-4**		**IM-1-2**		**IM-1-5**	**IM-1-5**			**IC-0**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***		***Chironomus riparius***		***Daphnia magna***	***Daphnia magna***		***Daphnia magna***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} 98 100	EC_{50} 19 000		EC_{50} 15 000		EC_{50} 25 000	NOEC 26 000		EC_{50} 95 100	EC_{50} 100 000
AF		100	100		100		100	10		100	100
RAC (µg/L)		981	190		150		250	2600		951	1000
FOCUS Scenario	**IM-1-4 PEC $_{gl\text{-}max}$ (µg/L)**			**IM-1-2 PEC $_{gl\text{-}max}$ (µg/L)**		**IM-1-5 PEC $_{gl\text{-}max}$ (µg/L)**			**IC-0 PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1											
-	7.8773	0.01	0.04	5.5680	0.04	0.9439	0.00	0.00	2.3540	0.00	0.00

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

For the intended use orchards (use no. 2, 4), calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

Table 9.5-~~108~~: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ASA-01 in orchards (use no. 3)

Group		**Fish acute**	**Inverteb. acute**		**Inverteb. acute**		**Inverteb. acute**	**Inverteb. prolonged**		**Inverteb. acute**	**Inverteb. acute**
Metabolite		**IM-1-4**	**IM-1-4**		**IM-1-2**		**IM-1-5**	**IM-1-5**			**IC-0**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***		***Chironomus riparius***		***Daphnia magna***	***Daphnia magna***		***Daphnia magna***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} 98 100	EC_{50} 19 000		EC_{50} 15 000		EC_{50} 25 000	NOEC 26 000		EC_{50} 95 100	EC_{50} 100 000
AF		100	100		100		100	10		100	100
RAC (µg/L)		981	190		150		250	2600		951	1000
FOCUS Scenario	**IM-1-4 PEC $_{gl\text{-}max}$ (µg/L)**			**IM-1-2 PEC $_{gl\text{-}max}$ (µg/L)**		**IM-1-5 PEC $_{gl\text{-}max}$ (µg/L)**			**IC-0 PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1											
-	18.9055	0.02	0.1	13.3632	0.09	2.2652	0.01	0.00	5.6495	0.01	0.01

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 use orchards (use no. 3, 5), calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

9.5.3 Overall conclusions

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance and metabolites. On the basis of PEC/RAC values it was concluded that the application of ASA-01 does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied in rape (use no. 1) and orchards (use no. 2, 3, 4, 5). ~~In case of rape (use no. 1) no risk mitigations measures are required.~~

rape 1 x 30 g as/ha (use no. 1)	
D2 ditch D2 stream D3 ditch D4 stream D5 stream R1 stream R3 stream	10mVFS +50%DRN or 20mVFS
D4 pond D5 pond R1 pond	NR
orchards 1 x 22.5 g as/ha (use no. 2)	
D3 ditch R1 stream R4 stream	20mVFS +90%DRN or 20mVFS+50mFS
D4 stream D5 stream R3 stream	20mVFS +90%DRN or 20mVFS+50mFS+50%DRN or 20mVFS+100mFS
D4 pond D5 pond R1 pond	5mFS+90%DRN or 10mFS+75%DRN or 15mFS+50%DRN or 20mFS
orchards 2 x 27 g as/ha (use no. 3)	
D4 stream D5 stream	20mVFS+50mFS+90%DRN or 20mVFS+100mFS
D3 ditch D4 pond D5 pond R1 pond R1 stream R3 stream R4 stream	20mVFS+90%DRN or 20mVFS+50mFS

FS – filter strip VFS – vegetated filter strip DRN – drift reducing nozzles

For Poland D3, D4 and R1 scenarios are characteristic so proposed risk mitigations measures are:

rape 1 x 30 g as/ha (use no. 1)	10mVFS +50%DRN or 20mVFS
orchards 1 x 22.5 g as/ha (use no. 2)	20mVFS +90%DRN or 20mVFS+50mFS+50%DRN or 20mVFS+100mFS
orchards 2 x 27 g as/ha (use no. 3)	20mVFS+50mFS+90%DRN or 20mVFS+100mFS

9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	The submitted risk assessment is based on SANCO guidance (2002) and new EU guidance (2013). The EU agreed endpoints were used in risk assessment. New studies for acute and chronic toxicity were submitted and accepted. Risk assessment performed in accordance with the SANCO guidance presented by the Applicant has been accepted. The hazard quotients are below the trigger value, indicating that the active substance and formulation pose an acceptable acute risk to bees. Therefore, an acceptable risk to bees is expected from the application of ASA-01. Taking into consideration the acute toxicity of formulation to bees and non-target arthropods other than bees, the negative effect on larvae pupation is not expected. In accordance with CZH conclusion, the risk assessment based on new, EFSA, 2013 guidance should be submitted.

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with acetamiprid and its representative formulation. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on bees of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	acetamiprid	Chronic, oral, 10 days	LDD_{50} = 11.7 µg a.s./bee/day	EFSA Journal 2016;14(11):4610
Apis mellifera	acetamiprid	Chronic larvae	EC_{10} = 1.3 µg/larvae/ developemental period	EFSA Journal 2016;14(11):4610
Apis mellifera	ASA-01	Oral, acute	**48 h LD_{50} = 16.3 µg/bee** (4.5 µg a.s./bee)	Stalmach M/ 2019/Study code: B/61/19 KCP 10.3.1.1.1/01
Apis mellifera	ASA-01	Contact, acute	**48 h LD_{50} > 200 µg/bee** (>55.1 µg a.s./bee)	Stalmach M/ 2019/Study code: B/62/19 KCP 10.3.1.1.2/01
Apis mellifera	ASA-01	Chronic, oral, 10 days	**LDD_{50} = 6.14 µg/bee/day** (1.69 µg a.s./bee/day)	Kulec-Płoszczyca E/2020/Study code: B/63/19 KCP 10.3.1.2/01

Apis mellifera	ASA-01	Single exposure, larvae	72 h LD_{50} = 33.05 µg/bee (9.1 µg a.s./bee)	Kulec-Płoszczyca E/2020/Study code: B/64/19 KCP 10.3.1.3/01
Apis mellifera	ASA-01	Chronic exposure, larvae	ED_{50} = 15.8 µg/larva (4.35 µg a.s./larva) **NOED = 3.3 µg/larva** (0.92 µg a.s./larva)	Kulec-Płoszczyca E/2020/Study code: B/65/19 KCP 10.3.1.4/01
Bombus spp	ASA-01	Oral, acute	**48 h LD_{50} = 93.4 µg/bee** (25.7 µg a.s./bee)	Mryczek E/2020/Study code: B/66/19 KCP 10.3.1.1.1/02
Bombus spp	ASA-01	Contact, acute	**48 h LD_{50} > 200 µg/bee** (>55.1 µg a.s./bee)	Mryczek E/2020/Study code: B/67/19 KCP 10.3.1.1.2/02
Higher-tier studies (tunnel test, field studies)				
Semi-field test (Cage and tunnel test) Five acceptable semi-field studies. Application during full flowering and bee flight at 1x 100-120 g a.s./ha, one study had an additional application one week before introduction of the bees. Generally, transient reduced foraging activity was seen. No increased mortality. No clear brood effects. Details per study are shown below: Due to concerns identified regarding the robustness and reliability of the semi-field and field studies, they could not be used to draw any conclusion, and in particular to exclude potential chronic effects and effects on the brood development.				

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.6-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.6.2 Risk assessment

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 no 3 also covers the risk for bees from all other intended uses (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Acute risk to adult honeybees

The acute risk to honeybees from use of ASA-01 was assessed using the maximum single application rate and the oral and contact LD_{50} values. A Hazard Quotient (HQ) of less than 50 indicates a low risk to bees.

Table 9.6-2: First-tier acute assessment of the risk for bees due to the use of ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)		
Product	ASA-01		
Application rate (kg/ha)	1 × 98.1*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Trigger HQ ≤ 50**
Oral toxicity	10.3	98.1*	9.52
Contact toxicity	>200		0.49

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* application rate of ASA-01 0.09 L/ha re-calculated with density of 1.0897 g/cm^3 (dRR Part B1-2, 4)

All Hazard Quotients are considerably less than 50, indicating that the active substance and formulated product ASA-01 does not pose an unacceptable acute risk to bees.

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

Not relevant.

9.6.3 Effects on bumble bees

Please refer to point 9.6.2.1.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

The acute risk of ASA-01 to honeybees was assessed from HQ between toxicity endpoints, estimated from acute oral and contact studies with formulated product as well as the maximum single application rate. The HQ values were considerably less than 5 that means product ASA-01 does not pose unacceptable acute oral and contact risk to honeybees. It can be concluded that ASA-01 used in accordance with GAP does not pose unacceptable risk to bees. No risk management measures are required.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology"was accepted. New studies for formulation were submitted. The risk envelope approach was used by the Applicant and it was accepted. The hazard quotients are below the trigger value (HQ ≤ 1), indicating that the active substance poses an acceptable risk to arthropods other than bees. Therefore, an acceptable risk to arthropods other than bees is expected if the application of the ASA-01 is in accordance with proposed pattern use. No mitigation measures are required.

9.7.1 Toxicity data

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

Effects on non-target arthropods of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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
Aged residue tests				
Typhlodromus pyri	ASA-01	Extended laboratory Aged residues	Mortality: 0 DAA LR_{50} = 236.64 ml/ha **220 ml/ha 43%** 270 ml/ha 70% 7 DAA LR_{50} = 275.51 ml/ha 220 ml/ha 42% 270 ml/ha 51% 14 DAA LR_{50} = 268.16 ml/ha 220 ml/ha 27% 270 ml/ha 51% 21 DAA 220 ml/ha 16% 270 ml/ha 23% Fecundity: 0 DAA ER_{50} = 105.96 ml/ha 220 ml/ha 3.14% 270 ml/ha 2.26% 7 DAA ER_{50} = 103.95 ml/ha 220 ml/ha 3% 270 ml/ha 2.25% 14 DAA 220 ml/ha 9.68% 270 ml/ha 8.93% 21 DAA 220 ml/ha 11.70% 270 ml/ha 11.47%	Artusio M/ 2021/Study code: 1017-1017ISAG20/r KCP 10.3.2.2/01
Aphidius rhopalosiphi	ASA-01	Extended laboratory Aged residues	Mortality: 0 DAA LR_{50} = 39.82 ml/ha 220 ml/ha 93.33% 270 ml/ha 100% 7 DAA LR_{50} = 58.33 ml/ha 220 ml/ha 76.67% 270 ml/ha 90%	Artusio M/ 2021/Study code: 1016-1016ISAG20/r KCP 10.3.2.2/02

			14 DAA LR_{50} = 51.28 ml/ha 220 ml/ha 76.67% 270 ml/ha 86.67% 21 DAA 220 ml/ha 76.67% 270 ml/ha 86.67% 35 DAA LR_{50} = 270.94ml/ha **220 ml/ha 43.33%** 270 ml/ha 63.33% 42 DAA 220 ml/ha 10% 270 ml/ha 13.33% <u>Reproduction</u>: 35 DAA 220 ml/ha 48.02% 42 DAA 220 ml/ha 1.56% 270 ml/ha 2.34%	
Coccinella septempunctata	ASA-01	Extended laboratory Aged residues	<u>Mortality</u>: 0 DAA LR_{50} = 92.9 ml/ha 220 ml/ha 87.50% 270 ml/ha 90% 7 DAA LR_{50} = 228.83 ml/ha 220 ml/ha 52.50% 270 ml/ha 75% 14 DAA LR_{50} = 1600.02 ml/ha **220 ml/ha 27.50%** 270 ml/ha 37.5% 21 DAA 220 ml/ha 5% 270 ml/ha 7.5% <u>Reproduction</u>: 7 DAA 220 ml/ha 10.11% 14 DAA 220 ml/ha 34.39% 270 ml/ha 33.33% 21 DAA 220 ml/ha 36.05% 270 ml/ha 35.47%	Artusio M/ 2021/Study code: 1015-1015ISAG20/r KCP 10.3.2.2/03
Chrysoperla carnea	ASA-01	Extended laboratory Aged residues	<u>Mortality</u>: 0 DAA LR_{50} = 177.78 ml/ha 220 ml/ha 53.33% 270 ml/ha 70% 7 DAA **220 ml/ha 30%** 270 ml/ha 30% 14 DAA 220 ml/ha 26.67% 270 ml/ha 33.33% 21 DAA 220 ml/ha 13.33% 270 ml/ha 23.33%	Artusio M/ 2021/Study code: 1018-1018ISAG20/r KCP 10.3.2.2/04

			Reproduction: 0 DAA 220 ml/ha 8.05% 7 DAA ER_{50} = 183.76 ml/ha 220 ml/ha 11.03% 270 ml/ha 8.06% 14 DAA ER_{50} = 261.18 ml/ha 220 ml/ha 15.06% 270 ml/ha 12.49% 21 DAA 220 ml/ha 27.94% 270 ml/ha 24.27%	
Field or semi-field tests				
-				

9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.7-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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 no 3 also covers the in-field risk for 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 ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)		
Product	ASA-01		
Application rate	2 × 0.09 L/ha		
MAF	1.7		
Test species **Higher tier**	**Rate with ≤ 50 % effect (ml/ha)**	**$PER_{in\text{-}field}$ (ml/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	220 at 0 DALT	153	yes
Aphidius rhopalosiphi	220 at 35 DALT		yes
Coccinella septempunctata	220 at 14 DALT		yes
Chrysoperla carnea	220 at 7 DALT		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.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use no. 3 also covers the off-field risk for arthropods from all other intended uses (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)
Product	ASA-01
Application rate	2 × 0.09 L/ha
MAF	1.7
VDF	5

Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	236.64	25.63	7.843	10	0.33
Aphidius rhopalosiphi	39.82				1.97
Coccinella septempunctata	92.9				0.84
Chrysoperla carnea	177.78				0.44

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.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in-field and off-field risk of ASA-01 to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from studies with the formulated product ASA-01 as well as in-field and off-field predicted environmental rate. No risk was determined in-field and off-field after application of ASA-01 in accordance with GAP. No risk management measures are required.

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

zRMS Comments:	The submitted information and justification were accepted. New studies were submitted and accepted. The max PECs values for active substance and its metabolites (see Section 8. Fate and behavior) were used for acute and long-term risk assessment An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the application of the ASA-01 is in accordance with proposed pattern use.

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 metabolite of acetamiprid. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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	IM-1-5	Homogenous mixing, chronic	**NOEC = 62.5 mg/kg d.w. soil** (growth, reproduction, behaviour)	EFSA Journal 2016;14(11):4610
Folsomia candida	IM-1-5	Homogenous mixing, chronic	NOECmortality = 62.7 mg/kg soil d.w. No EC values could be calculated as there were no effects below the highest tested value. **NOECreproduction = 12.5 mg/kg soil d.w.** No EC values were calculated as the data were not appropriate for modelling.	EFSA Journal 2016;14(11):4610
Eisenia fetida	ASA-01	Mixed into substrate 56 d, chronic 10 % peat content	Reproduction EC_{50} = 22.3 mg/kg dw (6.1 mg as/kg dw) **NOEC = 10 mg/kg dw (2.8 mg as/kg dw)** Survival LC_{50} = 27.4 mg/kg dw (7.5 mg as/kg dw) NOEC = 18 mg/kg dw (5 mg as/kg dw)	Pieczka P/2020/Study code: G/54/19 KCP 10.4.1.1/01
Hypoaspis aculeifer	ASA-01	Mixed into substrate 14 d, chronic 5 % peat content	Survival: LC_{50} >1000 mg/kg dw (>275.3 mg as/kg dw) NOEC ≥ 1000 mg/kg dw (≥275.3 mg as/kg dw) Reproduction: LC_{50} >1000 mg/kg dw (>275.3 mg as/kg dw) **NOEC = 560 mg/kg dw (154.2 mg as/kg dw)**	Wołany M/2020/Study code: G/56/19 KCP 10.4.2.1/01
Field studies				
Not relevant.				

Species	Substance	Exposure System	Results	Reference
Litter bag test				
Not relevant.				

~~* 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

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.8-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use no. 3 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 ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)		
Chronic effects on earthworms			
Active substance/metabolite	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
IM-1-5	62.5	0.023[1]	2717
acetamiprid (ASA-01)	2.8	0.024	116
Chronic effects on *Folsomia candida*			
Active substance/metabolite	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
IM-1-5	12.5	0.013[1]	961.5
Chronic effects on *Hypoaspis aculeifer*			
Active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
acetamiprid (ASA-01)	154.2	0.024	6425

TER values shown in bold fall below the relevant trigger
[1] $PEC_{s,accumulation}$

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk of ASA-01 to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* was assessed from toxicity exposure ratio (TER) between the selected toxicity endpoints for metabolite IM-1-5 s and the formulated product ASA-01 as well as the maximum soil PECs. The chronic TER values were greater than the trigger of 5, indicating an acceptable risk to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* following application of ASA-01 in accordance with GAP. No risk management measures are required.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	The submitted information and data were accepted. New study was submitted and accepted. An acceptable risk to soil microorganisms is expected if the ASA-01 formulation is applied in accordance with proposed pattern use.

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with representative formulation. Full details of these studies are provided in the respective EU RAR.

Effects on soil microorganisms of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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	ASA-01	28 d, aerobic	<25% effect at day 28 at: 0.14 mg/kg dw (0.04 mg a.s./kg dw) 0.72 mg/kg dw (**0.2 mg a.s./kg dw**)	Wołany M/2020/Study code: G/57/19 KCP 10.5/01

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.5-4. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 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 no. 3 also covers the risk for 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 ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)		
N-mineralisation			
Active substance/metabolite	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
acetamiprid (ASA-01)	0.2 (at 28 d)	0.024	yes

No toxicity data for metabolites IM-1-2, IM-1-4, IM-1-5 and IC-0 since the risk to soil micro-organisms from those metabolites is covered by evaluation performed for formulation ASA-01.

9.9.3 Overall conclusions

The risk of ASA-01 to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests and appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that ASA-01 does not pose unacceptable risk to soil micro-organisms following application in accordance with GAP. No risk management measures are required.

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

zRMS Comments:	New studies were submitted and accepted. The risk assessment is based on endpoints accepted in previous evaluation (CZ as zRMS, 2015). Based on studies results the HC_5 for seedling emergence and vegetative vigour were calculated. In the studies summary (Appendix 2) the relevant tables with observation results are presented. An acceptable risk to non-target terrestrial plants is expected if the ASA-01 formulation is applied in accordance with proposed pattern use. No mitigation measures are required.

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with the reference formulation. Full details of these studies are provided in the respective EU RAR.

Effects on non-target terrestrial plants of ASA-01 were not evaluated as part of the EU assessment of acetamiprid. 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
Sunflower Cabbage Pea Carrot Perrenial ryegrass Oats	ASA-01	21 d Seedling emergence	Plant number at the end of the experiment ER_{50} > 405 ml/ha Shoot length (plants without roots) ER_{50} > 405 ml/ha Plant dry weight (plants without roots) ER_{50} > 405 ml/ha	Pieczka P/2020/Study code: G/59/19 KCP 10.6/01
Sunflower Cabbage Pea Carrot Perrenial ryegrass Oats	ASA-01	21 d Vegetative vigour	Plant number at the end of the experiment ER_{50} > 405 ml/ha Shoot length (plants without roots) ER_{50} > 405 ml/ha Plant dry weight (plants without roots) ER_{50} > 405 ml/ha	Wołany M/2020/Study code: G/58/19 KCP 10.6/02

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ASA-01. Details of studies and results are included in Table 9.10-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use no. 3 also covers the off-field risk for non-target terrestrial plants from all other intended uses (see

9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ASA-01 in orchards (use no. 3)

Intended use	orchards (use no. 3)			
Product	ASA-01			
Application rate (ml/ha)	2 × 90			
MAF	1.7[1]			
Test species	**ER_{50} (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER criterion: TER ≥ 5**
NR	> 405 ml/ha	25.63	39.21	10.33

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1]MAF and drift rate from ESCORT 2 Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risk of ASA-01 to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation ASA-01 and off-field predicted environmental rate. The TER val-ues were greater than the trigger of 5, indicating an acceptable risk to non-target terrestrial plants following application of ASA-01 in accordance with GAP. No risk management measures are required.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

zRMS Comments:	The submitted information and data were accepted. The proposed classification and labelling was accepted. The precautionary statement P501 was added.

Justified proposals for classification and labelling

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

Table 9.13-1: Justified proposals for classification and labelling for ASA-01 according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Aquatic Chronic 1, H410
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS09
Signal word:	Warning
Hazard statement(s):	Very toxic to aquatic life with long lasting effects. [H410]
Precautionary statement(s):	-
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads). [SP 1] Collect spillage [P391] Dispose of contents/container in accordance with local regulation [P501]

Table 9.13-2: Summary of evaluation of the ecotoxicological studies for ASA-01

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	EC_{50} = 9.313 mg/L_{nom} LOEC = 6.250 mg/ L_{nom} NOEC = 3.130 mg/L_{nom}	YES	not classified	Kacperek-Karetta Z/ 2023/ Study code: W-44-22 KCP 10.2.1.2/01
Chronic toxicity to aquatic organisms	no data, extrapolation form active substance data	YES	Aquatic Chronic 1, H410	dRR Part C

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1.2/01	Kacperek-Karetta Z.	2023	ASA-01 *Daphnia magna*, Acute immobilisation test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: W-44-22 GLP: Y Published: N	N	XXXX
KCP 10.2.1.3/01	Janota D	2020	ASA-01 *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: W/50/19 GLP: Y Published: N	N	XXXX
	Janota D	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: W/50/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.1.1/01	Stalmach M	2019	ASA-01 Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/61/19	N	XXXX

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
			GLP: Y Published: N		
	Stalmach M	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/61/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.1.1/02	Myrczek E	2020	ASA-01 Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study Code: B/66/19 GLP: Y Published: N	N	XXXX
	Myrczek E	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study Code: B/66/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.1.2/01	Stalmach M	2019	ASA-01 Honeybees (Apis mellifera L.), Acute Contact Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/62/19 GLP: Y Published: N	N	XXXX
	Stalmach M	2020	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (Apis mellifera L.), Acute Contact Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/62/19 GLP: Y Published: N	N	XXXX

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 10.3.1.1.2/02	Myrczek E	2020	ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Lukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/67/19 GLP: Y Published: N	N	XXXX
	Myrczek E	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Lukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/67/19 GLP: Y Published: N	N	XXXX
	Myrczek E	2021	AMENDMENT NO. 2 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Lukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/67/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.2/01	Kulec-Płoszczyca E	2020	ASA-01 Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/58/19 GLP: Y Published: N	N	XXXX
		2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/58/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.3/01	Kulec-Płoszczyca E	2020	ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland	N	XXXX

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
			Study code: B/64/19 GLP: Y Published: N		
	Kulec-Płoszczyca E	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/64/19 GLP: Y Published: N	N	XXXX
KCP 10.3.1.4/01	Kulec-Płoszczyca E	2021	ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Repeated Exposure Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B/65/19 GLP: Y Published: N	N	XXXX
KCP 10.3.2.2/01	Artusio M	2021	Effects of ASA-01 (acetamiprid 300 g/L) on parasitoid Aphidius rhopalosiphi – Extended laboratory aged residue test – Year 2020 SAGEA Centro di Saggio s.r.l., Italy Study Code: 1016.I.SAG20/r GLP: Y Published: N	N	XXXX
KCP 10.3.2.2/02	Artusio M	2021	Effects of ASA-01 (acetamiprid 300 g/L) on *Typhlodromus pyri* – Extended laboratory aged residue test – 2020 SAGEA Centro di Saggio s.r.l., Italy Study Code: 1017.I.SAG20/r GLP: Y Published: N	N	XXXX
KCP 10.3.2.2/03	Artusio M	2021	Effects of ASA-01 (acetamiprid 300 g/L) on Coccinella Septempunctata – Extended laboratory aged residue test – 2020 SAGEA Centro di Saggio s.r.l., Italy Study code: 1015.I.SAG20/r	N	XXXX

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
			GLP: Y Published: N		
KCP 10.3.2.2/04	Artusio M	2021	Effects of ASA-01 (acetamiprid 300 g/L) on foliage dwelling predator Chrysoperla carnea in the laboratory – Extended laboratory aged resi-due study – Year 2020 SAGEA Centro di Saggio s.r.l., Italy Study code: 1018.I.SAG20/r GLP: Y Published: N	N	XXXX
KCP 10.4.1.1/01	Pieczka P	2020	ASA-01 Earthworm reproduction test (*Eisenia andrei*) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/54/19 GLP: Y Published: N	N	XXXX
	Pieczka P	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Earthworm reproduction test (*Eisenia andrei*) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/54/19 GLP: Y Published: N	N	XXXX
KCP 10.4.2.1/01	Wołany M	2020	ASA-01: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/56/19 GLP: Y Published: N	N	XXXX
	Czarnynoga M	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/56/19 GLP: Y Published: N	N	XXXX

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 10.5/01	Wołany M	2020	ASA-01 Soil Microorganisms: Nitrogen Transformation Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/57/19 GLP: Y Published: N	N	XXXX
	Czarnynoga M	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Soil Microorganisms: Nitrogen Transformation Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/57/19 GLP: Y Published: N	N	XXXX
KCP 10.6.2/01	Pieczka P	2020	ASA-01 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/59/19 GLP: Y Published: N	N	XXXX
	Pieczka P	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/59/19 GLP: Y Published: N	N	XXXX
KCP 10.6.2/02	Czarnynoga M	2020	ASA-01 Terrestrial Plant Test: Vegetative Vigour Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G/58/19 GLP: Y Published: N	N	XXXX
	Holewik P	2021	AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Terrestrial Plant Test: Vegetative Vigour Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland	N	XXXX

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
			Study code: G/58/19 GLP: Y Published: N		

* XXXX

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

Not relevant. No studies submitted. It is possible to extrapolate data from the active substance.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Not relevant. No studies submitted. It is possible to extrapolate data from the active substance.

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

Not relevant. No studies submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 KCP 10.2.1.1 Acute toxicity to fish

Not relevant. No studies submitted. It is possible to extrapolate data from the active substance.

A 2.2.1.2 KCP 10.2.1.2 Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study was accepted. The study was performed in accordance with GLP requirements and OECD 202 guidance. The validity criteria were met: • the percentage of immobilisation of Daphnia magna in the control was 0% (criterion: not more than 10%), • the dissolved oxygen concentrations in the test vessels were within the range of 8.1 – 8.7 mg/L (criterion: not less than 3 mg/L). No deviations were noted. The following endpoints were derived: • 48 h EC_{50} = 9.311 mg product/L, equivalent to 2.563 mg a.s./L • 48 h NOEC = 3.130 mg product/L, equivalent to 0.861 mg a.s./L

Reference: KCP 10.2.1.2/01

Report	ASA-01 *Daphnia magna*, Acute immobilisation test, Kacperek-Karetta Z.; 2023; Study code: W-44-22
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	the content of acetamiprid: 300 g/L
Description (physical state):	homogenous, flowing, slightly viscous liquid of white colour
Batch no.:	005-23-007
Production date:	01.2023
Expiration date:	01.2027

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

3. Test organism

Species:	*Daphnia magna* Straus
Source:	Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland
Age:	< 24 h old at exposure initiation, not first brood progeny
Feeding:	during the test daphnia were not fed
Test units:	glass beakers of volume 150 mL

4. Environmental conditions:

Medium:	Elendt M7 medium recommended by the OECD Guideline No. 202 prepared on the basis of deionized water by adding stock solutions of reagent-grade chemicals
pH:	control: 7.40 – 7.45
Dissolved oxygen:	control: 8.4 – 8.6 mg/L
Temperature:	20-21 °C
Lighting:	daily cycle 16 h light : 8 h dark; fluorescent light source

STUDY DESIGN AND METHOD

Immobilisation of *Daphnia magna* exposed to the test item, ASA-01, was investigated during a 48-hour a

static test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control per replicate. The definitive test was performed in the following range of the test item concentrations: 100, 50, 25, 12.5, 6.25 and 3.13 mg/L plus the control. Four replicates were used for the test item concentrations and the control, each with five Daphnia magna. The *Daphnia magna* were observed for immobilisation and any abnormal behavior or appearance after 24 and 48 h of exposure. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

Test design:	4 replicates per each test item concentration and the control; 5 *Daphnia magna* in each replicate
Stability of test compound:	in spent samples at exposure termination, the determined concentrations of acetamiprid were in the range of 96.0 – 98.7 of the nominal concentration; therefore, the concentrations of acetamiprid were stable during 48 h under test conditions
Stability of the compound:	the concentrations of acetamiprid were chemically determined using the validated high performance liquid chromatographic method with DAD detection, all fresh test item concentrations and the control, collected at exposure initiation and all spent test item concentrations collected at exposure termination were analysed, in samples at exposure termination, the determined concentrations of acetamiprid were in the range of 96.0 – 98.7% of the nominal concentration, therefore, the concentrations of acetamiprid were stable under test conditions
Type of the exposure:	static
Exposure time:	48 hours
Tested concentrations, definitive test:	100, 50, 25, 12.5, 6.25 and 3.13 mg/L plus the control (27.5, 13.8, 6.88, 3.44, 1.72 and 0.861 mg/L plus the control)
Dates:	start of the study 10.05.2023 start of the experimental part: 12.06.2023 end of the experimental part: 14.06.2023 end of the study: 04.08.2023
Statistic:	ToxRat Professional Version 3.3.0 commercial software
Validity of the test:	In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004): - the percentage of immobilisation of Daphnia magna in the control was 0% (criterion: not more than 10%), - the dissolved oxygen concentrations in the test vessels were within the range of 8.1 – 8.7 mg/L (criterion: not less than 3 mg/L).

RESULTS

In the definitive test, the recorded temperature during exposure was in the range of 20.0 – 21.0°C and constant within 1.0ºC. The measured pH values were between 7.40 – 7.49 at exposure initiation and in the range of 7.45 – 7.54 at exposure termination. The measured dissolved oxygen concentrations were in the ranges of 8.3 – 8.6 mg/L at exposure initiation and in the range of 8.2 – 8.6 mg/L at exposure termination.

In the control and in the test item concentration of 3.13 mg/L, no immobilisation of *Daphnia magna* was observed during exposure. At exposure termination in the test item concentrations of 100, 50, 25, 12.5 and 6.25 mg/L, the immobilisation of *Daphnia magna* was 100, 95, 90, 70 and 40%, respectively. At exposure termination in the concentrations of 100, 50, 25 and 12.5 mg/L undissolved particles were visible on the antennas of daphnids. No abnormal behavior of *Daphnia magna* was observed during exposure in the test item concentrations of 6.25 and 3.13 mg/L as well as in the control.

Table KCP 10.2.1.2-1: Immobilisation of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
3.13	20	0	0	0	0	0	0	0	0	0	0
6.25	20	0	0	0	0	2	3	1	2	0	40
12.5	20	0	0	0	0	4	4	3	3	0	70
25	20	1	0	0	0	4	5	4	5	5	90
50	20	0	1	0	1	5	5	4	5	10	95
100	20	1	2	1	2	5	5	5	5	30	100

The concentrations of acetamiprid were chemically determined using the validated liquid chromatography method with Diode Array Detection. Samples of all fresh test item concentrations and the control were analyzed at exposure initiation (fresh) and at exposure termination (spent). In fresh samples at exposure initiation, the determined concentrations of acetamiprid were in the range of 96.4 - 101.0% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. In spent samples at exposure termination, the determined concentrations of acetamiprid were in the range of 96.0 – 98.7 of the nominal concentration. Therefore, the concentrations of acetamiprid were stable during 48 h under test conditions. The endpoints value was determined based on the nominal test item concentration as well as on nominal concentrations of acetamiprid.

CONCLUSION

The endpoints value based on nominal test item concentration are summarised below.

Table KCP 10.2.1.2-2: Endpoint values based on the nominal test item concentrations, definitive test

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	>100	9.311 (7.125 – 11.957)
EC_{20}	72.435 (45.857 – 160.051)	4.845 (3.111 – 6.426)
EC_{10}	45.875 (19.031 – 71.174)	3.444 (1.943 – 4.823)
LOEC	>100	6.250
NOEC	>=100	3.130

Table KCP 10.2.1.2-3: Endpoint values based on the nominal concentrations of acetamiprid, definitive test

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	>27.500	2.563 (1.961 – 3.291)
EC_{20}	19.942 (12.626 – 44.093)	1.333 (0.856 – 1.768)
EC_{10}	12.634 (5.230 – 19.594)	0.947 (0.534 – 1.327)
LOEC	>27.500	1.720
NOEC	>=27.500	0.861

A 2.2.1.3 KCP 10.2.1.3 Effects on aquatic algae

Comments of zRMS:	The study was accepted. The study was performed in accordance with GLP requirements and OECD 201 guidance. The validity criteria were met: • the biomass in the control increased by a factor of 151.0 within the 72-hour test period (criterion: at least a 16-fold growth), • the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.6% (criterion: it must not exceed 7%), • the mean coefficient of variation for the section-by-section growth rate in the control culture was 33.4% (criterion: it must not exceed 35%). No deviations were noted.

	The following endpoints were derived: 72 h ErC_{50} > 100 mg/L; 72 h EyC_{50} > 100 mg/L NOEC was not assessed.

Reference:	KCP 10.2.1.3/01
Report	ASA-01 *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) Growth inhibition test; Janota D.; 2020; Study Code: W/50/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) Growth inhibition test; Janota D.; 2021; Study Code: W/50/19
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	SC for product Acetamiprid, 300 g/L
Description (physical state):	white liquid
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	*unicellular green algae, Raphidocelis subcapitata (*formerly *Pseudokirchneriella subcapitata* (Reinsch) Korshikov *(*syn. *Selenastrum capricornutum Prinz))* SAG 61.81
Source:	Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Toxicology, Poland; the algae, *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) on agar bevels were obtained from the Algae Collection, University Göttingen, Germany

Age:	72 hours
Test units:	flasks with a capacity of 250 mL containing 100 mL of either test item concentration or the control plugged with air permeable stoppers

4. Environmental conditions:

Medium:	APP
Medium temperature:	22.1 – 22.2°C
pH of the control:	7.52 – 8.75
Lighting:	mean light
	intensity: 7668 – 7703 lux; constant illumination

STUDY DESIGN AND METHOD

The growth of the green algae *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) exposed to the test item ASA-01, was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either test item concentration or the control per replicate. The initial density of the algae was 1 x 104 cells/mL. The definitive test was performed with a single test item concentration of 100 mg/L as a limit test plus the control. Six replicates were used for the test item concentration as well as for the control. An additional replicate without the algae was used as a background for spectrophotometric measurements.
Density of algae cells was determined in each replicate after 24, 48 and 72 h of exposure. Morphology observations of the algae were performed at exposure termination. In the test item concentration no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control. The concentrations of acetamiprid were chemically determined with a validated liquid chromatographic method with DAD detection.

Test design:	six replicates for the test item concentration and the ontrol; a background for the control and the test item concentration
Type of the exposure:	static
Exposure time:	72 hours
Inoculum:	10^4 cells/mL
Tested concentrations, definitive test:	100 mg/L plus the control
Stability of the test compound:	concentration and stability of acetamiprid in definitive test was checked at exposure initiation and termination
Dates:	start of the study 11.10.2019 start of the experimental part: 25.11.2019 end of the experimental part: 28.11.2019 end of the study: 22.01.2020
Statistic:	Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Two-sample t-test Procedure

Validity of the test:	In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) were met: – the biomass in the control increased by a factor of 151.0 within the 72-hour test period (criterion: at least a 16-fold growth), – the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.6% (criterion: it must not exceed 7%), – the mean coefficient of variation for the section-by-section growth rate in the control culture was 33.4% (criterion: it must not exceed 35%).

RESULTS

There was no growth rate and yield inhibition in the test item concentration of 100 mg/L. The concentrations of acetamiprid were determined using a validated 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 acetamiprid was 90.9% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. At exposure termination the determined concentration of acetamiprid were 92.4% of the nominal concentration. Therefore, the concentrations of acetamiprid was stable under test conditions.
The endpoint values were determined based on the nominal test item concentration.

CONCLUSION

The endpoint values based on the nominal test item concentration:
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata*, i.e. the **ErC50/72 h value is higher than 100 mg/L**.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata*, i.e. the **EyC50/72 h value is higher than 100 mg/L**.

The endpoint values based on the nominal concentrations of acetamiprid
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata*, i.e. the **ErC50/72 h value is higher than 27.54 mg/L**.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata*, i.e. the **EyC50/72 h value is higher than 27.54 mg/L**.

A 2.2.1.4 KCP 10.2.1.4 Effects on aquatic macrophytes

Not relevant. No studies submitted. It is possible to extrapolate data from the active substance.

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

Not relevant. No studies submitted. It is possible to extrapolate data from the active substance.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant. No studies submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 213 guideline. The 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%). Oral 96 h LD_{50} = 2.8 µg a.s./bee, equivalent to 10.3 µg formulation/bee.

Reference:	KCP 10.3.1.1.1/01
Report	ASA-01 Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test; Stalmach M.; 2019; Study Code: B/61/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test; Stalmach M..; 2021; Study Code: B/61/19
Guideline(s):	Yes, OECD 213
Deviations:	After consultation with the Sponsor, the test item name was changed from ASA-01 (Acetamiprid 300 SC) to ASA-01 which had no impact on the results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	an apiary at the Łukasiewicz Research Network – Instistute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies
Age:	3-week-old worker honeybees
Acclimation period:	-
Diet:	50% sucrose solution
Test units:	plastic cages (5 x 7 x 4.5 cm)

4. Environmental conditions:

Temperature:	25 – 27ºC
Relative humidity:	55 – 69%
Photoperiod:	dark room

STUDY DESIGN AND METHOD

The acute oral toxicity study of ASA-01 described in this report was conducted to determine the LD_{50} values for honeybees. Five doses of the test item were used. These included: 2.5, 5.0, 10.0, 20.0 and 40.0 µg/honeybee. The range of the 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 mentioned 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 tests 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 96-hour exposure.

Test design:	test item - number of doses: 5 doses and a control, number of replicates: 3 replicates, number of bees: 10 bees/replicate reference item - number of doses: 3 doses, number of replicates: 3 replicates, number of bees: 10 bees/replicate
Exposure time:	acute test, 96 h
Tested concentrations, definitive test:	2.5, 5.0, 10.0, 20.0 and 40.0 µg test item/bee and a control (0.0 µg/bee)
Stability of the test compound:	-
Dates:	start of the study 23.09.2019 start of the experimental part: 24.09.2019 end of the experimental part: 28.09.2019 end of the study: 06.12.2019
Statistic:	regression analysis using the log-probit method

Validity of the test:

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 LD_{50}/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

RRESULTS

The acute oral toxicity study of the test item, ASA-01 on honeybees (Apis mellifera L.) in the laboratory test are summarized below.

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

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 96 h			LD_{50} after 96 h [µg/bee]
		Total			
		[no.]	[%]	[%]*	
0.0 (Control)	30	1	**3.3**	**–**	**10.3** *(0.5 – 306.2)***
2.5	30	7	**23.3**	**20.1**	
5.0	30	6	**20.0**	**17.2**	
10.0	30	9	**30.0**	**27.6**	
20.0	30	24	**80.0**	**79.3**	
40.0	30	29	**96.7**	**96.6**	

*: mortality corrected according Abbott's equation [6]
**: the LD_{50} (with 95% confidence limits) was calculated with the log-probit method (ToxRat Proffesional 3.3.0. computer [SOP/B/67]).

CONCLUSION

The median lethal doses LD50/96 h is 10.3 µg/honeybee.

Calculated endpoint values based on the nominal test item and active ingredient concentration for mortality are given in table below.

Table KCP 10.3.1.1.1-2: ***Apis mellifera*** **acute oral toxicity test - endpoint values LD50 calculated based on the nominal concentration of the test item and active ingredient**

Endpoint value	test item	active ingredient
	ASA-01 [µg/bee]	acetamiprid [µg/bee]
LD_{50}/24h	18.9 *(15.5 – 23.5)**	5.2 *(4.3 – 6.5)**
LD_{50}/48h	16.3 *(13.0 – 21.0)**	4.5 *(3.6 – 5.8)**
LD_{50}/72h	13.9 *(7.3 – 32.8)**	3.8 *(2.0 – 9.0)**
LD_{50}/96h	10.3 *(0.5 – 306.2)**	2.8 *(0.1 – 84.3)**

*: The LD_{50} value (with 95% confidence limits)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 247 guideline. The validity criteria were met. • the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%). Oral 96 h LD_{50} = 24.50 µg a.s./bumblebee, equivalent to 89.0 µg formulation/ bumblebee; 96 h NOED = 13.8 µg a.s./bumblebee, equivalent to 50.0 µg formulation/ bumblebee.

Reference:	KCP 10.3.1.1.1/02
Report	ASA-01 Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Myrczek E; 2020; Study Code: B/66/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test; Myrczek E; 2021; Study Code: B/66/19
Guideline(s):	Yes, OECD 247
Deviations:	According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration. However, in the experiment they were replaced by glass calibrated pipettes. In the Study Plan the name of the test item was ASA-01, SC. However, at the Sponsor's request (e-mail from 23.09.2019) the formulation 'SC' was removed from the test item name. In the connection with above, there is a formal discrepancy in the name of the test item between Study Plan and Final Report. These deviations had no impact on the final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	bumbleebee (*Bombus* spp.)
Source:	commercial supplier: Koppert Polska sp. z o.o.
Age:	adult worker bumblebees
Acclimation period:	acclimatized to the test conditions for about 24 hours before starting the experiment
Diet:	50% sucrose solution
Test units:	a dark climate room

4. Environmental conditions:

Temperature:	25-26ºC
Relative humidity:	51-52%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The study was conducted to determine the acute oral toxicity of ASA-01 to bumblebees (*Bombus* spp.) with a laboratory method and to calculate the median lethal doses, i.e. the LD50 and NOED. Five doses of the test item, i.e. 200, 100, 50, 25 and 12.5 µg/bumblebee plus the control and the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary test results.
The bumblebees were exposed to the test item distributed in a 50% sucrose solution. The insects were selected for the exposure in terms of their sizes. The treated diet was provided in calibrated pipettes. Each pipette contained 40 µL of the sucrose solution with the test item at the tested dose. The insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 4 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4-5, 24, 48, 72 and 96 hours after the test/ reference item administration. The acute oral toxicity test finished after the 96-hour observation.

Test design:	the test item - number of doses: 5 and a control; number of replicates: 30; number of insects: 1 insect/replicate the reference item - number of doses: 1; number of replicates: 30; number of insects: 1 insect/replicate
Exposure time:	acute test, 96 h
Tested concentrations, definitive test:	200, 100, 50, 25, 12.5 µg test item/bumblebee and a control (0.0 µg/bumblebee)
Stability of the test compound:	the aim of the analytical part of the definitive test was to determine the concentrations of acetamiprid using a validated liquid chromatographic method with DAD detection; samples of the test item concentrations of 5.0 g/L (200 µg/40 µL), 0.3125 g/L (12.5 µg/40 µL) and the control (50% sucrose solution) at exposure initiation were chemically determined; the results confirm that the test item concentrations were prepared correctly
Dates:	start of the study 25.09.2019 start of the experimental part: 02.12.2019 end of the experimental part: 06.12.2019 end of the study: 30.03.2020
Statistic:	Probit analysis using max. likelihood regression, Chi2 2x2 Table with Bonferroni Correction, Step-down Cochran- Armitage Test Procedure
Validity of the test:	The following validity criteria were met: − Mortality of the control group was 0.0% at the end of the test (criterion: ≤ 10%). − Mortality in the toxic reference item group (dimethoate) at the end of the test was 100.0% (criterion: ≥ 50%).

RESULTS

Mortality of the control group was 0.0% after 96 hours of exposure. The percentage of mortality after 96 h hours of exposure to the test item at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bumblebee were 3.3, 0.0, 3.3, 70.0 and 90.0% respectively. The percentage of mortality after 96 h hours of exposure to the reference item at the dose of 4.0 µg/bumblebee was 100.0%. There were revealed statistically significant differences in mortality between the groups treated with the test item at the doses of 100.0 and 200 µg/bumblebee and the control group, after 24, 48, 72 and 96 hours of exposure (Step-down Cochran-Armitage Test Procedure). The mean weights of the bumblebees were: 0.217 g for the control group, for the groups treated with the test item: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bumblebee were 0.219, 0.215, 0.198, 0.200 and 0.208. In the group treated with the reference item weights of the bumblebees was: 0.211g.

Table KCP 10.3.1.1.1-3: *Bombus* spp. mortality after 24 hours of exposure and LD50/24 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]	NOED [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]		
0.0 (Control)	30	0	**0.0**	**110.8** *(94.4 - 131.3)*	**50.0**
12.5	30	0	**0.0**		
25	30	0	**0.0**		
50	30	1	**3.3**		
100+	30	14	**46.7**		
200+	30	26	**86.7**		
Reference item: dimethoate					
4.0	30	30	**100.0**	–	–

+: statistically significant differences

Table KCP 10.3.1.1.1-4: *Bombus* spp. mortality after 48 hours of exposure and LD50/48 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]	NOED [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]		
0.0 (Control)	30	0	**0.0**	**93.4** *(79.9 – 109.5)*	**50.0**
12.5	30	0	**0.0**		
25	30	0	**0.0**		
50	30	1	**3.3**		
100+	30	21	**70.0**		
200+	30	27	**90.0**		
Reference item: dimethoate					
4.0	30	30	**100.0**	–	–

* : the LD_{50} value (with 95% confidence limits)
n.d.: not determined
+ : statistical significant differences.

Table KCP 10.3.1.1.1-5: *Bombus* spp. mortality after 72 hours of exposure and LD50/72 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]	NOED [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]		
0.0 (Control)	30	0	**0.0**	**89.0** *(n.d.)**	**50.0**
12.5	30	1	**3.3**		
25	30	0	**0.0**		
50	30	1	**3.3**		
100+	30	21	**70.0**		
200+	30	27	**90.0**		
Reference item: dimethoate					
4.0	30	30	**100.0**	–	–

* : the LD_{50} value (with 95% confidence limits)
n.d.: not determined
+ : statistical significant differences

Table KCP 10.3.1.1.1-6: *Bombus* spp. mortality after 96 hours of exposure and LD50/96 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]	NOED [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]		
0.0 (Control)	30	0	**0.0**	**89.0** *(n.d.)**	**50.0**
12.5	30	1	**3.3**		
25	30	0	**0.0**		
50	30	1	**3.3**		
100+	30	21	**70.0**		
200+	30	27	**90.0**		
Reference item: dimethoate					
4.0	30	30	**100.0**	–	–

* : the LD_{50} value (with 95% confidence limits)
n.d.: not determined
+ : statistical significant differences
The exposure in the definitive test was performed between 02 – 06.12.2019

CONCLUSION

The median lethal doses after 24, 48, 72 and 96 h (LD50/24h, LD50/48h, LD50/72h, LD50/96h) are equal to 110.8, 93.4, 89.0 and 89.0 µg/bumblebee.

Table KCP 10.3.1.1.1-7: *Bombus* spp. acute oral toxicity test -final results

Endpoint value	test item	active ingredient
	ASA-01 [µg/bumblebee]	acetamiprid [µg/bumblebee]
LD_{50}/24h	110.8 *(94.4 – 131.3)**	30.5 *(26.0 – 36.2)**
LD_{50}/48h	93.4 *(79.9 – 109.5)**	25.7 *(22.0 – 30.1)**
LD_{50}/72h	89.0 *(n.d.)**	24.5 *(n.d.)**
LD_{50}/96h	89.0 *(n.d.)**	24.5 *(n.d.)**
NOED	50.0	13.8

*: The LD_{50} value (with 95% confidence limits)

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guidance 214 (contact toxicity). All validity criteria were met: • the average mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10%); • the LD50/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee). Some deviations were noted, which had no impact on the results. The following endpoint was derived: LD_{50} 48 h > 200 µg formulation/bee (higher than the highest dose used in the test, i.e. 200.0 µg/honeybee).

Reference:	KCP 10.3.1.1.2/01
Report	ASA-01 Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test; Stalmach M.; 2019; Study Code: B/62/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (Apis mellifera L.), Acute Contact Toxicity Test; Stalmach M.; 2020; Study Code: B/62/19
Guideline(s):	Yes, OECD 214
Deviations:	According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilisation. This deviation had no impact on the results.

	During the preliminary test there was short-term (below one hour) deviation in the temperature, which was below range from SOP/B/48 and method OECD Guideline No. 214 and EU Method C.17. (2008). It did not affect the results obtained in the study. After consultation with the Sponsor, the test item name was changed from ASA-01 (Acetamiprid 300 SC) to ASA-01 which had no impact on the results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

2. Vehicle and/or positive control: vehicle: water
positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera* strain: carnica
Source:	an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies
Age:	approximately 3 weeks
Acclimation period:	-
Diet:	sucrose
Test units:	test cages (5 x 7 x 4.5 cm)

4. Environmental conditions:

Temperature:	25-26ºC
Relative humidity:	58 – 63%
Photoperiod:	dark room

STUDY DESIGN AND METHOD

The aims of the study were to use a laboratory method to determine the acute contact toxicity ASA-01 to adult worker honeybees and to demonstrate that the LD50 values are higher than the highest dose used in the test. Mortality of honeybees, Apis mellifera, exposed to ASA-01 WG was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5; 25.0; 50.0; 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary test results. A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the application, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24, and 48 hours after the beginning of the treatment.

Test design:	the test item - number of doses: 5 doses and a control, number of replicates: 3 replicates, number of bees: 10 bees/replicate the reference item - number of doses: 3 doses, number of replicates: 3 replicates, number of bees: 10 bees/replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Stability of the test compound:	-
Dates:	start of the study 23.09.2019 start of the experimental part: 24.09.2019 end of the experimental part: 26.09.2019 end of the study: 12.12.2019
Statistic:	regression analysis using the log-probit method
Validity of the test:	The following validity criteria were met during the test: – the average mortality for the control was 0.0% after 48h (criterion: it must not exceed 10%), – the LD_{50}/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

RESULTS

The acute contact toxicity study of the test item, ASA-01 on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

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

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

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 µg/honeybee.

Table KCP 10.3.1.1.2-2: ***Apis mellifera*** **acute contact toxicity test - endpoint values LD50 calculated based on the nominal concentration of the test item and active ingredient**

Endpoint value	test item	active ingredient
	ASA-01 [µg/bee]	acetamiprid [µg/bee]
LD_{50}/24h	>200	>55.1
LD_{50}/48h	>200	>55.1

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guidance 246 (contact toxicity). All validity criteria were met: • mortality of the control groups was 0.0% after 48 h (criterion: ≤ 10%); • mortality in the toxic reference item group (dimethoate) at the end of the test was 90.0% (criterion: ≥ 50%). Some deviations were noted, which had no impact on the results. 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

Reference: KCP 10.3.1.1.2/02

Report	ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test; Myrczek E; 2020; Study Code: B/67/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test; Myrczek E; 2021; Study Code: B/67/19 AMENDMENT NO. 2 TO THE FINAL REPORT ASA-01 Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test; Myrczek E; 2021; Study Code: B/67/19
Guideline(s):	Yes, OECD 246
Deviations:	According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation. In the Study Plan the name of the test item was ASA-01, SC. However, at the Sponsor's request (e-mail from 23.09.2019) the formulation 'SC' was removed from the test item name. In the connection with above, there is a formal discrepancy in the name of the test item between Study Plan and Final Report. These deviations had no impact on the final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	
Description (physical state):	285 – 315 g acetamiprid/L
Batch no.:	homogeneous, lightly viscous fluent liquid, white colour
Production date:	20190212-01
Expiration date:	12.02.2019

2. Vehicle and/or positive control:	vehicle water + control with surfactant (distilled water with 1% of Triton(R)X-100) positive control: dimethoate

3. Test organism

Species:	bumbleebee (*Bombus* spp.)
Source:	commercial supplier: Koppert Polska sp. z o.o.
Age:	adult worker bumblebees

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

4. Environmental conditions:

Temperature:	25-25.5ºC
Relative humidity:	59-61%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The study was conducted to determine the acute contact toxicity of ASA-01 to bumblebees (Bombus spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200 µg/bumblebee plus the controls and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary test results.
The bumblebees were exposed to the test item diluted in distilled water with surfactant Triton-X and applied to the dorsal part of the thorax, using a microapplicator. The volume was 2 µL/bumblebee. The insects were selected for the exposure in terms of their sizes. After that, the insects were kept individually in isolators.
The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 10.0 µg/bumblebee.
The insects were observed for mortality and other signs of toxicity 4, 24 and 48 hours after the test/ reference item administration. The acute contact toxicity test finished after the 48-hour observation.

Test design:	the test item - number of doses: 1, a control and a control with surfactant; number of replicates: 50; number of insects: 1 insect/replicate the reference item - number of doses: 1; number of replicates: 30; number of insects: 1 insect/replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	200 µg/bumblebee (limit test)
Stability of the test compound:	the aim of the analytical part of the definitive test was to determine the concentrations of acetamiprid using a validated liquid chromatographic method with diode array detector; samples of the test item concentration of 100.0 g/L (i.e. 200 µg/2 µL) and the control (distilled water) at exposure initiation were chemically determined
Dates:	start of the study 25.09.2019 start of the experimental part: 18.12.2019 end of the experimental part: 20.12.2019 end of the study: 31.03.2020
Statistic:	Fisher's Exact Binomial Test, Chi2 rx2-Contingency Table

Validity of the test:

The following validity criteria were met:
- Mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 90.0% (criterion: ≥ 50%).

RESULTS

There is no statistically significant differences in mortality between control and control with surfactant 1% (Fisher's Exact Binomial Test). Mortality of the both control groups was 0.0% after 48 hours of exposure. The percentage of mortality after 48 h hours of exposure to the test item at the dose of 200 µg/bumblebee was 2%. During the experiment sublethal effects (toxic symptoms) were not observed. There is no statistically significant differences in mortality between the group treated with the test item at the dose of 200.0 µg/bumblebee and the control group, after 4, 24 and 48h (Chi2 rx2-Contingency Table). The percentage of mortality after 48 h hours of exposure to the reference item at the dose of 10.0 µg/bumblebee was 90.0%. The mean weights of the bumblebees in each group were: 0.216 g for the control group, 0,221 g for the control with surfactant, 0.205 g for the group treated with the test item and 0.194 g for the group treated with the reference item.

Table KCP 10.3.1.1.2-3: *Bombus* spp. mortality after 24 hours of exposure and LD50/24 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]	
Control	50	0	0.0	> 200.0
Control + surfactant 1%	50	0	0.0	
200.0 + surfactant 1%	50	0	0.0	
Reference item: dimethoate				
10.0 + surfactant 1%	30	18	60.0	–

Table KCP 10.3.1.1.2-4: *Bombus* spp. mortality after 48 hours of exposure and LD50/48 h

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality		LD_{50} [µg/bumblebee]
		Number of dead bumblebees [no.]	[%]	
Control	50	0	0.0	> 200.0
Control + surfactant 1%	50	0	0.0	
200.0 + surfactant 1%	50	1	2.0	
Reference item: dimethoate				
10.0 + surfactant 1%	30	27	90.0	–

CONCLUSION

The median lethal doses (LD50/24 h, LD50/48 h are higher than the dose used in the test, i.e. > 200 µg test item/bumblebee. Mortality of the both control groups was 0.0% after 48 hours of exposure. The percentage of mortality after 48 h hours of exposure to the test item at the dose of 200 µg/bumblebee was 2%. During the experiment sublethal effects (toxic symptoms) were not observed. There is no statistically significant differences in mortality between the group treated with the test item at the dose of 200.0 µg/bumblebee and the control group, after 4, 24 and 48h (Chi2 rx2-Contingency Table). The mean weights of the bumblebees in each group were: 0.216 g for the control group, 0,221 g for the control with surfactant, 0.205 g for the group treated with the test item and 0.194 g for the group treated with the reference item.

Table KCP 10.3.1.1.2-5: *Bombuss* spp. acute contact toxicity test - final results

Endpoint value	test item	active ingredient
	ASA-01 [µg/bumblebee]	acetamiprid [µg/bumblebee]
LD_{50}/24h	>200	>55.1
LD_{50}/48h	>200	>55.1

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: • mortality in control treatment group was being 3.3 % and therefore below the threshold of 15 % after 10 days of exposure and • average mortality in the reference substance treated group was 86.2 % at the end of the test (criterion given in the guideline: ≥50%). No significant deviations were noted. The following endpoints were calculated:

	LC_{50} = 268.8 mg formulation/kg food NOEC = 166.7 mg formulation/kg food; LDD_{50} = 6.14 µg formulation/bee NOEDD = 2.8 µg formulation/bee/day

Reference:	KCP 10.3.1.2/01
Report	ASA-01 Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test; Kulec-Płoszczyca E.; 2020; Study Code: B/63/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test; Kulec-Płoszczyca E.; 2021; Study Code: B/63/19
Guideline(s):	Yes, OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023
Stability of the test compound:	in the stability and definitive test, the concentrations of acetamiprid were chemically determined using a high performance liquid chromatography (HPLC) with DAD detection; the aim was to make sure that the solution of the test item was prepared properly

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

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies
Age:	freshly emerged young worker bees (max. 2 days old)

Acclimation period:	one day before the experiment, brood frames were transferred from the apiary to the experimental room, afterwards, they were placed in hatching boxes in an incubator, after hatching, the bees were acclimated to the test conditions for about one day.
Diet:	50% sucrose solution
Test units:	cages 8cm x 10cm x 6cm

4. Environmental conditions:

Temperature:	33.0 – 35.0ºC
Relative humidity:	50.1 – 69.9%
Photoperiod:	-

STUDY DESIGN AND METHOD

The aims of the study were to determine the chronic oral toxicity of the test item, ASA-01 to honeybees (Apis mellifera L.) and to determine the median lethal concentration, i.e. the LC50, median lethal dietary dose, i.e. LDD50, the no observed effect concentration (NOEC) and no observed dietary dose (NOEDD).

The mortality of honeybees exposed to ASA-01 was investigated during 10-days chronic oral toxicity test. Five doses of the test item were used. The nominal concentrations were 41.7, 83.3, 166.7, 333.3 and 666.7 mg/kg of diet (corresponding to the nominal doses of 1.25, 2.5, 5.0, 10.0 and 20.0 µg/30 mg/ day). Daily doses, consumed by the bees in the groups treated with the test item at the nominal concentrations of 41.7, 83.3, 166.7, 333.3 and 666.7 mg/kg (i.e. 1.25, 2.5, 5.0, 10.0 and 20.0 µg/30 mg/ day) were 0.9, 1.4, 2.8, 8.9 and 15.9 µg/bee/day, respectively. Daily doses were calculated on the basis of average consumption of a treated 50% sucrose solution and the nominal dose used for the treatment. The design of the definitive test was selected on the basis of the preliminary non-GLP test results. Each group of bees (3 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentrations of 41.7, 83.3, 166.7, 333.3 and 666.7 mg/kg, or 50% sucrose solution alone (control group) for 10 days.

Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per 10 bees) was fed the with 2 mL of a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.024 µg/30 mg/ day). Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10.

The insects were observed for mortality and behavioural abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure.

Average consumption of a 50% sucrose solution in the control group was 24.10 mg/bee/day. Average consumptions in the groups treated with the test item at the concentrations of 41.7, 83.3, 166.7, 333.3 and 666.7 mg/kg were 22.80, 17.11, 16.89, 26.61 and 23.78 mg/bee/day, respectively. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg was 16.01 mg/bee/day.

The concentrations of acetamiprid were chemically determined with a validated chromatographic method with DAD detection. Fresh samples of the test item concentrations of 666.7 and 41.7 mg/kg and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 41.7 mg/kg, the determined concentration of acetamiprid was 95.1% of the nominal concentration and in the fresh test item sample of 666.7 mg/kg, the determined concentration of acetamiprid was 90.1% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

After 4 days of the storage period, in the sample of the test item of 41.7 mg/kg, the determined concentration of acetamiprid was 94.4% and in the fresh test item sample of 666.7 mg/kg, the determined concentration of acetamiprid was 88.6% of the nominal concentration. Based on the results of chemical analyses, the concentration of acetamiprid were stable under storage conditions.

Test design:	tested dose and control in three replicates, 10 bees per replicate; reference item in three replicates, 10 bees per replicate
Exposure time:	chronic test, 10 days
Tested concentrations, definitive test:	nominal: 41.7, 83.3, 166.7, 333.3, 666.7 mg/kg (1.25, 2.5, 5.0, 10.0, 20.0 µg/bee/day) dose of the test item consumed by the bees: 0.9, 1.4, 2.8, 8.9 and 15.9 µg/bee/day
Dates:	start of the study: 15.06.2020 start of the experimental part: 23.06.2020 end of the experimental part: 03.07.2020 end of the study: 25.09.2020
Statistic:	ToxRat Professional
Validity of the test:	The following validity criteria were met during the test: - at the end of the experiment average mortality of the control groups was 3.3% (criterion: it must not exceed 15%), - after 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.024 µg/bee/day) was 86.2%.

CONCLUSION

The validity criterion concerning mortality was met, because mortality in the control was 3.3% after 10 days of exposure.

The percentages of mortality corrected according to the Abbott formula, of the honeybees exposed to the test item, ASA-01 at the concentrations of 41.7, 83.3, 166.7, 333.3 and 666.7 mg/kg (i.e. 1.25, 2.5, 5.0, 10.0 and 20.0 µg/30 mg/ day; dietary doses 0.9, 1.4, 2.8, 8.9 and 15.9 µg/bee/day) after 10 days of exposure, were -3.5, -3.5, 3.5, 79.3 and 100.0%, respectively. The negative values indicate that the mortality in the test item concentration was lower than in the control group.

On the basis of the obtained mortality results the LC50 is equal to 268.8 mg/kg, and the LDD50 value is equal to 6.14 µg/bee/day. The NOEC value is 166.7 mg/kg, while the NOEDD is equal to 2.8 µg/bee/day.

The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, since corrected mortality (according to the Abbott formula) was equal to 86.2% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

Table KCP 10.3.1.2-1: The effects of ASA-01 on mortality of honey bees

Nominal test item dose [µg/30 mg] [µg/bee/ day]	Nominal test item concentration [mg/kg]	Consumed[a] dose [µg/bee / day]	Number of tested bees [no]	Total mortality			LC_{50} [mg/kg]	LDD_{50} [µg/bee/ day]
				No.	[%]	Corr.[b] [%]		
ASA-01								
0.0 (Control)			30	1	3.3	–	268.8 (236.0 – 302.4)*	6.14 (5.0 – 7.3)*
1.25	41.7	0.9	30	0	0.0	-3.5		
2.5	83.3	1.4	30	0	0.0	-3.5		
5.0	166.7	2.8	30	2	6.7	3.5		
10.0	333.3+	8.9+	30	24	80.0	79.3		
20.0	666.7+	15.9+	30	30	100.0	100.0		
NOEC [mg/kg]			166.7					
NOEDD [µg/bee/day]			2.8					
Dimethoate (reference item)								
0.024	0.8	0.013	30	26	86.7	86.2	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and average sucrose solution consumption
[b]: mortality corrected to the formula of Abbott [8]
+: statistical significant difference [7], [SOP/B/67]
*: the LDD_{50}, LD_{50} values (with 59% confidence limits) [7], [SOP/B/67]

Table KCP 10.3.1.2-2: The effects of ASA-01 on mortality of honey bees – final results

Endpoint value	test item	active ingredient
	ASA-01	acetamiprid
LC_{50} [mg/kg]	268.8 *(236.0 – 302.4)**	74.0 *(65.0 – 83.3)**
NOEC [mg/kg]	166.7	45.9
LDD_{50} [µg/bee/day]	6.14 *(5.0 – 7.3)**	1.69 *(1.38 – 2.01)**
NOEDD [µg/bee/day]	2.80	0.77

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 acceptable. The study was conducted according to OECD guidance 237 The validity criteria were met: • larval mortality in the control: 13.9% at the end of the test (criterion: ≤ 15%), required ≤ 15 %; • corrected mortality of the larvae treated with the reference item (dimethoate) was 61.3% (criterion: ≥ 50%). Some negligible deviations from study plan were noted, but none of the deviations/ amendments impacted the quality and integrity of the study. The following endpoints for larval mortality were derived: • 72 h LD_{50} = 33.05 µg formulation/larva equivalent to 9.1 µg a.s./larva;

Reference:	KCP 10.3.1.3/01
Report	ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure; Kulec-Płoszczyca E.; 2020; Study Code: B/64/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure; Kulec-Płoszczyca E.; 2021; Study Code: B/64/19
Guideline(s):	Yes, OECD GD 237
Deviations:	According to the OECD Guideline No. 237 it is recommended to maintain the relative air humidity of 95%. However, the relative air humidity, in the definitive test, was in the range of 90.0 – 98.6%. This deviation had no impact on the final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

2. Vehicle and/or positive control: positive control: dimethoate

3. Test organism

Species: honeybee *Apis mellifera,* strain: carnica

Source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies; larvae were taken from three healthy, queen-right families (3 replicates) with known history and physiological status. Families had not been treated with chemical substances, such as antibiotics, anti-varroa, etc. for four weeks before the experiment

Age: one-day-old larvae

Preparation of larvae: on day 1 of the test combs with larvae were transferred from the apiary to the experimental room, each one-day-old larva, which had not formed a C shape yet, was transferred to a grafting cell, on the surface of diet A (20 µL), using a grafting tool, each cell was placed into a well of a 48-well plate, when a plate was filled with larvae, it was placed into a desiccator, which had previously been placed into an incubator

Acclimation period: -

Diet: The food was composed of the three following diets, adapted to the needs of the larvae at different stages of development [SPO/B/71]:
- Diet A (D1) in the volume of 20 µL per one larva: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.
- Diet B (D3) in the volume of 30 µL per one larva: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.
- Diet C (D4 in the volume of 30 µL per one larva, D5: 40 µL/ larva, D6 :50 µL/ larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.
The test and the reference items were given on D4 (three days after grafting the larvae). The volume of the test solution in the diet did not exceed 10% of the final volume of the diet.

Test units: well of a 48-well plate (EuroClone Primo), a piece of dental roll wetted with a 15% sterilising solution of glycerol
was placed at the bottom of the wells, the plates were placed into a hermetic desiccator, whereas the desiccator was placed in an incubator

4. Environmental conditions:

Temperature:	34.0 – 35.0ºC
Relative humidity:	90.0 – 98.6%

STUDY DESIGN AND METHOD

The aim of the study was to determine the median lethal dose, i.e. the LD50 after 72 h of exposure of honeybees (Apis mellifera L.) larvae to the test item ASA-01 under laboratory conditions. Five doses of the test item were used. These were 1.1, 3.3, 10.0, 30.0 and 90.0 µg/larva. They were selected on the basis of the preliminary non-GLP range-finding test results. On day 4 of the experiment, each larva (3 replicates; 12 larvae/replicate) was fed with 30 µL of treated diet. During the experiment, the larvae were kept in grafting cells placed into 48-well plates. The plates were kept in a desiccator, in an incubator. A toxic standard, i.e. dimethoate, was used to verify the sensitivity of the larvae and the precision of the test procedure. Mortality was recorded after 24, 48 and 72 hours of exposure (from day 5 (D5) – to day 7 (D7)).

Test design:	the test item - number of doses: 5 and a control; number of replicates: 3; number of larvae: 12/replicate the reference item - number of doses: 1; number of replicates: 3; number of larvae: 12/replicate
Exposure time:	72 hours
Tested concentrations, definitive test:	1.1, 3.3, 10.0, 30.0 and 90.0 µg/larva + control
Stability of the test compound:	In the definitive test, fresh samples of the control and the stock test item concentration of 900 µg/30 µL, i.e. 30.0 mg/mL, were chemically determined. The aim was to make sure that the stock test item concentration was prepared properly.
Dates:	start of the study 10.08.2020 start of the experimental part: 16.08.2020 end of the experimental part: 22.08.2020 end of the study: 02.10.2020
Statistic:	regression analysis using the log-probit method

CONCLUSION

Mortality of the control group at the end of the test was 13.9% (criterion: ≤ 15%). The percentages of corrected mortality of the honeybee larvae, exposed to the test item, ASA-01 at the doses of 1.1, 3.3, 10.0, 30.0 and 90.0 µg/larva were: 3.2, 0.0, 9.7, 32.3 and 74.2%, respectively.
The median lethal dose after 72 h of exposure, for the test item (LD50/72 h) is equal to 33.05 µg test item/larva (95% confidence limits: 21.34 – 54.64).
The effects of ASA-01 on mortality of honey bee larvae are summarized below:

Table KCP 10.3.1.3-1: Honeybees, Larval Toxicity Test, Single Exposure – final results

Dose [µg/larva]	Number of tested larvae [no.]	Mortality after 72 h of exposure (D7)			LD_{50} 72 h [µg/larva]
		Total			
		[no.]	[%]	Corrected[a] [%]	
ASA-01					
0.0 (Control)	36	5	**13.9**	–	**33.05** (21.34 – 54.64)*
1.1	36	6	**16.7**	**3.2**	
3.3	36	5	**13.9**	**0.0**	
10.0	36	8	**22.2**	**9.7**	
30.0	36	15	**41.7**	**32.3**	
90.0	36	28	**77.8**	**74.2**	
Dimethoate (reference item)					
8.8	36	24	**66.7**	**61.3**	not determined

[a]: Mortality corrected according to the Abbott formula
*: The LD_{50} value (with 95% confidence limits)

Endpoint value	test item	active ingredient
	ASA-01 [µg/larva]	**acetamiprid [µg/larva]**
LD_{50}/24h	> 90.0	>24.8
LD_{50}/48h	> 90.0	>24.8
LD_{50}/72h	33.05 *(21.34 – 54.64)**	9.1 *(5.9 – 15.0)**

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Comments of zRMS:	The study is acceptable. The study was conducted according to OECD guidance 239 The validity criteria were met: • cumulative larval mortality in the control group was 8.3% at day 8 (D8) (criterion: ≤ 15%). • Abbott corrected mortality of the larvae treated with the reference item at day 8 (D8) (dimethoate) was 93.9% (criterion: ≥ 50%). • emergence rate in the control group on D22 was 75.0% (criterion: ≥ 70%). No deviations were noted.

	The following endpoints for larval mortality on D22 were derived: • EC_{50} = 102.6 mg formulation/kg food • NOEC = 21.6 mg formulation/kg food • ED_{50} = 15.8 µg formulation/larva equivalent to 4.35 µg a.s./larva; • NOED = 3.3µg formulation/larva equivalent to 0.92 µg a.s./larva

Reference:	KCP 10.3.1.4/01
Report	ASA-01 Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Repeated Exposure; Kulec-Płoszczyca E.; 2021; Study Code: B/65/19
Guideline(s):	Yes, OECD GD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	honeybee *Apis mellifera*, strain: carnica
Source:	an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies
Age:	one-day-old larvae
Acclimation period:	-

Diet:	The food was composed of the three following diets, adapted to the needs of the larvae at different stages of development: • Diet A (D1) in the volume of 20 µL per one larva: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose. • Diet B (D3) in the volume of 20 µL per one larva: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose. • Diet C (D4 in the volume of 30 µL per one larva, D5: 40 µL/ larva, D6 :50 µL/ larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose. The fresh diet was prepared on each feeding day. The test and the reference items were given on D3, D4, D5 and D6. The volume of the test suspension/ solution in the diet did not exceed 10% of the final volume of the diet. Amount of the working test item suspensions and reference item solutions which was added to the diet B and C is presented in table 6 and 8, respectively.
Test units:	crystal polystyrene grafting cells with a diameter of 9 mm and depth of 8 mm, each cell was placed into a well of a 48-well plate (NEST), a piece of dental roll wetted with a 15% sterilising solution of glycerol was placed at the bottom of the wells, the plates were placed into a hermetic desiccator, whereas the desiccator was placed in an incubator
4. Environmental conditions:	
Temperature:	34-35ºC
Relative humidity:	D1 – D8: 90.2 – 98.5% D8 – D15: 90.2 – 98.5% D15 – D22: 64.1 – 79.9%
Photoperiod:	-

STUDY DESIGN AND METHOD

The larval toxicity test of ASA-01 was conducted to determine the median effective concentration/dose, i.e. EC_{50}/ED_{50} or any other effective concentration/dose, EC_x/ED_x, as well as the no observed effect concentration/dose (NOEC/NOED) after 22 days of the experiment. Five cumulative doses of the test item were used. These were 0.37, 1.1, 3.3, 10.0 and 30.0 µg/larva. They were selected on the basis of the preliminary non-GLP range-finding test.
From day 3 (D3) to day 6 (D6) of the experiment, each larva (3 replicates; 12 larvae/replicate) was fed with treated diet in the volume of 20, 30, 40 or 50 µL, respectively (total volume of treated diet was 140 µL). During the experiment, the larvae were kept in grafting cells placed into 48-well plates. The plates were kept in a desiccator, in an incubator.
A toxic standard, i.e. dimethoate, was used to verify the sensitivity of the larvae and the precision of the test procedure. Mortality of the larvae was recorded daily from day 4 (D4) – to day 8 (D8) and at day 10

and 11 (D10, D11). On day 15 (D15) mortality of pupae was recorded. The test was ended on day 22 when the emergence of adults was evaluated.

Test design:	the test item - number of doses: 5 and a control; number of replicates: 3; number of larvae: 12/replicate the reference item - number of doses: 1; number of replicates: 3; number of larvae: 12/replicate
Exposure time:	22 days
Tested concentrations, definitive test:	0.37, 1.1, 3.3, 10.0 and 30.0 µg/larva + control
Stability of the test compound:	The stability of stock solution of acetamirpid was tested at concentrations 1000 mg acetamiprid/L. Data for stability were obtained after 0 day, 6 days and 21 days of storage at cool temperature i.e. 20C – 80C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 21 days.
Dates:	start of the study 02.08.2021 start of the experimental part: 07.08.2021 end of the experimental part: 28.08.2021 end of the study: 22.09.2021
Statistic:	Probit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure, ToxRat Professional 3.3.0.

RESULTS

Mortality of the control group on day 8 (D8) of the test was 8.3% (criterion: ≤ 15%). The percentages of mortality corrected using Abbott's formula, of the honeybee larvae, exposed to the test item, ASA-01 at the doses of 0.37, 1.1, 3.3, 10.0 and 30.0 µg test item/larva at D8 were: (-3.0), 6.1, (-6.1), 39.4 and 57.6%, respectively. The negative values indicate that mortality in the groups treated with the test item was lower than in the control. The percentage of larval corrected mortality on D8 in the reference item group was 93.9%.

Pupal mortality of the control group on day 15 (D15) of the test was 19.4%. The percentages of corrected mortality of the honeybee pupae, exposed to the test item, ASA-01 at the doses of 0.37, 1.1, 3.3, 10.0 and 30.0 µg/larva at D15 were: (-13.8), 3.5, (-17.2), 44.8 and 62.1%, respectively. The negative values indicate that mortality in the treated groups was lower than in the control. The percentage of pupal mortality, corrected using Abbott's formula, on D15 in the reference item group was 100.0%.

The emergence of adults (emergence rate) at the end of the test (on D22) in the control group was 75.0%. In the groups treated with the test item at the doses of 0.37, 1.1, 3.3, 10.0 and 30.0 µg test item/larva the adult emergence rates were: 83.3, 75.0, 72.2, 41.7 and 25.0%, respectively.

The effects of ASA-01 on mortality of honey bee larvae are summarized below:

Table KCP 10.3.1.4-1: Honeybees, Larval Toxicity Test, Chronic Exposure – final results

Cumula-tive dose [µg test item/larva]	Concen-tration [mg test item/kg food]	Number of tested larvae [no.]	Total mortality (larval and pupal) on day 22 (D22)				
			Number [no.]	[%]	Corr[a] [%]	Number of emerged adults [No.]	Emergence rate [%]
Test item: ASA-01							
Control (0.0)		36	9	25.0	–	27	**75.0**
0.37	**2.4**	36	6	16.7	**-11.1****	30	**83.3**
1.1	**7.2**	36	9	25.0	**0.0**	27	**75.0**
3.3	**21.6**	36	10	27.8	**3.7**	26	**72.2**
10.0+	**64.9+**	36	21	58.3	**44.4**	15	**41.7**
30.0+	**194.8+**	36	27	75.0	**66.7**	9	**25.0**
ED_{50} [µg test item/larva]			**15.8** *(11.9 – 22.6)**				
EC_{50} [mg test item/kg]			**102.6** *(77.3 – 146.8)**				
NOED [µg test item/larva]			**3.3**				
NOEC [mg test item/kg]			**21.6**				
Reference item: Technical dimethoate mortality on day 8 (D8)							
7.39	**48.0**	36	34	94.4	**93.9**	not determined	

[a]: Mortality corrected according to the Abbott formula [7]
*: The ED_{50}/ EC_{50} value (with 95% confidence limits)
**: The negative value indicates that mortality in the treated group was lower than in the control
+: statistically significant difference

CONCLUSION

<u>The endpoint values determined based on nominal test item concentrations:</u>
– ED_{10} value is 4.1 µg test item/larva (95% confidence limits: 2.3 – 5.8), corresponding to EC_{10} 26.6 mg test item/kg (95% confidence limits: 14.9 – 37.7),
– ED_{20} value is 6.5 µg test item/larva (95% confidence limits: 4.3 – 8.8), corresponding to EC_{20} 42.2 mg test item/kg (95% confidence limits: 27.9 – 57.1),
– ED_{50} value is 15.8 µg test item/larva (95% confidence limits: 11.9 – 22.6), corresponding to EC_{50} 102.6 mg test item/kg (95% confidence limits: 77.3 – 146.8).
– The NOED value is equal to 3.3 µg test item/larva, corresponding to NOEC 21.6 mg test item/kg.

The endpoint values based on nominal concentrations of the active ingredient:
– ED_{10} value is 1.13 µg a.i./larva (95% confidence limits: 0.63 – 1.60), corresponding to EC_{10} 7.33 mg a.i./kg (95% confidence limits: 4.11 – 10.37),
– ED_{20} value is 1.79 µg a.i./larva (95% confidence limits: 1.18 – 2.42), corresponding to EC_{20} 11.62 mg a.i./kg (95% confidence limits: 7.69 – 15.73),
– ED_{50} value is 4.35 µg a.i./larva (95% confidence limits: 3.28 – 6.22), corresponding to EC_{50} 28.25 mg a.i./kg (95% confidence limits: 21.28 – 40.41).
– The NOED value is equal to 0.92 µg a.i./larva, corresponding to NOEC 5.96 mg a.i./kg.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not relevant. No studies submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not relevant. No studies submitted.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods

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

No new studies provided.

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

Comments of zRMS:	The extended laboratory aged residue study was accepted. The validity criteria were met: • mortality in control check: in the control unit, the mortality percentage after 48 hours was 3.33% (validity criterion ≤10%) (bioassay 1 - 0 DAA); • parasitisation in control check: the mean number of parasitized aphids per female was 16.07 (validity criterion ≥5) and there were no wasps producing zero values (validity criterion ≤2 (bioassay 1 - 0 DAA). • mortality in reference: mortality was 80.00% (bioassay 1 - 0 DAA). No deviation from study plan was noted. The following endpoints were derived: • for mortality LR_{50} =39.82 mL product/ha No NOER was assessed.

Reference:	KCP 10.3.2.2/01
Report	Effects of ASA-01 (acetamiprid 300 g/L) on parasitoid *Aphidius rhopalosiphi* – Extended laboratory aged residue test – Year 2020*;* Artusio M.; 2021; Study Code: 1016.I.SAG20/r

Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	SC, 300 g/L of acetamiprid
Description (physical state):	liquid
Batch no.:	19-03-19 YT1
Production date:	16.04.2020
Expiration date:	24.08.2020

2. Vehicle and/or positive control: vehicle: deionised water
positive control: dimetholate

3. Test organism

Species:	Parasitoids (Hymenoptera, Braconidae), *Aphidius rhopalosiphi*
Source:	Katz Biotech AG, Baruth, Germany
Age:	adult, not older than 48 hours
Acclimation period:	2 days under test conditions
Diet:	During the acclimation period a solution of 30% of honey in 100 mL of water was prepared and put on a cotton wool pad and given ad libitum to the insects. Before the application, barley plants for mortality assessment were lightly sprayed with a 10% w/w solution of sugar in water to provide both food and a foraging stimulus for the wasps; for the reproduction assessment a solution of 30% (by volume) of honey in water was put on a cotton wool.

Test units:	Mortality assessment: The test unit consisted of one pot (15.0 cm Ø) with barley seedlings (Hordeum vulgare; 10 seeds per pot), confined within a clear polyacrylic and transparent cylinder (22 cm high and 10 cm Ø). The cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction. After the introduction of the insects, this hole was plugged up with cotton wool. Reproduction assessment: Untreated pots (15.0 cm Ø) with barley seedlings (*Hordeum vulgare*; 30 seeds per pot) infested with ≥100 host aphids of all development stages (*Rhopalosiphum padi*; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (22 cm high and 10 cm Ø). The cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction. After the introduction of the insects, this hole was plugged up with a cotton wool. After the adult wasps were removed, the polyacrylic cylinders were left on the pots.
Plants:	Taxonomic group: Poaceae Common name: barley Species: *Hordeum vulgare* L. Variety: Cometa Stage at delivery: seed Source: Agricola Albese (Alba, CN) Cultivation substrate: artificial soil Grown site: open field under a rain cover (N 44°44’42.5’’ E 008°04’06.4’’) Stage for test start: BBCH 13-15 Maintenance: bottom watering two times a week Agrochemicals and/or fertilizers: none No. pots/treatment (mortality): 6 No. pots/treatment (reproduction): 15 Seeds/pot (mortality): 10 Seeds/pot (reproduction): 30

4. Environmental conditions:

Temperature:	20.287 °C
Relative humidity:	64.2 % RH
Light Intensity:	900 (mortality), 20000 (reproduction)
Light regime:	16 h light:8 h dark

STUDY DESIGN AND METHOD

Aim of the study was to determine the product persistence, intended as the decline rate of residues (fresh and aged) on barley plants treated once with the test item ASA-01 (acetamiprid 300 g/L), under rain-protected field conditions. Insects were exposed to fresh and aged residue of the test item at different timings (bioassay) after application (DAA).
The study encompassed 4 treatments (2 rates of the test item, control, reference item) with 6 replicates each containing 5 females of the parasitoid. The parasitoids were exposed to fresh and aged residue on barley

plants at 0, 7, 14, 21, 35 and 42 days after application (DAA). At each bioassay the location of the parasitoids, i.e. on plants, cylinder and sand, was recorded after 30, 60, 90, 120 and 150 minutes during the initial 3 hours after their release in the treated test units and survival parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, a minimum of 15 females per treatment, except the reference treatment, were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult females were removed after 24 hours and the aphid-infested plants left for a further 12 days before the number of aphid mummies that had developed was assessed. Mortality of exposed parasitoid; LR_{50}: lethal rate producing 50% mortality after 48h from exposure. Additionally, reproductive capacity for female survivors was assessed.

Test design:	mortality assessment: 6 replicates/group; 5 insects in each; fecundity assessment: 15 replicates/group; 1 females/replicate
Exposure time:	mortality phase: 48 hours + fecundity phase: 12 days
Tested concentrations, definitive test:	220 mL formulate product/ha (66 g acetamiprid /ha) 270 mL formulate product/ha (81 g acetamiprid /ha) 500 L water/ha
Stability of test compound:	
Dates:	start of the study: 09.06.2020 start of the experimental part: 24.08.2020 end of the experimental part: 20.10.2020 end of the study: 02.07.2021
Statistic:	Software used for statistical analysis was “R”, version 3.4.3. Software used for statistical analysis was “RStudio”, version 3.0.2. Mortality data were processed using the Fisher’s Exact Test, $\alpha \leq 0.05$ and LR50 was calculated. Correction for control mortality was processed using Schneider- Orelli's formula. The different regression tests were compared to each other and selected according to the best pseudoR2, AIC and lack of fit values. Therefore, the goodness of fit was also calculated. Reproduction data were analysed by Anova t-test, $\alpha \leq 0.05$. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were calculated. Software ARM 2020 by Gylling Data Management was used for statistical analysis of repellency data. The mean values were angularly transformed (square root arcsine) prior to comparison by one-way analysis of variance and Dunnett’s multiple comparison test.
Validity of the test:	The following criteria should be satisfied in the control for a test result to be considered valid: - mortality in the control treatment ≤ 10% after 48 hours; - mean number of parasitized aphids per female in the control treatment ≥ 5; - no more than two surviving wasps producing zero values in the control treatment; - corrected mortality > 50% in the reference item treatment.

RESULTS

Mean % mortality in the control were 3.33% (0 DAA), 6.67% (7 DAA), 3.33% (14 DAA), 0.00% (21 DAA), 10.00% (35 DAA) and 3.33% (42 DAA), while in the toxic reference treatment was 80.00% at 0 DAA. The mean number of mummies per female was 16.07 (0 DAA), 16.80 (35 DAA) and 17.07 (42 DAA).

Bioassay 1 (0 DAA)

Regarding repellency, the mean percentage of wasps settled on the treated plants showed significant differences between the control and treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha). Concerning the mean percentage of wasps settled on cylinder, statistically differences were noticed between the control and the treatment T2 (ASA-01 at 220 mL/ha), while a statistical difference was observed for the percentage of wasps deposited on the sand between control and the different dose rates of ASA-01. The mortality percentage observed at 2, 24 and 48 hours after the application (HAA) showed, at each assessment, significant differences between the treatments ASA-01 and the control. At each assessment, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality. Due to the similar mortality between the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value at 2 and 24 HAA. The estimated LR25 of ASA-01 at 48 HAA was 14.03 mL/ha (95% confidence intervals 4.00 – 49.17mL/ha) and the estimated LR50 of ASA-01 at 48 HAA was 39.82 mL/ha (95% confidence intervals 16.83 – 91.82 mL/ha). Reproduction evaluation was performed after 12 days and the mean number of mummies in the control was 16.07. Due to the high mortality in the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha) it was not possible to continue with the fecundity assessment.

Table KCP 10.3.2.2-1: Summary of results from the repellency assessment 0 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	67.33	a	25.33	a	7.33	a
T2	ASA-01 220 mL f.p./ha	66	25.33	b	50.00	b	24.67	b
T3	ASA-01 270 mL f.p./ha	81	26.67	b	38.67	a	34.67	b
T4	ROGOR L 40 ST 25 mL f.p./ha	10	46.67	a	36.67	a	16.67	a

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-2: Average percentage parasitoid mortality during the assessments 0 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality[c] (%)
T1	Control	-	0.00	-	0.00	-	3.33	± 3.33	-	-
T2	ASA-01 220 mL f.p./ha	66	30.00	***	90.00	***	93.33	± 6.67	***	93.10
T3	ASA-01 270 mL f.p./ha	81	43.33	***	96.67	***	100.00	± 0.00	***	100.00
T4	ROGOR L 40 ST 25 mL f.p./ha	10	13.33	n.s.	53.33	***	80.00	± 7.30	***	79.31

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test $\alpha \leq 0.05$ *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Table KCP 10.3.2.2-3: Mean number of parasitized aphids per female

Treatment number	Treatment	Rate (g a.i./ha)	Mean number of parasitized aphids per female	Standard error	Reduction of Parasitisation Efficiency (R%)
T1	Control	-	16.07	± 0.85	-

-, not applicable

Bioassay 2 (7 DAA)

Regarding repellency, the mean percentage of wasps settled on the treated plants showed significant differences between the control and treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha). Concerning the mean percentage of wasps settled on cylinder, statistically differences were noticed between the control and the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), while a statistical difference was observed for the percentage of wasps deposited on the sand between control and the treatment T3 (ASA-01 at 270 mL/ha). The mortality percentage observed at 2, 24 and 48 hours after the application (HAA) showed, at each assessment, significant differences between the treatments ASA-01 and the control. At each assessment, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality. Due to the similar mortality between the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value at 2 HAA. The estimated LR25 of ASA-01 at 24 HAA was 33.42 mL/ha (95% confidence intervals 10.21 – 109.39 mL/ha) and the estimated LR50 of ASA-01 at 24 HAA was 169.36 mL/ha (95% confidence intervals 99.95 – 286.95 mL/ha), while the estimated LR25 of ASA-01 at 48 HAA was 13.22 mL/ha (95% confidence intervals 3.71 – 47.04 mL/ha) and the estimated LR50 of ASA-01 at 48 HAA was 58.33 mL/ha (95% confidence intervals 28.30 – 120.23 mL/ha).

Table KCP 10.3.2.2-4: Summary of results from the repellency assessment 7 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	65.33	a	25.33	a	9.33	a
T2	ASA-01 220 mL f.p./ha	66	29.33	b	51.33	b	19.33	a
T3	ASA-01 270 mL f.p./ha	81	28.67	b	46.67	b	24.67	b
T4	ROGOR L 40 ST 25 mL f.p./ha	10	40.67	b	37.33	a	22.00	b

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-5: Average percentage parasitoid mortality during the assessments 7 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality[c] (%)
T1	Control	-	0.00	-	3.33	-	6.67	± 4.22	-	-
T2	ASA-01 220 mL f.p./ha	66	16.67	*	60.00	***	76.67	± 9.55	***	75.00
T3	ASA-01 270 mL f.p./ha	81	26.67	**	70.00	***	90.00	± 6.83	***	89.29
T4	ROGOR L 40 ST 25 mL f.p./ha	10	6.67	n.s.	40.00	***	60.00	± 7.30	***	57.14

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test α≤0.05 *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 3 (14 DAA)

Regarding repellency, the mean percentage of wasps settled on the treated plants showed significant differences between the control and treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha). Concerning the mean percentage of wasps settled on cylinder, statistically differences were noticed between the control and the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), while a statistical difference was observed for the percentage of wasps deposited on the sand between control and the treatment T3 (ASA-01 at 270 mL/ha). The mortality percentage observed at 2, 24 and 48 hours after the application (HAA) showed, at each assessment, significant differences between the treatments ASA-01 and the control. At each assessment, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality. At 2 HAA the LR25 estimated value was 217.44 mL/ha (95% confidence intervals 47.58 – 993.67 mL/ha) and the LR50 estimated value was 5028.40 mL/ha (95% confidence intervals 198.96 – 127086.91 mL/ha). The estimated LR25 of ASA-01 at 24 HAA was 40.33 mL/ha (95% confidence intervals 12.51 – 130.08 mL/ha) and the estimated LR50 of ASA-01 at 24 HAA was 164.85 mL/ha (95% confidence intervals 93.91 – 289.38

mL/ha), while the estimated LR25 of ASA-01 at 48 HAA was 12.05 mL/ha (95% confidence intervals 4.22 – 34.39 mL/ha) and the estimated LR50 of ASA-01 at 48 HAA was 51.28 mL/ha (95% confidence intervals 24.42 – 107.67 mL/ha). Due to the high mortality in the treatments, it was not possible to continue with the fecundity assessment.

Table KCP 10.3.2.2-6: Summary of results from the repellency assessment 14 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	67.33	a	24.00	a	8.67	a
T2	ASA-01 220 mL f.p./ha	66	26.67	b	54.67	b	18.67	a
T3	ASA-01 270 mL f.p./ha	81	28.67	b	44.00	b	27.33	b
T4	ROGOR L 40 ST 25 mL f.p./ha	10	42.67	b	28.00	a	29.33	b

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-7: Average percentage parasitoid mortality during the assessments 14 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality[c] (%)
T1	Control	-	3.33	-	3.33	-	3.33	± 3.33	-	-
T2	ASA-01 220 mL f.p./ha	66	26.67	*	46.67	***	76.67	± 13.08	***	75.87
T3	ASA-01 270 mL f.p./ha	81	26.67	*	70.00	***	86.67	± 9.89	***	86.21
T4	ROGOR L 40 ST 25 mL f.p./ha	10	6.67	n.s.	36.67	***	46.67	± 8.43	***	44.83

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test α≤0.05 *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 4 (21 DAA)

Regarding repellency, the mean percentage of wasps settled on the treated plants showed significant differences between the control and treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha). Concerning the mean percentage of wasps settled on cylinder, statistically differences were noticed between the control and the treatment T2 (ASA-01 at 220 mL/ha), while a statistical difference was observed for the percentage of wasps deposited on the sand between control and the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha). The mortality percentage observed at 2, 24 and 48 hours after the application (HAA) showed, at each assessment, significant differences between the treatments ASA-01 and

the control. At each assessment, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality. At 2 HAA the LR25 estimated value was 223.77 mL/ha (95% confidence intervals 193.40 – 258.90 mL/ha) and the LR50 estimated value was 270.00 mL/ha (95% confidence intervals 241.81 – 301.47 mL/ha). Due to the similar mortality between the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value at 24 and 48 HAA. Due to the high mortality in the treatments, it was not possible to continue with the fecundity assessment.

Table KCP 10.3.2.2-8: Summary of results from the repellency assessment 21 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	64.67	a	25.33	a	10.00	a
T2	ASA-01 220 mL f.p./ha	66	28.00	b	49.33	b	22.67	b
T3	ASA-01 270 mL f.p./ha	81	29.33	b	36.67	a	34.00	b
T4	ROGOR L 40 ST 25 mL f.p./ha	10	46.00	b	26.00	a	28.00	b

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-9: Average percentage parasitoid mortality during the assessments 21 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality[c] (%)
T1	Control	-	0.00	-	0.00	-	0.00	± 0.00	-	-
T2	ASA-01 220 mL f.p./ha	66	23.33	*	66.67	***	76.67	± 10.85	***	76.67
T3	ASA-01 270 mL f.p./ha	81	50.00	***	66.67	***	86.67	± 6.67	***	86.67
T4	ROGOR L 40 ST 25 mL f.p./ha	10	6.67	n.s.	23.33	**	36.67	± 6.15	***	36.67

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test $\alpha \leq 0.05$ *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 5 (35 DAA)

Regarding repellency, no statistical differences were showed between the control and the treatments about the mean percentage of wasps settled on the treated plants, cylinder and sand. The mortality percentage observed at 2 HAA showed significant differences between the treatment T3 (ASA-01 at 270 mL/ha) and the control, while at 24 and 48 hours after the application (HAA) significant differences were noticed

between all the treatments ASA-01 and the control. In the first assessment (2 HAA) the calculated LOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while the NOER value matched the test item rate of 220 mL/ha (i.e., treatment T2). In the assessment at 24 and 48 HAA the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2) and in was not possible to calculate the NOER due to the high mortality. Due to the similar mortality between the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value at 2 HAA. The estimated LR25 of ASA-01 at 24 HAA was 71.00 mL/ha (95% confidence intervals 20.61 – 244.68 mL/ha) and the estimated LR50 of ASA-01 at 24 HAA was 834.45 mL/ha (95% confidence intervals 163.56 – 4257.36 mL/ha), while the estimated LR25 of ASA-01 at 48 HAA was 23.19 mL/ha (95% confidence intervals 5.46 – 98.45 mL/ha) and the estimated LR50 of ASA-01 at 48 HAA was 270.94 mL/ha (95% confidence intervals 102.00 – 719.66 mL/ha). About reproduction significant differences were noticed between the treatment T2 (ASA-01 at 220 mL/ha) and the control. The calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value. Due to the high mortality in the treatment T3 (ASA-01 at 270 mL/ha) it was not possible to continue with the fecundity assessment and estimate the ER50 value.

Table KCP 10.3.2.2-10: Summary of results from the repellency assessment 35 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	67.33	a	22.00	a	10.67	a
T2	ASA-01 220 mL f.p./ha	66	55.33	a	26.00	a	18.67	a
T3	ASA-01 270 mL f.p./ha	81	56.00	a	20.00	a	24.00	a
T4	ROGOR L 40 ST 25 mL f.p./ha	10	55.33	a	28.00	a	16.67	a

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-11: Average percentage parasitoid mortality during the assessments 35 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality[c] (%)
T1	Control	-	0.00	-	6.67	-	10.00	± 4.47	-	-
T2	ASA-01 220 mL f.p./ha	66	13.33	n.s.	30.00	**	43.33	± 10.85	**	37.03
T3	ASA-01 270 mL f.p./ha	81	26.67	**	46.67	***	63.33	± 9.55	***	59.26
T4	ROGOR L 40 ST 25 mL f.p./ha	10	6.67	n.s.	20.00	n.s.	23.33	± 9.55	n.s.	14.81

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test α≤0.05 *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Table KCP 10.3.2.2-12: Mean number of parasitized aphids per female

Treatment number	Treatment	Rate (g a.i./ha)	Mean number of parasitized aphids per female	Standard error	Reduction of Parasitisation Efficiency (R%)	p^a
T1	Control	-	16.80	± 1.44	-	-
T2	ASA-01 220 mL f.p./ha	66	8.73	± 0.97	48.02	***

-, not applicable
n.s., not significantly different compared to the control
[a]Anova, α≤0.05 *,0.01 **, 0.001 ***

Bioassay 6 (42 DAA)

Regarding repellency, no statistical differences were showed between the control and the treatments about the mean percentage of wasps settled on the treated plants, cylinder and sand. The mortality percentage observed at 2, 24 and 48 hours after the application (HAA) showed, at each assessment, no significant differences between the treatments ASA-01 and the control. At each assessment, the calculated NOER matched the test item rate of 220 mL/ha (i.e., treatment T2). Due to the similar mortality between the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value at each assessment. About reproduction no significant differences were noticed between the treatments and the control. The calculated NOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while is not possible to calculate LOER value and the ER50 value.

Table KCP 10.3.2.2-13: Summary of results from the repellency assessment 42 DAA

Treatment number	Treatment name	Rate (g a.i./ha)	Mean % of wasps on treated plants		Mean % of wasps on cylinder		Mean % of wasps on sand	
T1	Control	-	68.67	a	21.33	a	10.00	a
T2	ASA-01 220 mL f.p./ha	66	67.33	a	23.33	a	9.33	a
T3	ASA-01 270 mL f.p./ha	81	66.00	a	22.67	a	11.33	a
T4	ROGOR L 40 ST 25 mL f.p./ha	10	66.67	a	20.00	a	13.33	a

-, not applicable
Means followed by same letter or symbol do not significantly differ (P=.05, Dunnett's vs. Control)

Table KCP 10.3.2.2-14: Average percentage parasitoid mortality during the assessments 42 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Check at 2 hours		Check at 24 hours		Check at 48 hours			
			Mortality (%)	p^a	Mortality (%)	p^a	Mortality (%)	SE [b]	p^a	Corrected mortality [c] (%)
T1	Control	-	0.00	-	0.00	-	3.33	± 3.33	-	-
T2	ASA-01 220 mL f.p./ha	66	3.33	n.s.	3.33	n.s	10.00	± 4.47	n.s.	6.90
T3	ASA-01 270 mL f.p./ha	81	0.00	n.s.	6.67	n.s.	13.33	± 4.22	n.s.	10.34
T4	ROGOR L 40 ST 25 mL f.p./ha	10	0.00	n.s.	3.33	n.s.	6.67	± 4.22	n.s.	3.46

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Test α≤0.05 *,0.01 **, 0.001 ***
[b], standard error from 6 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Table KCP 10.3.2.2-15: Mean number of parasitized aphids per female

Treatment number	Treatment	Rate (g a.i./ha)	Mean number of parasitized aphids per female	Standard error	Reduction of Parasitisation Efficiency (R%)	p^a
T1	Control	-	17.07	± 1.41	-	-
T2	ASA-01 220 mL f.p./ha	66	16.80	± 1.58	1.56	n.s.
T3	ASA-01 270 mL f.p./ha	81	16.67	± 0.91	2.34	n.s.

-, not applicable
n.s., not significantly different compared to the control
[a], Anova, α≤0.05 *,0.01 **, 0.001 ***

CONCLUSION

The obtained endpoints have been summarised below.

Table KCP 10.3.2.2-16: *Aphidius rhopalosiphi* - endpoints for the 0-DAA, 7-DAA, 14-DAA, 21-DAA, 35-DAA and 42-DAA bioassay

Endpoint (mL/ha)					
LR_{50} (0 DAA)	39.82	NOER	-	LOER	220
LR_{50} (7 DAA)	58.33	NOER	-	LOER	220
LR_{50} (14 DAA)	51.28	NOER	-	LOER	220
LR_{50} (21 DAA)	-	NOER	-	LOER	220
LR_{50} (35 DAA)	270.94	NOER	-	LOER	220
LR_{50} (42 DAA)	-	NOER	270	LOER	-
ER_{50} (0 DAA)	-	NOER	-	LOER	-
ER_{50} (35 DAA)	-	NOER	-	LOER	220
ER_{50} (42 DAA)	-	NOER	270	LOER	-

Table KCP 10.3.2.2-17: Mortality and fecundity efficiency of *Aphidius rhopalosiphi*

	T1 Control	T2 ASA-01 220 mL/ha	T3 ASA-01 270 mL/ha	T4 ROGOR L40 ST 25 mL/ha
Mortality 48 HAA (bioassay 1 – 0 DAA) [mean %]	3.33	93.33	100.00	80.00
Significance [a]	-	***	***	***
Mortality 48 HAA (bioassay 2 – 7 DAA) [mean %]	6.67	76.67	90.00	60.00
Significance [a]	-	***	***	***
Mortality 48 HAA (bioassay 3 – 14 DAA) [mean %]	3.33	76.67	86.67	46.67
Significance [a]	-	***	***	***
Mortality (bioassay 4 – 21 DAA) [mean %]	0.00	76.67	86.67	36.67
Significance [a]	-	***	***	***
Mortality (bioassay 5 –35 DAA) [mean %]	10.00	43.33	63.33	23.33
Significance [a]	-	**	***	n.s.
Mortality (bioassay 6 –42 DAA) [mean %]	3.33	10.00	13.33	6.67
Significance [a]	-	n.s.	n.s.	n.s.
Reproduction [mean mummies/females] (bioassay 1 - 0 DAA)	16.07	-	-	-
Significance [b]	-	-	-	-
Reproduction [mean mummies/females] (bioassay 5 - 35 DAA)	16.80	8.73	-	-
Significance [b]	-	***	-	-
Reproduction [mean mummies/females] (bioassay 6 - 42 DAA)	17.07	16.80	16.67	-
Significance [b]	-	-	-	-
Effect on reproduction [%] (bioassay 1 – 0 DAA)	-	-	-	-
Effect on reproduction [%] (bioassay 5 – 35 DAA)	-	48.02	-	-
Effect on reproduction [%] (bioassay 6 – 42 DAA)	-	1.56	2.34	-

Comments of zRMS:	The extended laboratory study was accepted. The validity criteria were met:

	• mortality in the control group: ≤ 20 % – observed: 3%. • corrected mortality in the reference group: 50 – 100 %, observed: 69%; • reproduction in the control group: ≥ 4 eggs per female (observed: 12.19 eggs per female); The following endpoints were derived: • for mortality LR_{50} = 236.64 mL product/ha in 200 L water/ha No NOER was assessed.

Reference:	KCP 10.3.2.2/02
Report	Effects of ASA-01 (acetamiprid 300 g/L) on *Typhlodromus pyri* – Extended laboratory aged residue test – 2020; Mautino G.; 2021; Study Code: 1017.I.SAG20/r
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	Change of Study Director
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	SC, 300 g/L of acetamiprid
Description (physical state):	liquid
Batch no.:	19-03-19 YT1
Production date:	16.04.2020
Expiration date:	24.08.2020

2. Vehicle and/or positive control: vehicle: distilled water
positive control: dimetholate

3. Test organism

Species:	Predatory mite (*Typhlodromus pyri*)
Source:	Katz Biotech AG, Baruth, Germany
Age:	protonymphs ≤24 hours old
Acclimation period:	1 day under test conditions in an incubator
Diet:	apple pollen, added at the day of the test start and at each assessment day, except for the last one

Test units:	bean leaf discs (44 mm Ø) leaning on a perforated (6 mm Ø) glass plate (54 mm Ø) blocked on a grid immerged in water, all systems were contained within a plastic container (250 × 250 × 80 mm)
Plants:	Taxonomic group: Fabaceae Common name: Bean Species: Phaseolus vulgaris L. Variety: Bingo Source: Agricola Albese (Alba, CN) Cultivation substrate: artificial soil Sowing date: 6 July 2020 Emergence date: 12 July 2020 Grown site: open field under a rain cover (N 44°44'42.5'' E 008°04'06.4''). Stage at test start: BBCH 13-15 Maintenance: bottom watering two times a week, none for agrochemical and fertilized

4. Environmental conditions:

Temperature:	26.275 °C
Relative humidity:	73.9 %
Photoperiod:	16 h light : 8 h dark, light intensity: 1000 lux

STUDY DESIGN AND METHOD

The study objective was to determine the product persistence, intended as the rate of decline of residues (fresh and aged) on bean leaves treated with test item ASA-01 (acetamiprid 300 g/L) under protected (with rain protection) field conditions. Residual toxicity was evaluated by assessing Typhlodromus pyri mortality and fecundity; mites were introduced at fixed intervals from the application onto leaf discs cut from the treated bean plants. Four bioassays were settled: Bioassay 1 where mites were introduced immediately after the application (when leaves were dried) (0 DAA); Bioassay 2 where mites were introduced after 7 days from application (7 DAA); Bioassay 3 where mites were introduced after 14 days from application (14 DAA) and Bioassay 4 where mites were introduced after 21 days from application (21 DAA).

The study encompassed 4 treatments (2 rates of the test item, control with deionised water, reference item) with 5 replicates each containing 20 mites. Protonymphs were exposed to fresh and aged residue on bean leaf discs at 0, 7, 14 and 21 days after application (DAA). Survival of the mites was assessed after 3 and 7 days to determine their percent mortality. After mortality assessment, the reproduction was checked. Surviving mites from the control and from all test item groups were sexed and the number of eggs per females was recorded in 3 assessments days within 8 days.

Test design:	tested concentrations, reference item and control in 5 replications, number of mites: 20 mites/replicate
Exposure time:	14 days (7 days of mortality phase + 7 days of fecundity test)
Tested concentrations, definitive test:	220 mL formulate product/ha (66 g as /ha) – T2 270 mL formulate product/ha (81 g as /ha) – T3 400 L water/ha
Stability of test compound:	-

Dates:	start of the study: 09.06.2020 start of the experimental part: 20.07.2020 end of the experimental part: 24.08.2020 end of the study: 02.07.2021
Statistic:	Software used for statistical analysis was "R", version 3.4.3. Mortality data were processed using the Fisher's Exact Test (one-sided greater, α=0.05) and LRx after 7 days of the exposure was calculated. Correction for control mortality was processed using Schneider-Orelli's formula. Different regression tests were compared each other and those with the best pseudoR2, AIC and lack of fit values were selected. Therefore, the goodness of fit was also calculated. Fecundity data were analysed by Dunnett's t-test, α=0.001 and the ER50 calculated. In case of lack of homogeneity of variances, a weighted leastsquares regression was performed on data. Also, in this case, the goodness of fit was calculated by the lack of fit method. In case of outliers, they were taken into account for calculation. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were provided.
Validity of the test:	The following criteria should be satisfied in the control for a test result to be considered valid: - mortality in the control check ≤ 20% on day-7; - mean cumulative number of eggs per female in control check ≥ 4; - correct mortality of between 50% and 100% in the toxic reference treatment on day seven.

RESULTS

All study validity criteria were met.

Bioassay 1 mortality (0 DAA)

The mortality percentage for the untreated control was 3.00%, while the percentage of test item were 43.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 41.24%) and 70.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 69.07%). Significant differences were noticed between the treatments and the control. The reference item ROGOR L 40 ST was significantly different with a mortality value of 69.00% (corrected mortality: 68.04%). A dose-response effect on Typhlodromus pyri mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 191.80 mL/ha (95% confidence intervals are 161.31 – 228.06 mL/ha) and the estimated LR50 was 236.64 mL/ha (95% confidence intervals are 220.50 – 253.96 mL/ha).

Table KCP 10.3.2.2-18: Average percentage of mite mortality during the assessments (Bioassay 1 at 0 DAA)

Date: 27 Jul 2020							
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Check at 3 days**	**Check at 7 days**			
			Mortality (%)	**Mortality**		***p*[a]**	**Corrected mortality (%)[c]**
				(%)	**SE[b]**		
T1	Control	-	1.00	3.00	± 0.40	-	0.00
T2	ASA-01 220 mL/ha	66.0 acetamiprid	20.00	43.00	± 2.01	**	41.24
T3	ASA-01 270 mL/ha	81.0 acetamiprid	27.00	70.00	± 1.14	***	69.07
T4	ROGOR L 40 ST 15 mL/ha	6.0 dimethoate	39.00	69.00	± 1.39	***	68.04

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 5 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 2 mortality (7 DAA)

The mortality percentage for the untreated control was 4.00%, while the percentage of the test item were 42.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 39.58%) and 51.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 48.96%). Significant differences were noticed between the treatments and the control. The reference item ROGOR L 40 ST was significantly different with a mortality value of 63.00% (corrected mortality: 61.46%). A dose-response effect on Typhlodromus pyri mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 160.02 mL/ha (95% confidence intervals are 88.01 – 290.95 mL/ha) and the estimated LR50 was 275.51 mL/ha (95% confidence intervals are 221.48 – 342.72 mL/ha).

Table KCP 10.3.2.2-19: Average percentage of mite mortality during the assessments (Bioassay 2 at 7 DAA)

Date: 03 Aug 2020							
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Check at 3 days**	**Check at 7 days**			
			Mortality (%)	**Mortality**		***p*[a]**	**Corrected mortality (%)[c]**
				(%)	**SE[b]**		
T1	Control	-	0.00	4.00	± 0.37	-	0.00
T2	ASA-01 220 mL/ha	66.0 acetamiprid	22.00	42.00	± 1.21	**	39.58
T3	ASA-01 270 mL/ha	81.0 acetamiprid	41.00	51.00	± 1.11	**	48.96
T4	ROGOR L 40 ST 15 mL/ha	6.0 dimethoate	48.00	63.00	± 1.29	***	61.46

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 5 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 3 mortality (14 DAA)

The mortality percentage for the untreated control was 1.00%, while the percentage of the test item were 27.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 26.26%) and 51.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 50.51%). Significant differences were noticed between the treatments and the control. The reference item ROGOR L 40 ST was significantly different with a mortality value of 36.00% (corrected mortality: 35.35%). A dose-response effect on Typhlodromus pyri mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 271.63 mL/ha (95% confidence intervals are 197.61 – 239.68 mL/ha) and the estimated LR50 was 268.16 mL/ha (95% confidence intervals are 251.44 – 286.01 mL/ha).

Table KCP 10.3.2.2-19: Average percentage of mite mortality during the assessments (Bioassay 3 at 14 DAA)

Date: 10 Aug 2020							
Treatment number	Treatment	Rate (g a.i./ha)	Check at 3 days	Check at 7 days			
			Mortality (%)	Mortality		p^a	Corrected mortality (%)[c]
				(%)	SE[b]		
T1	Control	-	0.00	1.00	± 0.20	-	0.00
T2	ASA-01 220 mL/ha	66.0 acetamiprid	25.00	27.00	± 0.68	**	26.26
T3	ASA-01 270 mL/ha	81.0 acetamiprid	40.00	51.00	± 0.66	***	50.51
T4	ROGOR L 40 ST 15 mL/ha	6.0 dimethoate	22.00	36.00	±0.97	**	35.35

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 5 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 4 mortality (21 DAA)

The mortality percentage for the untreated control was 0.00%, while the percentage of the test item were 16.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 16.00%) and 23.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 23.00%). Significant differences were noticed between treatment T3 (ASA-01 at 270 mL/ha) and the control. The reference item ROGOR L 40 ST was significantly different with a mortality value of 22.00% (corrected mortality: 22.00%). A dose-response effect on Typhlodromus pyri mortality was observed; therefore, the calculated LOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while the calculate NOER matched the test item rate of 220 mL/ha (i.e., treatment T2). Due to the very low mortality it was not possible to calculate the LR25 and LR50 value.

Table KCP 10.3.2.2-20: Average percentage of mite mortality during the assessments (Bioassay 4 at 21 DAA)

Date: 10 Aug 2020							
Treatment number	Treatment	Rate (g a.i./ha)	Check at 3 days	Check at 7 days			
			Mortality (%)	Mortality		p^a	Corrected mortality (%)[c]
				(%)	SE[b]		
T1	Control	-	0.00	0.00	± 0.00	-	0.00
T2	ASA-01 220 mL/ha	66.0 acetamiprid	13.00	16.00	± 1.02	n.s.	16.00
T3	ASA-01 270 mL/ha	81.0 acetamiprid	7.00	23.00	± 1.21	*	23.00
T4	ROGOR L 40 ST 15 mL/ha	6.0 dimethoate	14.00	22.00	±0.75	*	22.00

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 5 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 1 fecundity (0 DAA)

The mean cumulative number of eggs per female in the water control was 12.19, while treatments values were 3.14 in treatment T2 (ASA-01 at 220 mL/ha) and 2.26 in treatment T3 (ASA-01 at 270 mL/ha). Significant differences were noticed between the treatments and the control. A dose-response effect on Typhlodromus pyri fecundity was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value. The estimated ER25 of ASA-01 was 43.98 mL/ha (95% confidence intervals are 29.36 – 58.60 mL/ha) and the estimated ER50 was 105.96 mL/ha (95% confidence intervals are 70.73 – 141.19 mL/ha).

Table KCP 10.3.2.2-21: Mean cumulative number of eggs and juveniles stages per female (Bioassay 1 at 0 DAA)

Date: from 27 Jul to 03 Aug 20						
Treatment number	Treatment	Rate (g a.i./ha)	Mean cumulative number of eggs per female	Standard error	Percent reduction (Pr%) of fecundity	p^a
T1	Control	-	12.19	± 0.8	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	3.14	± 0.64	74.23	***
T3	ASA-01 270 mL/ha	81.0 acetamiprid	2.26	± 1.24	81.48	***

-, not applicable
n.s., not significantly different compared to the control
[a], Dunnett's test, α=0.001 ***, 0.01 **, 0.05 *

Bioassay 2 fecundity (7 DAA)

The mean cumulative number of eggs per female in the water control was 13.00, while treatments values were 3.00 in treatment T2 (ASA-01 at 220 mL/ha) and 2.25 in treatment T3 (ASA-01 at 270 mL/ha).
Significant differences were noticed between the treatments and the control. A dose-response effect on Typhlodromus pyri fecundity was observed; therefore, the calculated LOER matched the test item rate of

220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value. The estimated ER25 of ASA-01 was 43.14 mL/ha (95% confidence intervals are 27.06 – 59.23 mL/ha) and the estimated ER50 was 103.95 mL/ha (95% confidence intervals are 65.20 – 142.71 mL/ha).

Table KCP 10.3.2.2-22: Mean cumulative number of eggs and juveniles stages per female (Bioassay 2 at 7 DAA)

Date: from 03 to 10 Aug 20						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mean cumulative number of eggs per female**	**Standard error**	**Percent reduction (Pr%) of fecundity**	***p*[a]**
T1	Control	-	13.00	± 1.50	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	3.00	± 0.27	76.91	***
T3	ASA-01 270 mL/ha	81.0 acetamiprid	2.25	± 0.82	82.73	***

-, not applicable
n.s., not significantly different compared to the control
[a], Dunnett's test, α=0.001 ***, 0.01 **, 0.05 *

Bioassay 3 fecundity (14 DAA)

The mean cumulative number of eggs per female in the water control was 12.02, while treatments values were 9.68 in treatment T2 (ASA-01 at 220 mL/ha) and 8.93 in treatment T3 (ASA-01 at 270 mL/ha). The dose effect on mites was not statistically significant (ANOVA, P>0.05), therefore, it was not possible to calculate LOER, NOER and the ER50.

Table KCP 10.3.2.2-23: Mean cumulative number of eggs and juveniles stages per female (Bioassay 3 at 14 DAA)

Date: from 10 to 17 Aug 20						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mean cumulative number of eggs per female**	**Standard error**	**Percent reduction (Pr%) of fecundity**	***p*[a]**
T1	Control	-	12.02	± 1.03	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	9.68	± 1.11	19.46	n.s.
T3	ASA-01 270 mL/ha	81.0 acetamiprid	8.93	± 1.65	25.65	n.s.

-, not applicable
n.s., not significantly different compared to the control
[a], Anova, α= 0.05 *

Bioassay 4 fecundity (21 DAA)

The mean cumulative number of eggs per female in the water control was 15.80, while treatments values were 11.70 in treatment T2 (ASA-01 at 220 mL/ha) and 11.47 in treatment T3 (ASA-01 at 270 mL/ha). The dose effect on mites was not statistically significant (ANOVA, P≤0.05), therefore, it was not possible to calculate LOER, NOER and the ER50.

Table KCP 10.3.2.2-24: Mean cumulative number of eggs and juveniles stages per female (Bioassay 4 at 21 DAA)

Date: from 17 to 24 Aug 20						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mean cumulative number of eggs per female**	**Standard error**	**Percent reduction (Pr%) of fecundity**	***p*[a]**
T1	Control	-	15.80	± 2.95	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	11.70	± 1.46	25.95	n.s.
T3	ASA-01 270 mL/ha	81.0 acetamiprid	11.47	± 1.88	27.38	n.s.

-, not applicable
n.s., not significantly different compared to the control
[a]Anova, α= 0.05 *

CONCLUSION

The obtained endpoints have been summarised below.

Table KCP 10.3.2.2-25: Mortality after 7 days of exposure and fecundity of predator mite Typhlodromus pyri for each bioassay

Treatment	T1 Control	T2 ASA-01 220 mL/ha	T3 ASA-01 270 mL/ha	T4 ROGOR L40 ST 15 mL/ha
Mortality (bioassay 1 – 0 DAA) [mean %]	3.00	43.00	70.00	69.00
Significance [a]	-	**	***	***
Mortality (bioassay 2 – 7 DAA) [mean %]	4.00	42.00	51.00	63.00
Significance [a]	-	**	**	***
Mortality (bioassay 3 – 14 DAA) [mean %]	1.00	27.00	51.00	36.00
Significance [a]	-	*	***	**
Mortality (bioassay 4 – 21 DAA) [mean %]	0.00	16.00	23.00	22.00
Significance [a]	-	n.s.	*	*
Fecundity [mean eggs/female] (bioassay 1 - 0 DAA)	12.19	3.14	2.26	-
Significance [b]	-	***	***	-
Pr % (bioassay 1 - 0 DAA)	-	74.23	81.48	-
Fecundity [mean eggs/female] (bioassay 2 - 7 DAA)	13.00	3.00	2.25	-
Significance [b]	-	***	***	-
Pr % (bioassay 2 - 7 DAA)	-	76.91	82.73	-
Fecundity [mean eggs/female] (bioassay 3 - 14 DAA)	12.02	9.68	8.93	-
Significance [c]	-	n.s.	n.s.	-
Pr % (bioassay 3 - 14 DAA)	-	19.46	25.65	-

Fecundity [mean eggs/female] (bioassay 4 - 21 DAA)		15.80	11.70	11.47	-
Significance [c]		-	n.s.	n.s.	-
Pr % (bioassay 4 - 21 DAA)		-	25.95	27.38	
Endpoint (mL/ha)					
LR_{50} (0 DAA)	236.64	NOER	-	LOER	220
LR_{50} (7 DAA)	275.51	NOER	-	LOER	220
LR_{50} (14 DAA)	268.16	NOER	-	LOER	220
LR_{50} (21 DAA)	-	NOER	220	LOER	270
ER_{50} (0 DAA)	105.96	NOER	-	LOER	220
ER_{50} (7 DAA)	103.95	NOER	-	LOER	220
ER_{50} (14 DAA)	-	NOER	-	LOER	-
ER_{50} (21 DAA)	-	NOER	-	LOER	-

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], Dunnett's test, α=0.001 ***, 0.01 **, 0.05 *
[c], Anova, α= 0.05 *
DAA = Days After Application
Pr %, Percent reduction of fecundity

Comments of zRMS:	The extended laboratory study was accepted. The validity criteria were met: • Mortality in control check: Mortality was 10.00% (bioassay 1 - 0 DAA). • Reproduction in control check The mean number of eggs per female per day was 37.76 (bioassay 0 DAA). • Mortality in reference Mortality was 82.50% (bioassay 0 DAA) in the toxic reference treatment. No deviation from study plan was noted. The following endpoints were derived: • for mortality LR_{50} = 92.9 mL product/ha

Reference:	KCP 10.3.2.2/03
Report	Effects of ASA-01 (acetamiprid 300 g/L) on *Coccinella Septempunctata* – Extended laboratory aged residue test – 2020; Mautino G.; 2021; Study Code: 1015.I.SAG20/r
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	Change of Study Director
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	SC, 300 g/L of acetamiprid
Description (physical state):	liquid
Batch no.:	19-03-19 YT1
Production date:	16.04.2020
Expiration date:	24.08.2020

2. Vehicle and/or positive control: vehicle: deionised water
positive control: dimetholate

3. Test organism

Species:	Coleoptera, Coccinellidae, *Coccinella septempunctata* L.
Source:	Katz Biotech AG, Baruth, Germany
Age:	first instar larvae, 3-day old
Acclimation period:	3 days before test start
Diet:	larvae were fed ad libitum with aphids (*Acyrthosiphon pisum* Mordv.) of mixed stages provided by the same supplier, from hatching until one day before the application
Test units:	Mortality assessment: Cylinder (45.8 mm Ø x 50 mm) of transparent plastic provided with PTFE (politetrafluoroetilene), a perforated lid (28 mm Ø) and with an insect proof net. The cylinder was inserted into a plate provided with a leaf disc of bean (50 mm Ø) leaning on two filter paper layers and blocked to it by rubbers, arranged in a cross design. Fecundity assessment Transparent plastic boxes (145 × 135 × 85 mm) with a perforated lid provided with an insect proof net for aeration. At the box bottom one layer of filter paper, while inside, three pieces of bubble wrap (PE) and a dark plastic cylinder, as oviposition substrate. Egg clutches were stored in individually labelled plastic containers (60 mL in volume) until larval hatch, over a wet layer of filter paper.

Plants:	Taxonomic group: Fabaceae Common name: bean Species: Phaseolus vulgaris L. Variety: Bingo Source: Agricola Albese (Alba, CN) Cultivation substrate: natural soil Sowing date: 23 June 2020 Emergence date: 30 June 2020 Grown site: open field under a rain cover (N 44°44'42.5'' E 008°04'06.4''). Stage for test start: BBCH 13-15 Maintenance: bottom watering two times a week, none for agrochemical and fertilized

4. Environmental conditions:

Temperature:	25.452 °C
Relative humidity:	68.9%
Photoperiod:	16 hours light : 8 hours dark, light intensity 1500 lx

STUDY DESIGN AND METHOD

The study objective was to determine the product persistence, intended as the rate of decline of residues (fresh and aged) on bean leaves treated with test item ASA-01 (acetamiprid 300 g/L) under protected field conditions. Residual toxicity was evaluated by assessing *Coccinella septempunctata* mortality and fecundity; ladybugs were introduced after 0, 7, 14 and 21 days from the application, on leaf discs cut from the treated bean plants. Four bioassays were settled: Bioassay 1 where insects were introduced immediately after the application (when leaves were dried) (0 DAA); Bioassay 2 where insects were introduced after 7 days from application (7 DAA); Bioassay 3 where insects were introduced after 14 days from application (14 DAA) and Bioassay 4 where insects were introduced after 21 days from application (21 DAA).

The study encompassed 4 treatments (2 rates of the test item, control, reference item) with 40 replicates each containing 1 larva. The larvae were exposed to fresh and aged residue on bean leaf discs at 0, 7, 14 and 21 days after application (DAA) and daily observed for mortality until the adult's emergence. At the end of this period, the observations consisted in recording percent mortality; when the survived pupae were hatch in the control, females and males (adults) were sexed, assessed for their reproductive performance and transferred to the mass-rearing units. The reproduction test started one week after the first egg laying observation. Insects oviposition was checked daily for up to 15 days. The eggs-hatching was assessed. Alive beetles was recorded.

Test design:	tested concentrations, reference item and control in 40 replicates per group, 1 larva per replicate
Tested concentrations, definitive test:	220 mL formulate product/ha (66 g as /ha) – T2 270 mL formulate product/ha (81 g as /ha) – T3 400 L water/ha
Stability of test compound:	
Dates:	start of the study: 09.06.2020 start of the experimental phase: 08.04.2020 end of the experimental phase: 14.09.2020 end of the study: 02.07.2021

Statistic:	Software used for statistical analysis was "R", version 3.4.3. Software used for statistical analysis was "RStudio", version 3.0.2. Mortality data were processed using the Fisher's Exact Test ($\alpha \leq 0.05$) and LR50 was calculated. Correction for control mortality was processed using Schneider-Orelli's formula. The different regression tests were compared each other and it was selected the ones with the best: pseudoR2, AIC and lack of fit values. Therefore, the goodness of fit was also calculated. The reproductive performance was evaluated only qualitatively as described in the IOBC/WPRS guideline. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality were provided.
Validity of the test:	The following criteria should be satisfied in the control for a test result to be considered valid: - mean mortality of larvae in the water treated control should not exceed 30%; - mean mortality of larvae in the toxic reference treatment should be higher than 40%; - no. 2 fertile eggs per viable female per day.

RESULTS

All study validity criteria were met.

Bioassay 1 mortality (0 DAA)

The mortality percentage for the test item were 87.50% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 86.11%) and 90.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 88.89%). Significant differences were noticed between the treatments and the control. The reference item ROGOR L 40 ST was significantly different with a mortality value of 82.50% (corrected mortality: 80.56%). A dose-response effect on Coccinella septempunctata mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 53.00 mL/ha (95% confidence intervals are 0.65 – 4306.28 mL/ha) and the estimated LR50 was 92.90 mL/ha (95% confidence intervals are 5.78 – 1493.45 mL/ha).

Table KCP 10.3.2.2-26: Average percentage of *Coccinella septempunctata* mortality (Bioassay 1 at 0 DAA)

Date: 09 to 29 Jul 2020						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mortality (%) ±SE[b]**		***p*[a]**	**Corrected mortality[c] (%)**
T1	Control	-	10.00	± 0.05	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	87.50	± 0.05	***	86.11
T3	ASA-01 270 mL/ha	81.0 acetamiprid	90.00	± 0.05	***	88.89
T4	ROGOR L 40 ST 30 mL/ha	12.0 dimethoate	82.50	± 0.06	***	80.56

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 40 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 2 mortality (7 DAA)

The mortality percentage for the test item were 52.50% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 45.71%) and 75.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 71.43%). Significant differences were noticed between the treatments and the control (mortality 12.50%). The reference item ROGOR L 40 ST was significantly different with a mortality value of 50.00% (corrected mortality: 42.86%). A dose-response effect on Coccinella septempunctata mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 181.10 mL/ha (95% confidence intervals are 139.30 – 235.45 mL/ha) and the estimated LR50 was 228.83 mL/ha (95% confidence intervals are 206.49 – 253.58 mL/ha).

Table KCP 10.3.2.2-27: Average percentage of *Coccinella septempunctata* mortality (Bioassay 2 at 7 DAA)

Date: 16 Jul to 05 Aug 2020						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mortality (%) ±SE[b]**		***p*[a]**	**Corrected mortality[c] (%)**
T1	Control	-	12.50	± 0.05	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	52.50	± 0.08	***	45.71
T3	ASA-01 270 mL/ha	81.0 acetamiprid	75.00	± 0.07	***	71.43
T4	ROGOR L 40 ST 30 mL/ha	12.0 dimethoate	50.00	± 0.08	***	42.86

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 40 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 3 mortality (14 DAA)

The mortality percentage for the test item were 27.50% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 21.62%) and 37.50% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 32.43%). Significant differences

were noticed between the treatments and the control (mortality 7.50%). The reference item ROGOR L 40 ST was not significantly different with a mortality value of 22.50% (corrected mortality: 16.22%). A dose-response effect on Coccinella septempunctata mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while is not possible to calculate NOER value due to the high mortality compared to the control. The estimated LR25 of ASA-01 was 85.00 mL/ha (95% confidence intervals are 23.77 – 304.00 mL/ha) and the estimated LR50 was 1600.02 mL/ha (95% confidence intervals are 183.46 – 13954.63 mL/ha).

Table KCP 10.3.2.2-28: Average percentage of *Coccinella septempunctata* mortality (Bioassay 3 at 14 DAA)

Date: 23 Jul to 07 Aug 2020						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mortality (%) ±SE[b]**		***p*[a]**	**Corrected mortality[c] (%)**
T1	Control	-	7.50	± 0.04	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	27.50	± 0.07	*	21.62
T3	ASA-01 270 mL/ha	81.0 acetamiprid	37.50	± 0.08	***	32.43
T4	ROGOR L 40 ST 30 mL/ha	12.0 dimethoate	22.50	± 0.07	n.s.	16.22

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 40 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 4 mortality (21 DAA)

The mortality percentage for the test item were 5.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 5.00%) and 7.50% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 7.50%). No Significant differences were noticed between the treatments and the control (mortality 0.00%). The reference item ROGOR L 40 ST was not significantly different with a mortality value of 5.00% (corrected mortality: 5.00%). No dose-response effect on Coccinella septempunctata mortality was observed; therefore, the calculated NOER matched the test item rate of 220 mL/ha (i.e., treatment T2). Due to the very low mortality, it was not possible to calculate the LR25 and LR50 value.

Table KCP 10.3.2.2-29: Average percentage of *Coccinella septempunctata* mortality (Bioassay 4 at 21 DAA)

Date: 23 Jul to 07 Aug 2020						
Treatment number	**Treatment**	**Rate (g a.i./ha)**	**Mortality (%) ±SE[b]**		***p*[a]**	**Corrected mortality[c] (%)**
T1	Control	-	0.00	± 0.00	-	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	5.00	± 0.03	n.s.	5.00
T3	ASA-01 270 mL/ha	81.0 acetamiprid	7.50	± 0.04	n.s.	7.50
T4	ROGOR L 40 ST 30 mL/ha	12.0 dimethoate	5.00	± 0.03	n.s.	5.00

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
[b], standard error from 40 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 1 fecundity (0 DAA)

Due to the high mortality in the treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha) it was not possible to continue with the fecundity assessment. The mean number of eggs per female per day in the water control was 37.76.

Table KCP 10.3.2.2-30: Fecundity of *Coccinella septempunctata* mortality (Bioassay 1 at 0 DAA)

Treatment name	**Treatment**	**Rate (g a.i./ha)**	**Mean number of females**	**Mean no. of eggs**	**Mean no. of hatched eggs**	**Mean number of eggs per female per day (RrX)**	**SE[a]**	**Egg-hatching (%)**
T1	Control	-	4.86	183.18	139.39	37.76	±1.70	76.00 [b]

[a], standard error (SE) from the number of breeding boxes.
[b], mean values from four breeding boxes.

Bioassay 2 fecundity (7 DAA)

Due to the high mortality in the treatment T3 (ASA-01 at 270 mL/ha) it was not possible to continue with the fecundity assessment for that treatment. The mean number of eggs per female per day in the water control was 40.81, while in the treatment T2 (ASA-01 at 220 mL/ha) was 10.11.

Table KCP 10.3.2.2-31: Fecundity of *Coccinella septempunctata* mortality (Bioassay 2 at 7 DAA)

Treatment name	**Treatment**	**Rate (g a.i./ha)**	**Mean number of females**	**Mean no. of eggs**	**Mean no. of hatched eggs**	**Mean number of eggs per female per day (RrX)**	**SE[a]**	**Egg-hatching (%)**	**Pr (%)**
T1	Control	-	4.27	173.25	129.73	40.81	±2.32	74.81 [b]	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	5.00	50.55	34.23	10.11	±1.73	68.09 [c]	8.98

[a], standard error (SE) from the number of breeding boxes.
[b], mean values from four breeding boxes.
[c], mean values from two breeding boxes.

Bioassay 3 fecundity (14 DAA)

The mean number of eggs per female per day was 34.39 in T2 (ASA-01 at 220 mL/ha) and 33.33 in T3 (ASA-01 at 270 mL/ha) in comparison to the untreated control where 36.37 eggs per female per day were recorded. The hatching rate was 70.75 % in treatment T2 (ASA-01 at 220 mL/ha) and 74.31% in treatment T3 (ASA-01 at 270 mL/ha), while in the control it was equal to 83.94%. The mean number of eggs counted in 15 days was 159.70 in treatment T2 (ASA-01 at 220 mL/ha) and 133.33 in treatment T3 (ASA-01 at 270 mL/ha).

Table KCP 10.3.2.2-32: Fecundity of *Coccinella septempunctata* mortality (Bioassay 3 at 14 DAA)

Treatment name	Treatment	Rate (g a.i./ha)	Mean number of females	Mean no. of eggs	Mean no. of hatched eggs	Mean number of eggs per female per day (RrX)	SE[a]	Egg-hatching (%)	Pr (%)
T1	Control	-	4.45	155.36	130.43	36.37	±5.32	83.94 [b]	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	4.61	159.70	112.76	34.39	±2.03	70.75 [c]	15.71
T3	ASA-01 270 mL/ha	81.0 acetamiprid	4.00	133.33	99.24	33.33	±2.18	74.31 [c]	11.47

[a], standard error (SE) from the number of breeding boxes.
[b], mean values from four breeding boxes.
[c], mean values from three breeding boxes.

Bioassay 4 fecundity (21 DAA)

The mean number of eggs per female per day was 36.05 in T2 (ASA-01 at 220 mL/ha) and 35.47 in T3 (ASA-01 at 270 mL/ha) in comparison to the untreated control where 39.12 eggs per female per day were recorded. The hatching rate was 82.50 % in treatment T2 (ASA-01 at 220 mL/ha) and 82.69% in treatment T3 (ASA-01 at 270 mL/ha), while in the control it was equal to 83.59%. The mean number of eggs counted in 15 days was 109.73 in treatment T2 (ASA-01 at 220 mL/ha) and 84.18 in treatment T3 (ASA-01 at 270 mL/ha).

Table KCP 10.3.2.2-33: Fecundity of *Coccinella septempunctata* mortality (Bioassay 4 at 21 DAA)

Treatment name	Treatment	Rate (g a.i./ha)	Mean number of females	Mean no. of eggs	Mean no. of hatched eggs	Mean number of eggs per female per day (RrX)	SE[a]	Egg-hatching (%)	Pr (%)
T1	Control	-	4.11	160.93	134.34	39.12	±1.86	83.59 [b]	-
T2	ASA-01 220 mL/ha	66.0 acetamiprid	3.23	109.73	90.70	36.05	±2.07	82.50 [b]	1.30
T3	ASA-01 270 mL/ha	81.0 acetamiprid	2.57	84.18	69.70	35.47	±2.19	82.69 [b]	1.07

[a], standard error (SE) from the number of breeding boxes.
[b], mean values from four breeding boxes.

CONCLUSION

All study validity criteria were met.
Mean % mortality in the control were 10.00% (0 DAA), 12.50% (7 DAA), 7.50% (14 DAA) and 0.00 (21 DAA), while in the toxic reference treatment was 82.50% at 0 DAA. The mean number of eggs per female per day were 37.76 (0 DAA), 40.18 (7 DAA), 36.37 (14 DAA) and 39.12 (21 DAA).

Table KCP 10.3.2.2-34: Mortality and fecundity efficiency of *Coccinella septempunctata*

	T1 Control	T2 ASA-01 220 mL/ha	T3 ASA-01 270 mL/ha	T4 ROGOR L40 ST 30 mL/ha
Mortality (bioassay 1 – 0 DAA) [mean %]	10.00	87.50	90.00	82.50
Significance [a]	-	***	***	***
Mortality (bioassay 2 – 7 DAA) [mean %]	12.50	52.50	75.00	50.00
Significance [a]	-	***	***	***
Mortality (bioassay 3 – 14 DAA) [mean %]	7.50	27.50	37.50	22.50
Significance [a]	-	*	***	n.s.
Mortality (bioassay 4 – 21 DAA) [mean %]	0.00	5.00	7.50	5.00
Significance [a]	-	n.s.	n.s.	n.s.
Reproduction [mean eggs/female/day] (bioassay 1 - 0 DAA)	37.76	-	-	-
Reproduction [mean eggs/female/day] (bioassay 2 - 7 DAA)	40.81	10.11	-	-
Reproduction [mean eggs/female/day] (bioassay 3 - 14 DAA)	36.37	34.39	33.33	-
Reproduction [mean eggs/female/day] (bioassay 4 - 21 DAA)	39.12	36.05	35.47	-
% egg-hatching (bioassay 1 - 0 DAA)	76.00	-	-	-
% Pr (bioassay 1 - 0 DAA)	-	-	-	-
% egg-hatching (bioassay 2 - 7 DAA)	74.81	68.09	-	-
% Pr (bioassay 2 - 7 DAA)	-	8.98		
% egg-hatching (bioassay 3 - 14 DAA)	83.94	70.75	74.31	-
% Pr (bioassay 3 - 14 DAA)	-	15.71	11.47	-

% egg-hatching (bioassay 4 - 21 DAA)		83.59	82.50	82.69	-
% Pr (bioassay 4 – 21 DAA)		-	1.30	1.07	-
Endpoint (mL/ha)					
LR_{50} (0 DAA)	92.90	NOER	-	LOER	220
LR_{50} (7 DAA)	228.83	NOER	-	LOER	220
LR_{50} (14 DAA)	1600.02	NOER	-	LOER	220
LR_{50} (21 DAA)	-	NOER	220	LOER	-

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.001 ***, 0.01 **, 0.05 *
DAA = Days After Application
%Pr, percent reduction in reproduction

Comments of zRMS:	The extended laboratory study was accepted. The validity criteria were met: • mortality in the control: ≤ 20 % (observed: 16.67 %); • mortality in the reference item group: > 50 % (observed: 70 %); • reproduction in the control: ≥ 15 (mean number of eggs/female and day; observed: 24.97); • hatching rate in the control group was 74.18%. The following endpoint was derived: LR_{50} = 177.78 mL product/ha. No NOER was assessed.

Reference:	KCP 10.3.2.2/04
Report	Effects of ASA-01 (acetamiprid 300 g/L) on foliage dwelling predator *Chrysoperla carnea* in the laboratory – Extended laboratory aged residue study – Year 2020 Mautino G.; 2021; Study Code: 1018.I.SAG20/r
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	Change of Study Director
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): ASA-01

Formulation: SC, 300 g/L of acetamiprid

Description (physical state): liquid

Batch no.:	19-03-19 YT1
Production date:	16.04.2020
Expiration date:	24.08.2020

2. Vehicle and/or positive control: vehicle: deionised water
positive control: dimetholate

3. Test organism

Species:	Neuroptera, Chrysopidae, *Chrysoperla carnea* Stephens
Source:	Katz Biotech AG, Baruth, Germany
Age:	first instar larvae
Acclimation period:	2 days under test conditions (same feeding)
Diet:	larvae fed ad libitum with with Sitotroga sp.' eggs (provided by the same supplier), from hatching until one day before the application
Test units:	Mortality assessment: Cylinder (45.8 mm Ø x 50 mm) of transparent plastic provided with PTFE (politetrafluoroetilene), a perforated lid (28 mm Ø) and with an insect proof net. The cylinder was inserted into a plate provided with a leaf disc of bean (50 mm Ø) leaning on two filter paper layers and blocked to it by rubbers, arranged in a cross design. Fecundity assessment Glass cylinder (5000 cc, 16 cm Ø, 29 cm) provided with an insect proof net, blocked to it by rubbers.
Plants:	Taxonomic group: Fabaceae Common name: bean Species: Phaseolus vulgaris L. Variety: Bingo Source: Agricola Albese (Alba, CN) Cultivation substrate: natural soil Sowing date: 23 Sept. 2020 Emergence date: 29 Sept. 2020 Grown site: open field under a rain cover (N 44°44’42.5’’ E 008°04’06.4’’) Stage for test start: BBCH 13-15 Maintenance: bottom watering two times a week, none for agrochemical and fertilized

4. Environmental conditions:

Temperature:	26.090 °C
Relative humidity:	67.4%
Photoperiod:	16 hours light : 8 hours dark, light intensity 1500 lx

STUDY DESIGN AND METHOD

The aim of the study was to determine the product persistence, intended as the rate of decline of residues (fresh and aged) on bean leaves treated with test item ASA-01 (acetamiprid 300 g/L) under field conditions (with rain protection). Residual toxicity was evaluated observing the effects on *Chrysoperla carnea* mortality over time and to evaluate the sub-lethal effects on insect fecundity subsequent to their exposure to the test item applied once on bean plants compared to a water-treated control and to a reference item.

Insects were introduced after 0, 7, 14 and 21 days from the application. Four bioassays were settled: Bioassay 1 where insects were introduced immediately after the application (when leaves were dried) (0 DAA); Bioassay 2 where insects were introduced after 7 days from application (7 DAA); Bioassay 3 where insects were introduced after 14 days from application (14 DAA) and Bioassay 4 where insects were introduced after 21 days from application (21 DAA).
The study encompassed 4 treatments (2 rates of the test item, control, reference item) with 30 replicates each containing 1 larva. The larvae were exposed to fresh and aged residue on bean leaf discs at 0, 7, 14 and 21 days after application (DAA) and observed regularly, three times per week. At the end of this period, the observations consisted in giving percent mortality; ≥ 50% of larvae exposed to the test item survived and successfully completed their metamorphosis, females and males (adults) were sexed, assessed for their reproductive performance and transferred to the mass-rearing units. The reproduction test started one week after the first egg laying observation. Samples of two laid eggs, covering an oviposition period of 24 hours, were taken in one week. Larvae hatching and lacewings survival were assessed.

Test design:	tested concentrations, reference item and control in 30 replicates per group, 1 larva per replicate
Tested concentrations, definitive test:	220 mL formulate product/ha (66 g as /ha) – T2 270 mL formulate product/ha (81 g as /ha) – T3 400 L water/ha
Stability of test compound:	
Dates:	start of the study: 09.06.2020 start of the experimental phase: 07.10.2020 end of the experimental phase: 16.12.2020 end of the study: 02.07.2021
Statistic:	Software used for statistical analysis was "R", version 3.4.3. Mortality data were processed using the Fisher's Exact Test, $\alpha \leq 0.05$ and LR50 was calculated. Correction for control mortality was processed using Schneider-Orelli's formula. The different regression tests were compared each other and it was selected the ones with the best: pseudoR2, AIC and lack of fit values. Therefore, the goodness of fit was also calculated. Fecundity data were analysed by Anova and Dunnett's t-test, $\alpha \leq 0.05$ and the ER50 calculated. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were calculated.

RESULTS

All study validity criteria were met.

Bioassay 1 mortality (0 DAA)
The mortality percentage for the test item was 53.33% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 44.00%) and 70.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 64.00%). Significant differences were noticed between the treatments and the control, showing a 16.67% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 70.00% (corrected mortality: 64.00%). A dose-response effect on Chrysoperla carnea mortality was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while it was not possible to calculate the NOER value. The estimated LR25 of ASA-01 was 8.61 mL/ha (95% confidence intervals are 1.42 –

52.332 mL/ha) and the estimated LR50 was 177.78 mL/ha (95% confidence intervals are 63.03 – 501.45 mL/ha).

Table KCP 10.3.2.2-35: Average percentage of *Chrysoperla carnea* mortality during the assessments 0 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Mortality (%)		p^a	Corrected mortality[c] (%)
T1	Control	-	16.67	± 0.07 [b]	-	-
T2	ASA-01 220 mL/ha	66	53.33	± 0.09 [b]	**	43.99
T3	ASA-01 270 mL/ha	81	70.00	± 0.09 [b]	***	64.00
T4	ROGOR L 40 ST 110 mL/ha	44	70.00	± 0.09 [b]	***	64.00

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.05 *, 0.01 **, 0.001 ***
[b], standard error from 30 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 2 mortality (7 DAA)

The mortality percentage for the test item was 30.00% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 19.23%) and 30.00% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 19.23%). No significant differences were noticed between the treatments and the control, showing a 13.33% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 66.67% (corrected mortality: 61.54%). No dose-response effect on Chrysoperla carnea mortality was observed; therefore, the calculated NOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while it was not possible to calculate the LOER value. Due to the low mortality of treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value.

Table KCP 10.3.2.2-36: Average percentage of *Chrysoperla carnea* mortality during the assessments 7 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Mortality (%)		p^a	Corrected mortality[c] (%)
T1	Control	-	13.33	± 0.06 [b]	-	-
T2	ASA-01 220 mL/ha	66	30.00	± 0.09 [b]	n.s	19.23
T3	ASA-01 270 mL/ha	81	30.00	± 0.09 [b]	n.s	19.23
T4	ROGOR L 40 ST 110 mL/ha	44	66.67	± 0.09 [b]	***	61.54

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.05 *, 0.01 **, 0.001 ***
[b], standard error from 30 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 3 mortality (14 DAA)

The mortality percentage for the test item was 26.67% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 15.38%) and 33.33% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 23.08%). No Significant

differences were noticed between the treatments and the control, showing a 13.33% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 66.67% (corrected mortality: 61.54%). No dose-response effect on Chrysoperla carnea mortality was observed; therefore, the calculated NOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while it was not possible to calculate the LOER value. Due to the low mortality of treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value.

Table KCP 10.3.2.2-37: Average percentage of *Chrysoperla carnea* mortality during the assessments 14 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Mortality (%)		p^a	Corrected mortality[c] (%)
T1	Control	-	13.33	± 0.06[b]	-	-
T2	ASA-01 220 mL/ha	66	26.67	± 0.08[b]	n.s	15.39
T3	ASA-01 270 mL/ha	81	33.33	± 0.09[b]	n.s	23.08
T4	ROGOR L 40 ST 110 mL/ha	44	66.67	± 0.09[b]	***	61.54

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.05 *, 0.01 **, 0.001 ***
[b], standard error from 30 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 4 mortality (21 DAA)

The mortality percentage for the test item was 13.33% in T2 (ASA-01 at 220 mL/ha, corrected mortality: 3.70%) and 23.33% in T3 (ASA-01 at 270 mL/ha, corrected mortality: 14.81%). No significant differences were noticed between the treatments and the control, showing a 10.00% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 53.33% (corrected mortality: 48.14%). No dose-response effect on Chrysoperla carnea mortality was observed; therefore, the calculated NOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while it was not possible to calculate the LOER value. Due to the low mortality of treatments T2 (ASA-01 at 220 mL/ha) and T3 (ASA-01 at 270 mL/ha), it was not possible estimate the LR50 value.

Table KCP 10.3.2.2-38: Average percentage of *Chrysoperla carnea* mortality during the assessments 21 DAA

Treatment number	Treatment	Rate (g a.i./ha)	Mortality (%)		p^a	Corrected mortality[c] (%)
T1	Control	-	10.00	± 0.06[b]	-	-
T2	ASA-01 220 mL/ha	66	13.33	± 0.06[b]	n.s	3.70
T3	ASA-01 270 mL/ha	81	23.33	± 0.08[b]	n.s	14.81
T4	ROGOR L 40 ST 110 mL/ha	44	53.33	± 0.09[b]	***	48.14

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, α=0.05 *, 0.01 **, 0.001 ***
[b], standard error from 30 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

Bioassay 1 fecundity (0 DAA)

The mean number of eggs per female per day was 8.05 (T2 – ASA-01 at 220 mL/ha). Significant differences were noticed between the treatment T2 (ASA-01 at 220 mL/ha) and the control, showing a mean number of eggs per female per day of 24.97. The hatching rate was 67.42% in treatment T2 (ASA-01 at 220 mL/ha), while in the control it was equal to 74.18%. Due to the high mortality in the treatment T3 (ASA-01 at 270 mL/ha) it was not possible to continue with the fecundity assessment. A dose-response effect on Chrysoperla carnea reproductive performance was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while it was not possible to calculate the NOER value. Due to the presence of only two treatments tested it is not possible to estimate the ER50 value.

Table KCP 10.3.2.2-39: Mean number of eggs per female per day during the assessments 0 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		Mean number of eggs per female per day	SE	p^a
			Females	Eggs	Females	Eggs			
T1	Control	I	8	195	8	218	24.97	±0.84	-
		II	8	199	8	187			
T2	ASA-01 220 mL/ha	I	5	43	5	35	8.05	±0.25	***
		II	5	36	5	47			

-, not applicable
n.s., not significantly different compared to the control,
[a]Anova, α=0.05 *, 0.01 **, 0.001 ***

Table KCP 10.3.2.2-40: Average percentage of egg hatching during the assessments 0 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		% egg-hatching
			Eggs	Larvae	Eggs	Larvae	
T1	Control	I	195	141	218	155	74.18
		II	199	159	187	137	
T2	ASA-01 220 mL/ha	I	43	38	35	23	67.42
		II	36	22	47	25	

Bioassay 2 fecundity (7 DAA)

The mean number of eggs per female per day was 11.03 (T2 – ASA-01 at 220 mL/ha) and 8.06 (T3 – ASA-01 at 270 mL/ha) in comparison to the untreated control where 27.82 eggs per female per day were recorded. Significant differences were noticed between the treatments and the control. The hatching rate was 65.87% in treatment T2 (ASA-01 at 220 mL/ha) and 63.78% in treatment T3 (ASA-01 at 270 mL/ha), while in the control it was equal to 72.83%. A dose-response effect on Chrysoperla carnea reproductive performance was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while it was not possible to calculate the NOER value. The estimated ER25 of ASA-01 was 114.69 mL/ha (95% confidence intervals are 85.14 – 314.53 mL/ha) and the estimated ER50 was 183.76 mL/ha (95% confidence intervals are 59.73 – 307.78 mL/ha).

Table KCP 10.3.2.2-40: Mean number of eggs per female per day during the assessments 7 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		Mean number of eggs per female per day	SE	p^a
			Females	Eggs	Females	Eggs			
T1	Control	I	7	171	7	186	27.82	±1.77	-
		II	6	156	6	164			
		III	5	154	5	159			
T2	ASA-01 220 mL/ha	I	8	113	8	89	11.03	±1.59	**
		II	8	86	8	65			
T3	ASA-01 270 mL/ha	I	7	63	7	55	8.06	±0.37	**
		II	8	56	8	67			

-, not applicable
n.s., not significantly different compared to the control,
[a]Dunnet, α=0.05 *, 0.01 **, 0.001 ***

Table KCP 10.3.2.2-41: Average percentage of egg hatching during the assessments 7 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		% egg-hatching
			Eggs	Larvae	Eggs	Larvae	
T1	Control	I	171	115	186	146	72.83
		II	156	126	164	102	
		III	154	140	159	92	
T2	ASA-01 220 mL/ha	I	113	78	89	57	65.87
		II	86	46	65	52	
T3	ASA-01 270 mL/ha	I	63	53	55	39	63.78
		II	56	53	67	28	

Bioassay 3 fecundity (14 DAA)

The mean number of eggs per female per day was 15.06 (T2 – ASA-01 at 220 mL/ha) and 12.49 (T3 – ASA-01 at 270 mL/ha). Significant differences were noticed between the treatments and the control, showing a mean number of eggs per female per day of 25.83. The hatching rate was 64.05% in treatment T2 (ASA-01 at 220 mL/ha) and 63.05% in treatment T3 (ASA-01 at 270 mL/ha), while in the control it was equal to 68.93%. A dose-response effect on Chrysoperla carnea reproductive performance was observed; therefore, the calculated LOER matched the test item rate of 220 mL/ha (i.e., treatment T2), while it was not possible to calculate the NOER value. The estimated ER25 of ASA01 was 149.06 mL/ha (95% confidence intervals are 69.88 – 367.99 mL/ha) and the estimated ER50 was 261.18 mL/ha (95% confidence intervals are 160.14 – 362.22 mL/ha).

Table KCP 10.3.2.2-41: Mean number of eggs per female per day during the assessments 14 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		Mean number of eggs per female per day	SE	p^a
			Females	Eggs	Females	Eggs			
T1	Control	I	6	125	6	153	25.83	±1.34	-
		II	6	162	6	167			
		III	5	136	5	133			
T2	ASA-01 220 mL/ha	I	8	162	8	106	15.06	±1.69	*
		II	8	96	8	118			
T3	ASA-01 270 mL/ha	I	7	101	7	107	12.49	±2.37	*
		II	8	86	8	76			

-, not applicable
n.s., not significantly different compared to the control,
[a]Dunnet, α=0.05 *, 0.01 **, 0.001 ***

Table KCP 10.3.2.2-42: Average percentage of egg hatching during the assessments 14 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		% egg-hatching
			Eggs	Larvae	Eggs	Larvae	
T1	Control	I	125	95	153	126	68.93
		II	162	122	167	106	
		III	136	77	133	79	
T2	ASA-01 220 mL/ha	I	162	91	106	77	64.05
		II	96	66	118	74	
T3	ASA-01 270 mL/ha	I	101	82	107	75	63.05
		II	86	47	76	35	

Bioassay 4 fecundity (21 DAA)

The mean number of eggs per female per day was 27.94 (T2 – ASA-01 at 220 mL/ha) and 24.27 (T3 – ASA-01 at 270 mL/ha). No significant differences were noticed between the treatments and the control, showing a mean number of eggs per female per day of 31.03. The hatching rate was 68.00% in treatment T2 (ASA-01 at 220 mL/ha) and 69.52% in treatment T3 (ASA-01 at 270 mL/ha), while in the control it was equal to 69.79%. No dose-response effect on Chrysoperla carnea reproductive performance was observed; therefore, the calculated NOER matched the test item rate of 270 mL/ha (i.e., treatment T3), while it was not possible to calculate the LOER value and estimate the ER50 value.

Table KCP 10.3.2.2-43: Mean number of eggs per female per day during the assessments 21 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		Mean number of eggs per female per day	SE	p^a
			Females	Eggs	Females	Eggs			
T1	Control	I	7	195	7	193	31.03	±2.60	-
		II	7	192	7	217			
		III	6	236	6	198			
T2	ASA-01 220 mL/ha	I	7	202	7	163	27.94	±1.04	n.s.
		II	6	169	6	187			
		III	6	191	6	146			
T3	ASA-01 270 mL/ha	I	6	166	6	153	24.27	±1.23	n.s.
		II	6	142	6	144			
		III	5	89	5	135			

-, not applicable
n.s., not significantly different compared to the control,
[a]Anova, α=0.05 *, 0.01 **, 0.001 ***

Table KCP 10.3.2.2-44: Average percentage of egg hatching during the assessments 21 DAA

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		% egg-hatching
			Eggs	Larvae	Eggs	Larvae	
T1	Control	I	195	125	193	116	69.79
		II	192	159	217	170	
		III	236	171	198	119	
T2	ASA-01 220 mL/ha	I	202	149	163	125	68.00
		II	169	109	187	106	
		III	191	145	146	86	
T3	ASA-01 270 mL/ha	I	166	113	153	134	69.52
		II	142	107	144	88	
		III	89	60	135	81	

CONCLUSION

All study validity criteria were met. Mean % mortality in the control were 16.67% (0 DAA), 13.33% (7 DAA), 13.33% (14 DAA) and 10.00% (21 DAA), while in the toxic reference treatment was 70.00% at 0 DAA. The mean number of eggs per female was 24.97 (0 DAA), 27.82 (7 DAA), 25.83 (14 DAA) and 31.03 (21 DAA).

Table KCP 10.3.2.2-45: Mortality and fecundity efficiency of *Chrysoperla carnea*

	T1 Control deionised water	T2 ASA-01 220 mL/ha	T3 ASA-01 270 mL/ha	T4 ROGOR L40 ST 110 mL/ha
Mortality (bioassay 1 – 0 DAA) [mean %]	16.67	53.33	70.00	70.00
Significance [a]	-	**	***	***
Mortality (bioassay 2 – 7 DAA) [mean %]	13.33	30.00	30.00	66.67
Significance [a]	-	n.s.	n.s.	***
Mortality (bioassay 3 – 14 DAA) [mean %]	13.33	26.67	33.33	66.67
Significance [a]	-	n.s.	n.s.	***
Mortality (bioassay 4 – 21 DAA) [mean %]	10.00	13.33	23.33	53.33
Significance [a]	-	n.s.	n.s.	***
Reproduction [mean eggs/females] (bioassay 1 - 0 DAA)	24.97	8.05	-	-
Significance [b]	-	***	-	-
Reproduction [mean eggs/females] (bioassay 2 - 7 DAA)	27.82	11.03	8.06	-
Significance [c]	-	**	**	-
Reproduction [mean eggs/females] (bioassay 3 - 14 DAA)	25.83	15.06	12.49	-
Significance [c]	-	*	*	
Reproduction [mean eggs/females] (bioassay 4 - 21 DAA)	31.03	27.94	24.27	-
Significance [b]	-	n.s.	n.s.	-

% egg-hatching (bioassay 1 - 0 DAA)		74.18	67.42	-	-
% egg-hatching (bioassay 2 - 7 DAA)		72.83	65.87	63.78	-
% egg-hatching (bioassay 3 - 14 DAA)		68.93	64.05	63.05	-
% egg-hatching (bioassay 4 - 21 DAA)		69.79	68.00	69.52	-
Endpoint (mL/ha)					
LR_{50} (0 DAA)	177.78	NOER	-	LOER	220
LR_{50} (7 DAA)	-	NOER	270	LOER	-
LR_{50} (14 DAA)	-	NOER	270	LOER	-
LR_{50} (21 DAA)	-	NOER	270	LOER	-
ER_{50} (0 DAA)	-	NOER	-	LOER	220
ER_{50} (7 DAA)	183.76	NOER	-	LOER	220
ER_{50} (14 DAA)	261.18	NOER	-	LOER	220
ER_{50} (21 DAA)	-	NOER	270	LOER	-

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test, $\alpha \leq 0.001$ ***, 0.01 **, 0.05 *
[b], Anova, $\alpha \leq 0.05$ *,0.01 **, 0.001 ***
[c], Dunnet, $\alpha \leq 0.05$ *,0.01 **, 0.001 ***
DAA = Days After Application

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 submitted study was accepted. The validity criteria were met: • adult mortality 4 weeks: less than10 % (being 1.3 % after 4 weeks); • number of juveniles per replicate: more than 30; (being 81.9) • coefficient of variation of reproduction: less than 30 % (being 21 %) No significant deviations from study plan were noted: The following endpoints for mortality were derived: NOEC = 18 mg formulation/kg d.w., equivalent to 5.0 mg a.s./kg dw LC_{50} = 27.4 mg formulation/kg d.w., equivalent to 7.6 mg a.s./kg dw and for reproduction the following endpoints were derived: NOEC = 10 mg formulation/kg d.w, equivalent to 2.8 mg a.s./kg dw EC_{10} = 11.3 mg formulation/kg d.w, equivalent to 3.1 mg a.s./kg dw EC_{50} = 22.3 . formulation/kg d.w, equivalent to 6.1 mg a.s./kg dw

Reference:	KCP 10.4.1.1/01
Report	ASA-01 Earthworm reproduction test (*Eisenia andrei*); Pieczka P.; 2020; Study Code: G/54/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Earthworm reproduction test (*Eisenia andrei*); Pieczka P.; 2021; Study Code: G/54/19
Guideline(s):	Yes, OECD 222
Deviations:	Due to the Sponsor's request, the name of the test item was changed from ASA-01, SC to ASA-01. The deviation did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	earthworm *Eisenia andrei*
Source:	cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology
Age:	about 7 months old
Acclimation period:	24 hours acclimatization
Diet:	during the experiment, the earthworms were fed on air-dried finely ground cow manure; one day after the beginning of the experiment, it was spread on the soil surface (5 g food/ container) and moistened; the food prepared in this way was provided once a week during the four-week period (5 g food/container); after 4 weeks (when the adult earthworms were removed from the soil), the juvenile earthworms were fed only once (5 g food/container)

Test units:	plastic containers (204 cm2) with a capacity of about 1.4L, containers were covered with perforated transparent foil in order to prevent the earthworms from escaping, to allow gaseous exchange, and to provide access to light

4. Environmental conditions:

Temperature:	18-21°C
Soil:	artificial soil 10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
pH:	pH at the beginning of the experiment: 5.54 – 5.56; pH at the end of the experiment: 5.54 – 5.59;
Soil moisture content:	soil moisture content at the beginning of the experiment: 21.4 – 22.4% (54.4 – 56.9% of the maximum water holding capacity) soil moisture content at the end of the experiment: 19.8–21.5% (50.3 – 54.6% of the maximum water holding capacity)
Photoperiod:	light-dark cycle: 16h : 8h light intensity at the beginning of the experiment: 593 – 746 lux light intensity at the end of the experiment: 698 – 738 lux

STUDY DESIGN AND METHOD

The aims of the study were to assess the impact of ASA-01 on reproduction of the earthworm, Eisenia andrei and to determine the EC10, EC20, EC50, and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. The concentrations of the test item were 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also untreated control group (with deionised water and without test item) divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined.
The impact of the test item on reproduction was evaluated after an additional 4-week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Test design:	number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms /replicate
Exposure time:	8 weeks
Tested concentrations, definitive test:	0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg/kg dry weight of the artificial soil

Stability of the test compound:	in order to verify the nominal soil concentration of the test item, the analytical measurements of the artificial soil treated with the test item at the highest concentrations (i.e. 32 mg of the test item/kg dry weight of the artificial soil) were performed at the beginning, during (after 4 weeks) and at the end of the test; there was 1 additional container for each analysed concentration and the control group
Dates:	start of the study: 12.12.2019 start of the experimental part: 18.12.2019 end of the experimental part: 15.02.2020 end of the study: 04.03.2020
Statistic:	EC_{10}, EC_{20}, EC_{50}, LC_{50} – logit analysis using linear max. likelihood regression, NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Dunnett's Multple t-test Procedure NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software
Validity of the test:	The results are considered valid because the following criteria were satisfied in the controls: - each replicate produced 81.9 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment), - the coefficient of variation of reproduction was 21.0% (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 1.3% (criterion: ≤ 10%).

RESULTS

After 4 weeks of the experiment, at the concentrations ranging from 0.056 to 32 mg/kg dry weight of artificial soil, the mortality of adult earthworms was between 0.0 and 62.5%. Mortality in the control group was equal to 1.3%.

Table KCP 10.4.1.1-1: Mortality of the adult earthworms *(Eisenia andrei)* after 4 weeks of the experiment

Concentration [mg of the test item/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Number of alive earthworms [no.]	Total mortality	
				no.	%
0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 10 10 10 10 10 10 9	1	1.3
0.056	1 2 3 4	10 10 10 10	10 10 10 9	1	2.5
0.10	1 2 3 4	10 10 10 10	10 10 10 9	1	2.5
0.18	1 2 3 4	10 10 10 10	10 10 10 9	1	2.5
0.32	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
0.56	1 2 3 4	10 10 10 10	10 10 9 10	1	2.5
1.00	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
1.8	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
3.2	1 2 3 4	10 10 10 10	10 10 10 9	1	2.5
5.6	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
10	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
18	1 2 3 4	10 10 10 10	9 10 10 8	3	7.5
32	1 2 3 4	10 10 10 10	6 0 3 6	25*	62.5

The results of the observations of the earthworms for changes in behaviour and in morphology are presented below. After 4 weeks of the experiment, the treated alive earthworms did not exhibit any changes in appearance and behaviour.

Table KCP 10.4.1.1-2: Results of the observations of the adult earthworms (*Eisenia andrei*) for changes in behaviour and in morphology.

Concentration [mg of the test item/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Changes in behaviour and in morphology
0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 nc 10 nc 10 nc 10 nc 10 nc 10 nc 10 nc 9 nc 1 d
0.056	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 9 nc 1 d
0.10	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 9 nc 1 d
0.18	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 9 nc 1 d
0.32	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 10 nc
0.56	1 2 3 4	10 10 10 10	10 nc 10 nc 9 nc 1 d 10 nc
1.00	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 10 nc
1.8	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 10 nc
3.2	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 9 nc 1 d
5.6	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 10 nc
10	1 2 3 4	10 10 10 10	10 nc 10 nc 10 nc 10 nc
18	1 2 3 4	10 10 10 10	9 nc 1 d 10 nc 10 nc 8 nc 2 d
32	1 2 3 4	10 10 10 10	6 nc 4 d 10 d 3 nc 7 d 6 nc 4 d

nc – no changes;
d – dead

After the application of the test item at the concentrations ranging from 0.056 to 18 mg of the test item/kg dry weight of artificial soil, the body weight decrease was between 4.0 and 11.9%. As for the control group, the body weight decrease was equal to 4.7%.

After the application of the test item at the concentrations ranging from 0.056 to 32 mg of the test item/kg dry weight of the artificial soil, the mean number of juveniles was between 20.0 – 106.3 per replicate. The mean number of juveniles in the control group was equal to 81.9 per replicate. After 8 weeks of the experiment, it was concluded that ASA-01 had a statistically significant impact on reproduction of the earthworms at concentrations ranging from 18 to 32.0 mg of the test item/kg dry weight of artificial soil.

Table KCP 10.4.1.1-3: Number of juvenile earthworms (*Eisenia andrei*) after 8 weeks of the experiment

Concentration [mg of the test item/kg dry weight of the artificial soil]	Replicate	Number of juveniles [no.]	Mean ±SD	Comparison to the control [%]	CV* [%]
0 (control)	1 2 3 4 5 6 7 8	89 80 93 99 45 72 94 83	81.9 ± 17.2	-	21.0
0.056	1 2 3 4	67 76 108 79	82.5 ± 17.7	100.8	21.5
0.10	1 2 3 4	104 106 110 87	101.8 ± 10.1	124.3	10.0
0.18	1 2 3 4	129 99 100 76	101.0 ± 21.7	123.4	21.5
0.32	1 2 3 4	115 125 103 75	104.5 ± 21.6	127.6	20.7
0.56	1 2 3 4	95 106 88 66	88.8 ± 16.9	108.4	19.0
1.00	1 2 3 4	102 124 102 97	106.3 ± 12.1	129.8	11.4
1.8	1 2 3 4	104 71 88 93	89.0 ± 13.7	108.7	15.4
3.2	1 2 3 4	85 78 71 85	79.8 ± 6.7	97.4	8.4
5.6	1 2 3 4	65 72 90 87	78.5 ± 12.0	95.9	15.2
10	1 2 3 4	90 81 64 80	78.8 ±10.8	96.2	13.7
18	1 2 3 4	57 64 44 49	53.5 ± 8.8+	65.3	16.5
32	1 2 3 4	27 3 12 38	20.0 ± 15.6+	24.4	77.8

* - coefficient of variation

+- statistically significant difference between the control and the treatment group (Dunnett's Multiple t-test Procedure, significance level = 0.05, one-sided smaller)

Table KCP 10.4.1.1-4: Results of the observations for changes in behaviour and in morphology of the juveniles earthworms (*Eisenia andrei*)

Concentration [mg of the test item/kg dry weight of the artificial soil]	Replicate	Number of juveniles after 8 weeks of the experiment [no.]	Changes in behaviour and in morphology*
0 (control)	1	89	nc
	2	80	nc
	3	93	nc
	4	99	nc
	5	45	nc
	6	72	nc
	7	94	nc
	8	83	nc
0.056	1	67	nc
	2	76	nc
	3	108	nc
	4	79	nc
0.10	1	104	nc
	2	106	nc
	3	110	nc
	4	87	nc
0.18	1	129	nc
	2	99	nc
	3	100	nc
	4	76	nc
0.32	1	115	nc
	2	125	nc
	3	103	nc
	4	75	nc
0.56	1	95	nc
	2	106	nc
	3	88	nc
	4	66	nc
1.00	1	102	nc
	2	124	nc
	3	102	nc
	4	97	nc
1.8	1	104	nc
	2	71	nc
	3	88	nc
	4	93	nc
3.2	1	85	nc
	2	78	nc
	3	71	nc
	4	85	nc
5.6	1	65	nc
	2	72	nc
	3	90	nc
	4	87	nc
10	1	90	nc
	2	81	nc
	3	64	nc
	4	80	nc
18	1	57	nc
	2	64	nc
	3	44	nc
	4	49	nc
32	1	27	nc
	2	3	nc
	3	12	nc
	4	38	nc

* nc – no changes

CONCLUSION

The concentration of the test item causing 50% mortality of the adult earthworms (LC_{50}) is equal to 27.4 mg of the test item/kg dry weight of artificial soil (7.6 mg of acetamiprid /kg dry weight of artificial soil). The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

Table KCP 10.4.1.1-5: Earthworm reproduction test – final results

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of acetamiprid/kg dry weight of artificial soil]
EC_{10}	11.3 (10.3 – 12.2)	3.1 (2.8 – 3.4)
EC_{20}	14.5 (13.6 – 15.4)	4.0 (3.7 – 4.2)
EC_{50}	22.3 (21.5 – 23.2)	6.1 (5.9 – 6.4)
NOEC (reproduction)	10.0	2.8
LOEC (reproduction)	18.0	5.0
LC_{50}	27.4	7.5
NOEC (survival)	18.0	5.0
LOEC (survival)	32.0	8.8

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not relevant. No studies submitted.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: • mean mortality of adult females: ≤ 20 %; observed 2.5 %; • mean number of juveniles per replicate: ≥ 50; observed 113.6; • coefficient of variation (mean number of juveniles per replicate): ≤ 30 %; observed 12.4 %; Some deviations from OECD 226 guidance were noted: 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test. 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution.

	3. Due to the use of the temperature extraction method, there was no impossible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. These deviations did not affect on final results and conclusion. The following endpoints were derived: • mortality: NOEC ≥1000 mg formulation/kg d.s., equivalent to ≥ 275.3 mg a.s./kg ds; LC_{50} > 1000 mg formulation/kg d.s., equivalent to > 275.3 mg a.s./kg ds • reproduction: NOEC = 560 mg formulation/kg d.s., equivalent to 154.2 mg a.s./kg ds EC_{50} > 1000 mg formulation/kg d.s., equivalent to 275.3 mg a.s./kg ds EC_{10} = 841.1 mg formulation/kg d.s., equivalent to 231.6 mg a.s./kg ds

Reference:	KCP 10.4.2.1/01
Report	ASA-01: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil; Wołany M.; 2020; Study Code: G/56/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil; Czarnynoga M.; 2021; Study Code: G/56/19
Guideline(s):	Yes, OECD 226
Deviations:	There are three deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test. 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution. 3. Due to the use of the temperature extraction method, there was no impossible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. Deviations from the Study Plan: Due to the Sponsor's request, the name of the test item was changed from ASA-01, SC to ASA-01.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	SC (suspension concentrate) 285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

Species:	the predatory mites, *Hypoaspis (Geolaelaps) aculeifer*
Source:	laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology, Poland
Age:	7 – 14 days after becoming adult
Sex:	female
Diet:	during the experiment, the mites were fed with the cheese mite, *Tyrophagus putrescentiae* obtained from a standard laboratory culture at the Łukasiewicz Research Network Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology, the mites were fed at the beginning of the test and then every 3 days, food was introduced into each test vessel using a small brush
Test units:	glass vessels with a capacity of 50 mL (diameter: 3.5 cm; height of soil: about 2 cm) with covers

4. Environmental conditions:

Temperature:	18.0 – 20.0°C
Soil:	artificial soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand
Stability of test compound:	the analytical measurements of the artificial soil treated with the test item at the highest concentration (1000.0 mg/kg dry weight of the artificial soil) was provided at the beginning, during (after 7 days) and at the end of the test, the control and the treated group (1000.0 mg/kg dry weight of the artificial soil) for chemical analysis were divided into 2 replicates, therefore there were 6 additional containers for analysed concentration and control group

WHC:	soil moisture content at the beginning of the test: 13.3 – 14.4% (41.3 – 44.7% of the maximum water holding capacity) soil moisture content in the middle of the test: 13.6 – 14.7% (42.2 – 45.6% of the maximum water holding capacity) soil moisture content at the end of the test: 13.4 – 14.6% (41.6 – 45.3% of the maximum water holding capacity)
pH:	pH at the beginning of the test: 5.52– 5.59 pH at the end of the test: 5.50 – 5.65
Photoperiod:	light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 647 – 672 lux light intensity at end of the test: 683 – 702 lux

STUDY DESIGN AND METHOD

The aims of the study were to assess the effects of the test item on the reproductive output of the predatory mite, Hypoaspis aculeifer and to determine the EC10, EC20, EC50, and NOEC.
Nine concentrations of the test item were used. These included: 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of aqueous suspension was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.

Test design:	number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Exposure time:	14 days
Tested concentrations, definitive test:	a control, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg test item/kg dry weight of the artificial soil
Dates:	start of the study: 13.12.2019 start of the experimental part: 20.01.2020 end of the experimental part: 06.02.2020 end of the study: 04.03.2020
Statistic:	EC_{10}, EC_{20}, EC_{50} – a probit analysis using linear max. likelihood regression LC_{10}, LC_{20}, LC_{50} – a probit analysis using linear max. likelihood regression NOEC: - offspring number – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure - survival – Fisher's Exact Binomial Test with Bonferroni Correction

Validity of the test:	The results are considered valid because the following criteria were satisfied in the control: -mean adult mortality: 2.5% (criterion: ≤ 20%), - the mean number of juveniles per vessel at the end of the test: 113.6 (criterion: ≥ 50 juveniles at the end of the test, - the coefficient of variation for the number of juveniles: 12.4 (criterion: ≤ 30%).

RESULTS

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 10.0 to 1000.0 mg/kg dry weight of the artificial soil was between 0% and 7.5%. Mortality of the control group was equal to 2.5%. Mortality of adult mites after 14 days of the experiment is presented below.

Table KCP 10.4.2.1-1: Mortality of soil mite *Hypoaspis (Geolaelaps) aculeifer* after 14 days of the experiment

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested mites	Number of alive mites after 14 days [no.]	Mortality	
				no.	%
0.0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	9 10 10 10 10 10 9 10	2	2.5
10.0	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
18.0	1 2 3 4	10 10 10 10	10 10 10 9	1	2.5
32.0	1 2 3 4	10 10 10 10	10 9 10 10	1	2.5
56.0	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
100.0	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
180.0	1 2 3 4	10 10 10 10	10 10 8 10	2	5.0
320.0	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
560.0	1 2 3 4	10 10 10 10	10 10 7 10	3	7.5
1000.0	1 2 3 4	10 10 10 10	9 10 9 10	2	5.0

After the application of the test item at the concentrations ranging from 10.0 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 93.0 – 120.0 per replicate. The mean number of juveniles in the control group was equal to 113.6 per replicate. The number of juveniles at the end of the test is presented below.

Table KCP 10.4.2.1-2: Number of juvenile mites (*Hypoasis aculeifer*) after 14 days of the experiment

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV* [%]
0.0 (control)	1 2 3 4 5 6 7 8	135 107 97 124 119 98 104 125	113.6 ± 14.0	-	12.4
10	1 2 3 4	116 89 122 128	113.8 ± 17.2	100.1	15.1
18	1 2 3 4	123 92 105 125	111.3 ± 15.7	97.9	14.1
32	1 2 3 4	129 104 123 116	118.0 ± 10.7	103.9	9.1
56	1 2 3 4	126 118 101 135	120.0 ± 14.5	105.6	12.0
100	1 2 3 4	111 105 128 106	112.5 ± 10.7	99.0	9.5
180	1 2 3 4	93 118 123 113	111.8 ± 13.2	98.3	11.8
320	1 2 3 4	104 116 131 128	119.8 ± 12.3	105.4	10.3
560	1 2 3 4	104 115 89 119	106.8 ± 13.4	93.9	12.6
1000	1 2 3 4	86 104 89 93	93.0^{+} ± 7.9	81.8	8.5

* - coefficient of variation

$^{+}$ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller)

CONCLUSION

The endpoints values are presented in tables below.

Table KCP 10.4.2.1-3: Endpoint values – the impact of the test item on survival of adult females *(Hypoaspis aculeifer)*

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of acetamiprid/ kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 275.3
LC_{20}	> 1000.0	> 275.3
LC_{50}	> 1000.0	> 275.3
NOEC	≥ 1000.0	≥ 275.3

Table KCP 10.4.2.1-4: Endpoint values - the impact of the test item on reproduction of the predatory mites *(Hypoaspis aculeifer)*

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of acetamiprid / kg dry weight of the artificial soil]
EC_{10}	**841.1** (267.9 – >1000.0)	**231.6** (73.8 – >275.3)
EC_{20}	> 1000.0	> 275.3
EC_{50}	> 1000.0	> 275.3
NOEC	560.0	154.2

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not relevant. No studies submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. The coefficients of variation (CV) in the control group were 9.4, 6.2, 0.8 and 3.5%, 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%. The insignificant deviation from OECD 216 guideline was noted: *According to 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, with no impact on quality and integrity of the study. On the basis of the results, it was concluded that ASA-01 at the concentration corresponding to the PEC: 0.14 mg of the test item / kg dry weight of soil (0.04 mg of acetamiprid / kg dry weight of soil), and 5 x PEC: 0.72 mg of the test item / kg dry weight of soil (0.2 mg of acetamiprid / kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference:	KCP 10.5/01
Report	ASA-01 Soil Microorganisms: Nitrogen Transformation Test; Wołany M.; 2020; Study Code: G/57/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Soil Microorganisms: Nitrogen Transformation Test; Czarnynoga M.; 2021; Study Code: G/57/19
Guideline(s):	Yes, OECD 216
Deviations:	According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm 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. Due to Sponsor's request, the name of the test item was changed from ASA-01, SC to ASA-01. These deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	285 – 315 g acetamiprid/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test organism

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

4. Environmental conditions:

Temperature:	19.6 – 21.2°C
Soil moisture:	40.5% – 46.0% of the maximum water holding capacity
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The aim of the study was to detect long-term adverse effects ASA-01 the processes of nitrogen transformation in aerobic surface soils. Agricultural soil was used. It was manually cleared of large objects and sieved to a particle size of 2 mm. The concentrations of the test item were:
- PEC: 0.14 mg of the test item / kg dry weight of soil (0.04 mg of a.s. / kg dry weight of soil)
- 5 x PEC: 0.72 mg of the test item / kg dry weight of soil (0.2 mg of a.s. / kg dry weight of soil)

The treated and the control soils were divided into three replicates. On days 0, 7, 14, 28, 42 and 56 of incubation, soil samples were collected to determine the quantities of nitrate. The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using deionised water. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used. The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

Test design:	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 500 g); the soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil.
Exposure time:	28 days

Tested concentrations, definitive test: PEC: 0.14 mg of the test item / kg dry weight of soil (0.04 mg of a.s. / kg dry weight of soil) and 5 x PEC: 0.72 mg of the test item / kg dry weight of soil (0.02 mg of a.s. / kg dry weight of soil)

Stability of the test compound: -

Dates: start of the study 13.12.2019
start of the experimental part: 21.01.2020
end of the experimental part: 19.02.2020
end of the study: 04.03.2020

Statistic: Shapiro-Wilk's test on Normal Distribution
Levene's Test on Variance Homogeneity (with Residuals)
Williams Multiple Sequential t-test Procedure

Validity of the test: The coefficients of variation (CV) in the control group were 9.4, 6.2, 0.8 and 3.5%, 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%.

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: 0.14 mg of the test item / kg dry weight of soil (0.04 mg of acetamiprid / kg dry weight of soil), and 5 x PEC: 0.72 mg of the test item / kg dry weight of soil (0.2 mg of acetamiprid / kg dry weight of soil) did not exceed 25% on 28 day of analysis.

Table KCP 10.5.-1: Nitrate formation rate [mg nitrate/kg dry weight of soil/day] for selected time intervals.

Time interval [d]	Control				PEC				5 x PEC			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 – 7	-6,450	-7,058	-13,093	**-8.867 ± 3.67**	-5,608	-4,350	0,942	**-3.005 ± 3.48**	7,580	17,438	8,080	**11.033⁺ ± 5.55**
0 – 14	-10,558	-10,201	-10,244	**-10.335 ± 0.20**	-13,512	-13,862	-13,276	**-13.550⁺ ± 0.29**	-9,150	-7,825	-8,204	**-8.393⁺ ± 0.68**
0 – 28	-5,132	-5,255	-5,884	**-5.424 ± 0.40**	-7,073	-6,147	-6,064	**-6.428⁺ ± 0.56**	-4,419	-4,546	-4,048	**-4.338⁺ ± 0.26**

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14 and 28 day

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure. significance level = 0.05. two sided)

Table KCP 10.5.-2: Deviations from the control based on nitrate formation rate for selected time intervals [%]

Time interval [d]	PEC	5 x PEC
0 – 7	66.1	224.4
0 – 14	-31.1	18.8
0 – 28	-18.5	20.0

"-" – nitrate formation rate in the treatment group was lower than in the control group

Values obtained using ToxRat 2.10. computer software.

CONCLUSION

On the basis of the results, it was concluded that ASA-01 at the concentration corresponding to the PEC: 0.14 mg of the test item / kg dry weight of soil (0.04 mg of acetamiprid / kg dry weight of soil), and 5 x PEC: 0.72 mg of the test item / kg dry weight of soil (0.2 mg of acetamiprid / kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen 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. The validity criteria were fulfilled: • a minimum of 70 % emergence in the control, • the seedlings exhibited no visible phytotoxic effects (e.g., chlorosis, necrosis, wilting and stem deformations) and exhibited only normal variation in growth and morphology in the control, • the mean survival of the control plants was at least 90 % at the end of the test in the control. A deviation from OECD 208 guideline was noted: 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 64.50 – 189.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.

	The deviation did not affect the results of the study. 1. The test item, i.e. ASA-01 applied at rates ranging from 25.4 and 405 mL of the test item/ha had no impact on the seedling emergence of all analysed species. 2. Plants of all analysed species emerged at all of analysed rates. The delayed seedling emergence and mortality of plants related to the test item was not observed. 3. On the basis of NOER, ER25 and ER50 values determined from the shoot length and dry shoot weight it was proved that the test item slightly inhibited the process of growth of pea. 4. Phytotoxic symptom as stunted growth was observed in cultivation of pea. The ER_{50} was determined to be >405 mL product/ha for seedling emergence. The results of observation are presented at the end of the study summary.

Reference:	KCP 10.6.2/01
Report	ASA-01 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Pieczka P.; 2020; Study Code: G/59/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; Pieczka P.; 2021; Study Code: G/59/19
Guideline(s):	Yes, OECD 208
Deviations:	According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 64.50 – 189.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	nominal concentration of acetamiprid: 300 g/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

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

3. Test plants:	sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea* var. *capitata*), pea (*Pisum sativum*), carrot (*Daucus carota*), Oats (*Avena sativa*), perennial ryegrass (*Lolium perenne*)
Soil:	sandy loam, collected from the place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, (49˚ 59', 780 N; 18˚55', 190 E) where no plant protection products or organic and inorganic fertilizers had been used; the soil was collected from a depth of 20 cm, it was manually cleared of large objects, e.g. plant residuals or stones, and sieved to 2 mm particle size
Test containers:	plastic pots (diameter: 15 cm; 177 cm^2)
4. Environmental conditions:	
Temperature:	17.7 – 26.5°C
Relative humidity:	48.4 – 86.5%
Photoperiod:	lighting: 16 h light : 8 h dark; light intensity: 64.5– 189.7 μE/m2/s
CO_2 concentration:	318 – 341ppm

STUDY DESIGN AND METHODS

The study, aimed at evaluating the effect of ASA-01 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. Five application rates were used for sunflower, cabbage, pea, carrot, perennial ryegrass and oats. There was also a concurrent control group. Selected number of plants per pot provide the adequate growth conditions and avoid overcrowding during the experiment.
The number of seeds per pot as well as the total number of seeds per rate for each of the tested species is presented below:
- sunflower: 3 seeds/pot – 21 seeds/rate (7 pots/rate);
- cabbage: 3 seeds/pot – 21 seeds/rate (7 pots/rate);
- pea: 3 seeds/pot – 21 seeds/rate (7 pots/rate);
- carrot: 5 seeds/pot – 20 seeds/rate (4 pots/rate);
- perennial ryegrass: 5 seeds/pot – 20 seeds/rate (4 pots/rate);
- oats: 5 seeds/pot – 20 seeds/rate (4 pots/rate).

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_{25}, ER_{50} and NOER.

Test design:	number of rates: 5 application rates + control for sunflower, cabbage, pea, carrot, perennial ryegrass and oats; number of replicates: 4 replicates/rate for carrot, perennial ryegrass, oats; 7 replicates/rate for cabbage, sunflower and pea. the total number of plants per application rate – 20 for carrot, perennial ryegrass, oats and 21 for cabbage,

	sunflower and pea test termination: 14 days after the emergence of 50% of the control seedlings
Exposure time:	14 days since emergence of 50% seeds in control
Tested concentrations, definitive test:	control, 25.4, 50.7, 101.3, 202.1, 405 ml/ha; volume of deionised water used to prepare the highest rate: 500 L/ha
Stability of test compound:	the concentration of acetamiprid in water suspensions was determined with a validated analytical method
Dates:	start of the study 31.12.2019 start of the experimental part: 03.02.2020 end of the experimental part: 29.02.2020 end of the study: 30.03.2020
Statistic:	ER_{25}, ER_{50} : probit analysis. NOER: emergence, shoot length and shoot dry weight – 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 of the test:	On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of ASA-01 on seedling emergence and seedling growth of terrestrial plants were met: - the seedling emergence in the control (validity criterion: at least 70%) was as follows: 100.0% - sunflower, 100.0% - cabbage, 100.0% - pea, 90.0% – carrot, 100.0% – perennial ryegrass, 100.0% – oats, - the mean survival of the emerged control seedlings (validity criterion: at least 90%) was as follows: 100.0% - sunflower, 100.0% - cabbage, 100.0 % - pea, 100.0% – carrot, 100.0% – perennial ryegrass, 100.0% – oats, - the control seedlings did not exhibit any visible phytotoxic effects; - environmental conditions for all plants of the same species were identical.

RESULTS

Sunflower (*Helianthus annuus*)

After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, seedling emergence of sunflower was not delayed by one day when compared with the control. At the rates ranging from 25.4 to 405 mL of the test item/ha the death of sunflower plants was not observed.
At the control group, 100% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha, total number of plants at the end of the experiment was equal to 100% in comparison to the control group.
After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the sunflower shoot length was between 90.1 and 105.4% of the control shoot length.
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the sunflower shoot weight was between 90.6 and 107.7% of the control shoot weight.
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the plant damage was not observed.

Table KCP 10.6.2-1: Sunflower (*Helianthus annuus*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	3	3	3	3	3	21	100.0	100.0
405	21	3	3	3	3	3	3	3	21	100.0	100.0

Table KCP 10.6.2-2: Sunflower (*Helianthus annuus*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	134.0	127.0	165.7	133.0	139.0	100.7	156.7	136.6	21.0	-
25.4	108.7	130.7	109.7	134.7	117.3	132.0	128.3	123.0	11.0	90.1
50.7	113.7	125.0	151.7	132.0	118.7	118.3	123.3	126.1	12.7	92.3
101.3	119.3	127.0	155.7	131.7	108.7	150.0	151.0	134.8	17.9	98.7
202.1	123.0	115.7	123.7	127.3	147.3	123.0	130.7	127.2	10.0	93.2
405	150.0	133.0	146.3	170.7	129.0	122.3	156.0	143.9	16.9	105.4

Table KCP 10.6.2-3: Sunflower (*Helianthus annuus*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	106.0	102.0	107.3	122.7	129.3	83.3	132.7	**111.9**	**17.4**	**-**
25.4	96.7	107.7	101.7	96.3	98.7	99.7	109.0	**101.4**	**5.1**	**90.6**
50.7	90.7	99.0	93.0	104.0	121.7	105.3	102.0	**102.2**	**10.2**	**91.4**
101.3	121.0	106.7	126.0	129.7	87.0	137.3	135.7	**120.5**	**18.0**	**107.7**
202.1	98.0	114.7	114.0	129.7	110.3	127.0	112.7	**115.2**	**10.6**	**102.9**
405	105.0	100.7	122.3	139.7	116.7	105.7	138.3	**118.3**	**15.9**	**105.7**

Table KCP 10.6.2-4: Sunflower (*Helianthus annuus*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		
25.4	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		
50.7	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		
101.3	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		
202.1	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		
405	1	0	0.0	nc	0	0.0	nc
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
	6	0			0		
	7	0			0		

nc - no changes

Cabbage *(Brassica oleracea ver. capitata)*

After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, seedling emergence of cabbage was not delayed when compared with the control. The death of cabbage plants was not observed.

At the control group, 100.0% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha total number of plants at the end of the experiment was equal to 100.0% in comparison to the control group.

After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the cabbage shoot length was between 93.2 and 96.5% of the control shoot length.

After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the cabbage shoot weight was between 90.8 and 113.2% of the control shoot weight.
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the plant damage was not observed.

Table KCP 10.6.2-5: Cabbage (*Brassica oleracea ver. capitata*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	3	3	3	3	3	21	100.0	100.0
405	21	3	3	3	3	3	3	3	21	100.0	100.0

Table KCP 10.6.2-6: Cabbage (*Brassica oleracea ver. capitata*) – shoot length

Application rate [g of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	87.3	78.0	85.3	78.7	83.7	78.3	77.3	81.2	4.1	-
25.4	71.7	84.7	77.3	79.0	84.3	71.7	78.0	78.1	5.3	96.1
50.7	74.3	85.3	83.3	79.0	72.0	79.0	72.0	77.9	5.3	95.8
101.3	76.3	77.3	82.0	78.0	79.3	70.7	85.0	78.4	4.5	96.5
202.1	70.3	63.3	79.7	83.7	79.0	77.0	79.3	76.0	6.9	93.6
405	76.7	71.3	77.7	74.7	67.3	86.7	75.7	75.7	6.0	93.2

Table KCP 10.6.2-7: Cabbage (*Brassica oleracea ver. capitata*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	38.0	41.7	50.3	35.3	33.3	34.7	42.3	**39.4**	**5.9**	**-**
25.4	36.0	42.3	45.3	34.3	41.7	44.0	43.0	**41.0**	**4.2**	**104.0**
50.7	34.0	34.3	42.0	31.7	33.3	45.3	29.7	**35.8**	**5.7**	**90.8**
101.3	40.0	36.0	50.0	44.7	43.3	40.7	44.0	**42.7**	**4.4**	**108.3**
202.1	35.0	28.0	45.3	54.0	45.3	34.0	54.7	**42.3**	**10.3**	**107.5**
405	45.3	46.3	43.0	47.3	35.0	50.3	44.7	**44.6**	**4.8**	**113.2**

Table KCP 10.6.2-8: Cabbage (*Brassica oleracea ver. capitata*) – plant damage

Application rate [g of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
25.4	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
50.7	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
101.3	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
202.1	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
405	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc

nc – no changes

Pea (*Pisum sativum*)

After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, seedling emergence of pea was not delayed when compared with the control. The death of plants was not observed (Table 16).
At the control group, 100.0% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha total number of plants at the end of the experiment was ranging from 81.0 to 100.0% in comparison to the control group (Table 17).

After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the pea shoot length was between 80.4 and 93.4% of the control shoot length (Table 18).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the pea shoot weight was between 81.2 and 98.1% of the control shoot weight (Table 19).
After the application of the test item at the rate equal to 405 mL of the test item/ha, the plant damage as stunted growth was observed (Table 20).

Table KCP 10.6.2-9: Pea (*Pisum sativum*) – plant number at the end of the experiment

Application rate [mL of the test/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	2	3	2	2	2	17	81.0+	81.0
405	21	2	3	2	3	3	2	3	18	85.7+	85.7

Table KCP 10.6.2-10: Pea (*Pisum sativum*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	295.3	273.7	215.3	192.3	248.3	177.3	292.0	242.0	47.9	-
25.4	287.7	184.7	244.7	235.3	148.7	240.7	185.0	218.1	47.2	90.1
50.7	220.0	214.3	215.7	207.3	265.0	207.3	227.3	222.4	20.0	91.9
101.3	249.3	197.0	162.0	231.0	227.3	257.7	258.3	226.1	35.5	93.4
202.1	239.7	250.0	211.5	179.7	194.0	194.5	241.0	215.8	27.8	89.1
405	167.5	206.0	202.0	167.7	197.0	223.5	199.3	194.7+	20.4	80.4

Table KCP 10.6.2-11: Pea (*Pisum sativum*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	208.3	157.3	198.7	132.3	167.7	162.3	213.7	**177.2**	**30.2**	**-**
25.4	195.3	129.7	170.0	196.3	131.7	164.7	150.0	**162.5**	**27.3**	**91.7**
50.7	177.3	154.7	172.7	151.7	164.3	152.3	155.3	**161.2**	**10.4**	**91.0**
101.3	173.0	178.7	154.0	174.0	162.3	157.7	217.7	**173.9**	**21.3**	**98.1**
202.1	179.3	192.0	185.0	111.7	140.0	161.0	184.0	**164.7**	**29.4**	**93.0**
405	159.0	146.7	178.5	101.3	96.7	186.0	139.3	**143.9+**	**34.8**	**81.2**

Table KCP 10.6.2-12: Pea (*Pisum sativum*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
25.4	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
50.7	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
101.3	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
202.1	1 2 3 4 5 6 7	0 0 0 0 0 0 0	0.0	nc	0 0 0 0 0 0 0	0.0	nc
405	1 2 3 4 5 6 7	10 10 10 10 10 10 10	10.0	sg	15 15 20 30 20 20 20	20.0	sg

nc – no changes, sg – stunted growth

Carrot (*Daucus carota*)

After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, seedling emergence of carrot was not delayed when compared with the control. The accidental death of plant was observed at the rate equal to 50.7 mL of the test item/ha (Table 21).

At the control group, 90.0% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha total number of plants at the end of the experiment was ranging from 83.3 to 111.1% in comparison to the control group (Table 22).

After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the carrot shoot length was between 92.7 and 98.9% of the control shoot length (Table 23).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the carrot shoot weight was between 88.6 and 97.3% of the control shoot weight (Table 24).
After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the plant damage was not observed (Table 25).

Table KCP 10.6.2-13: Carrot (*Daucus carota*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	4	5	4	5	18	90.0	-
25.4	20	5	5	5	4	19	95.0	105.6
50.7	20	2	4	5	4	15	75.0	83.3
101.3	20	5	5	5	3	18	90.0	100.0
202.1	20	5	5	5	5	20	100.0	111.1
405	20	4	5	4	3	16	80.0	88.9

Table KCP 10.6.2-14: Carrot (*Daucus carota*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	62.0	55.6	64.0	60.2	60.5	3.6	-
25.4	57.2	49.4	67.6	54.3	57.1	7.7	94.5
50.7	54.5	53.3	53.2	63.3	56.1	4.8	92.7
101.3	50.2	72.2	58.0	48.0	57.1	10.9	94.5
202.1	59.4	57.8	58.4	63.6	59.8	2.6	98.9
405	50.3	61.6	56.3	61.7	57.4	5.4	95.0

Table KCP 10.6.2-15: Carrot (*Daucus carota*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	6.0	5.0	5.8	5.8	**5.6**	**0.4**	-
25.4	4.4	5.2	5.6	6.8	**5.5**	**1.0**	**97.3**
50.7	5.5	5.0	5.2	5.5	**5.3**	**0.2**	**94.0**
101.3	5.6	5.0	4.8	5.3	**5.2**	**0.4**	**91.9**
202.1	4.8	5.2	4.8	5.2	**5.0**	**0.2**	**88.7**
405	5.3	5.4	5.0	4.3	**5.0**	**0.5**	**88.6**

Table KCP 10.6.2-16: Carrot (*Daucus carota*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
25.4	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
50.7	1 2 3 4	0 0 0 20	5.0	nc, d	0 0 0 20	5.0	nc, d
101.3	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
202.1	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
405	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc

nc – no changes, d – dead plant

Perennial ryegrass (*Lolium perenne*)

The seedling emergence of perennial ryegrass was not delayed when compared with the control group at the rates between 25.4 and 405 mL of the test item/ha. The death of plants was not observed (Table 26).

At the control group, 100.0% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha total number of plants at the end of the experiment ranged from 85.0 to 100.0% in comparison to the control group (Table 27).
After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the perennial ryegrass shoot length was between 93.2 and 109.2% of the control shoot length (Table 28).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the perennial ryegrass shoot weight was between 98.9 and 105.6% of the control shoot weight (Table 29).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the plant damage was not observed Table 30.

Table KCP 10.6.2-17: Perennial ryegrass (*Lolium perenne*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	-
25.4	20	5	5	5	5	20	100.0	100.0
50.7	20	5	5	5	5	20	100.0	100.0
101.3	20	5	5	4	4	18	90.0	90.0
202.1	20	4	4	5	5	18	90.0	90.0
405	20	4	4	5	4	17	85.0	85.0

Table KCP 10.6.2-18: Perennial ryegrass (*Lolium perenne*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	204.6	168.0	204.4	193.4	192.6	17.2	-
25.4	194.0	190.2	167.6	194.6	186.6	12.8	96.9
50.7	172.6	148.2	203.8	193.6	179.6	24.6	93.2
101.3	199.0	228.6	216.3	197.5	210.3	14.9	109.2
202.1	141.0	219.3	204.2	185.6	187.5	33.9	97.4
405	189.3	208.8	196.4	242.5	209.2	23.6	108.6

Table KCP 10.6.2-19: Perennial ryegrass (*Lolium perenne*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	8.4	6.2	9.0	8.6	8.1	1.3	-
25.4	9.0	8.8	7.4	8.0	8.3	0.7	103.1
50.7	7.8	6.2	10.0	9.6	8.4	1.8	104.3
101.3	7.8	10.4	8.0	6.3	8.1	1.7	100.8
202.1	7.5	8.8	8.0	7.6	8.0	0.6	98.9
405	7.5	8.0	8.0	10.5	8.5	1.4	105.6

Table KCP 10.6.2-20: Perennial ryegrass (*Lolium perenne*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
25.4	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
50.7	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
101.3	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
202.1	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
405	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc

nc – no changes

Oats (*Avena sativa*)

After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, seedling emergence of oats was not delayed when compared with the control. The death of plants was not observed (Table 31).

At the control group, 100.0% of plants emerged. At rates ranging from 25.4 to 405 mL of the test item/ha, total number of plants at the end of the experiment ranged from 90.0 to 100.0% in comparison to the control group (Table 32).
After the application of the test item at the rates between 25.4 and 405 mL of the test item/ha, the oats shoot length was between 89.0 and 98.0% of the control shoot length (Table 33).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the oats shoot weight was between 90.6 and 102.0 of the control shoot weight (Table 34).
After the application of the test item at the rates ranging from 25.4 to 405 mL of the test item/ha, the plant damage was not observed (Table 35).

Table KCP 10.6.2-21: Oats (*Avena sativa*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	-
25.4	20	5	5	5	5	20	100.0	100.0
50.7	20	5	5	3	5	18	90.0	90.0
101.3	20	5	3	5	5	18	90.0	90.0
202.1	20	5	4	5	5	19	95.0	95.0
405	20	5	5	5	5	20	100.0	100.0

Table KCP 10.6.2-22: Oats (*Avena sativa*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	339.0	327.8	353.6	351.0	342.9	11.9	-
25.4	340.6	349.8	310.6	342.6	335.9	17.3	98.0
50.7	306.0	329.4	279.7	305.8	305.2	20.3	89.0
101.3	324.4	300.0	307.0	336.2	316.9	16.5	92.4
202.1	326.6	338.8	303.4	326.0	323.7	14.7	94.4
405	335.8	293.8	282.8	325.0	309.4	25.1	90.2

Table KCP 10.6.2-23: Oats (*Avena sativa*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	66.2	63.0	77.2	79.6	71.5	8.1	-
25.4	74.0	68.8	70.4	75.2	72.1	3.0	100.8
50.7	70.4	57.6	78.3	55.8	65.5	10.7	91.7
101.3	74.8	86.0	75.4	55.6	73.0	12.7	102.0
202.1	66.2	83.0	61.0	70.0	70.1	9.4	98.0
405	66.8	53.4	68.4	70.4	64.8	7.7	90.6

Table KCP 10.6.2-24: Oats (*Avena sativa*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects					
		Day 7			Day 14		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
25.4	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
50.7	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
101.3	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
202.1	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc
405	1 2 3 4	0 0 0 0	0.0	nc	0 0 0 0	0.0	nc

nc – no changes

CONCLUSION

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

Table KCP 10.6.2-25: Seedling emergence and seedling growth test – final results (g of test item/ha)

Endpoint	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 405	> 405	> 405	> 405	> 405	> 405
NOER	> 405	> 405	101.3	≥ 405	≥ 405	≥ 405
Shoot length (plants without roots)						
ER_{50}	> 405	> 405	> 405	> 405	> 405	> 405
NOER	≥ 405	≥ 405	202.1	≥ 405	≥ 405	≥ 405
Plant dry weight (plants without roots)						
ER_{50}	> 405	> 405	> 405	> 405	> 405	> 405
NOER	≥ 405	≥ 405	202.1	≥ 405	≥ 405	≥ 405

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

Table KCP 10.6.2-26: Seedling emergence and seedling growth test – final results (g of as/ha)

Endpoint	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	> 121.5	> 121.5	30.4	≥ 121.5	≥ 121.5	≥ 121.5
Shoot length (plants without roots)						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	≥ 121.5	≥ 121.5	60.6	≥ 121.5	≥ 121.5	≥ 121.5
Plant dry weight (plants without roots)						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	≥ 121.5	≥ 121.5	60.6	≥ 121.5	≥ 121.5	≥ 121.5

The test item, i.e. ASA-01 applied at rates ranging from 25.4 and 405 mL of the test item/ha had no impact on the seedling emergence of all analyzed species.

Plants of all analyzed species emerged at all of analyzed rates. The delayed seedling emergence and mortality of plants related to the test item was not observed.

On the basis of NOER, ER25 and ER50 values determined from the shoot length and dry shoot weight it was proved that the test item slightly inhibited the process of of growth of pea.

Phytotoxic symptom as stunted growth was observed in cultivation of pea.

Comments of zRMS:	The study is considered acceptable. All validity criteria were fulfilled: • the seedling emergence of > 90% was observed (validity criterion: at least 70%), • plants in the control group exhibited no visible phytotoxic effects • the mean survival of the plant in the control group was of 100% (validity criterion: at least 90 %). A deviation from OECD 208 guideline was noted: 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 80.3 – 148.8 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The ER_{50} was determined to be >405 mL product/ha for vegetative vigour for all species for shoot height and shoot fresh weight. The results of observation are added at the end of the study summary

Reference:	KCP 10.6.2/02
Report	ASA-01 Terrestrial Plant Test: Vegetative Vigour Test; Czarnynoga M.; 2020; Study Code: G/58/19 AMENDMENT NO. 1 TO THE FINAL REPORT ASA-01 Terrestrial Plant Test: Vegetative Vigour Test; Holewik P.; 2021; Study Code: G/58/19
Guideline(s):	Yes, OECD 227
Deviations:	According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 80.3 – 148.8 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	ASA-01
Formulation:	nominal concentration of acetamiprid: 300 g/L
Description (physical state):	homogeneous, lightly viscous fluent liquid, white colour
Batch no.:	20190212-01
Production date:	12.02.2019
Expiration date:	12.02.2023

2. Vehicle and/or positive control:	vehicle control: water positive control: not relevant
3. Test plants:	sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea var. capitata*), pea (*Pisum sativum*), carrot (*Daucus carota*), perennial ryegrass (*Lolium perenne*), oats (*Avena sativa*)
Seed sowing:	sunflower - 3 plants/pot – 21 plants/concentration (7 pots/concentration); cabbage - 3 plants/pot – 21 plants/concentration (7 pots/concentration); pea - 3 plants/pot – 21 plants /concentration (7 pots/concentration); carrot - 5 plants/pot – 20 plants/concentration (4 pots/concentration) - perennial ryegrass - 5 plants/ pot - 20 plants/concentration (4 pots/concentration) - oats - 5 plants/pot – 20 plants /concentration (4 pots/concentration)
Soil:	sandy loam taken from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna (49˚ 59', 780 N; 18˚55', 190 E); the site chosen for soil collection had not been treated with any plant protection products or organic and inorganic fertilisers; the soil was collected from a depth of 20 cm. It was sieved to 2 mm particle size to homogenize it and remove coarse particles
Test containers:	plastic pots (pot's diameter – 15 cm, pot's surface area – about 177 cm^2)

4. Environmental conditions:

Temperature:	18.5 – 26.0°C
Relative humidity:	47.4 – 89.4%
Photoperiod:	16h light and 8h dark, light intensity: 80.3 – 148.8 µE/m2/s
CO_2 concentration:	327 – 346 ppm

STUDY DESIGN AND METHODS

The study, aimed at evaluating the effect of ASA-01 on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (10 seeds/pot for carrot, oats, perennial ryegrass and 6 seeds/pot for sunflower, cabbage, pea). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. As a result, the number of plants per pot as well as the total number of plants per concentration were:
- sunflower: 3 plants/pot – 21 plants/concentration (7 pots/concentration);
- cabbage: 3 plants/pot – 21 plants/concentration (7 pots/concentration);
- pea: 3 plants/pot – 21 plants/concentration (7 pots/concentration);
- carrot: 5 plants/pot – 20 plants/concentration (4 pots/concentration);
- perennial ryegrass - 5 plants/pot – 20 plants/concentration (4 pots/concentration)
- oats - 5 plants/pot – 20 plants/concentration (4 pots/concentration).

The pot is defined as a replicate. The test item was sprayed onto the plants. For each species, five application rates were used. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for carrot, oats, perennial ryegrass and 7 replicates for sunflower, cabbage and pea. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed. The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER25, ER50, and NOER.

Test design:	number of rates: 5 application rates + control; number of replicates: 4 replicates/rate for carrot, oats, perennial ryegrass and 7 replicates/rate for sunflower, cabbage, pea. The total number of plants per application rate – 20 for carrot, perennial ryegrass and oats, 21 for sunflower, pea, cabbage
Exposure time:	21 days after the spraying
Tested concentrations, definitive test:	25.4, 50.7, 101.3, 202.1 and 405.0 mL of the test item/ha – sunflower, cabbage, pea, carrot, perennial ryegrass and oats; volume of deionised water used to prepare the highest rate: 500 L/ha
Stability of test compound:	The concentration of acetamiprid in water was determined with a validated analytical method, all test aqueous suspension (application rates) of 100 mL volume each were subjected to chemical analysis
Dates:	start of the study 30.12.2019 start of the experimental part: 13.02. 2020 end of the experimental part: 07.03.2020 end of the study: 26.03.2020
Statistic:	ER_{25}, ER_{50} – probit analysis NOER (shoot length and shoot dry weight) - 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 of the test:

The following validity criteria were met:
- the seedling emergence (validity criterion: at least 70%) was as follows:
83.3 – 90.5% – sunflower,
85.7 – 90.5% – cabbage,
85.7 – 92.9% – pea,
75.0 – 90.0% – carrot,
85.0 – 95.0% – perennial ryegrass,
92.5 – 95.0% – oats,
- the mean survival of the emerged control seedlings was 100% in sunflower, cabbage, pea, carrot,
perennial ryegrass and oats,
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

RESULTS

Sunflower (*Helianthus annuus*)
After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the sunflower shoot length was between 95.0 – 114.6% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the sunflower shoot dry weight was between 98.0 – 120.7% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage were not observed.

Table KCP 10.6.2-27: Sunflower (*Helianthus annuus*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	3	3	3	3	3	21	100.0	100.0
405.0	21	3	3	3	3	3	3	3	21	100.0	100.0

Table KCP 10.6.2-28: Sunflower (*Helianthus annuus*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	267.7	291.7	298.3	288.3	328.7	306.3	249.3	**290.0**	**25.8**	-
25.4	292.7	260.7	295.7	310.7	337.3	251.0	281.0	**289.9**	**29.4**	**99.9**
50.7	280.0	264.7	298.3	286.0	274.0	275.7	249.7	**275.5**	**15.5**	**95.0**
101.3	311.7	313.0	353.3	311.7	278.7	329.7	281.7	**311.4**	**26.0**	**107.4**
202.1	258.0	274.0	279.3	291.7	297.0	320.7	324.0	**292.1**	**24.2**	**100.7**
405.0	332.3	303.3	273.3	322.3	367.3	381.3	347.0	**332.4**	**37.1**	**114.6**

Table KCP 10.6.2-29: Sunflower (*Helianthus annuus*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	201.0	323.7	411.7	381.3	403.0	375.3	374.7	**353.0**	**72.7**	-
25.4	343.3	273.3	359.0	434.3	354.0	279.7	376.7	**345.8**	**55.8**	**98.0**
50.7	374.7	341.0	350.0	359.3	301.7	398.7	352.3	**354.0**	**29.9**	**100.3**
101.3	373.3	437.7	346.0	430.0	391.3	429.3	424.3	**404.6**	**34.9**	**114.6**
202.1	328.3	380.7	462.7	487.0	449.0	431.0	442.3	**425.9**	**54.0**	**120.7**
405.0	406.0	433.7	368.3	409.3	405.3	436.7	425.7	**412.1**	**23.3**	**116.8**

Table KCP 10.6.2-30: Sunflower (*Helianthus annuus*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic effects								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		

nc – no changes

Cabbage (*Brassica oleracea var. capitata*)

After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the cabbage shoot length was between 95.3 – 106.2% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the cabbage shoot dry weight was between 93.1 – 115.2% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage were not observed.

Table KCP 10.6.2-31: Cabbage (*Brassica oleracea var. capitata*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	3	3	3	3	3	21	100.0	100.0
405.0	21	3	3	3	3	3	3	3	21	100.0	100.0

Table KCP 10.6.2-32: Cabbage (*Brassica oleracea var. capitata*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	144.7	125.3	141.0	145.3	139.3	140.7	173.7	144.3	14.6	-
25.4	137.0	136.7	147.7	125.3	133.0	149.3	134.0	137.6	8.4	95.3
50.7	150.7	144.0	145.0	175.0	157.7	154.3	146.3	153.3	10.8	106.2
101.3	155.0	144.3	140.7	143.0	156.7	157.0	151.0	149.7	6.9	103.7
202.1	129.0	150.0	127.0	152.0	141.7	135.3	136.3	138.8	9.7	96.2
405.0	139.3	145.3	136.3	140.0	147.3	151.3	139.3	142.7	5.4	98.9

Table KCP 10.6.2-33: Cabbage (*Brassica oleracea var. capitata*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	388.3	468.7	467.3	371.7	454.3	539.0	401.3	**441.5**	**58.3**	**-**
25.4	516.7	489.0	479.3	455.7	496.7	337.3	421.0	**456.5**	**60.9**	**103.4**
50.7	457.3	437.3	376.3	348.3	474.7	420.0	363.3	**411.0**	**48.9**	**93.1**
101.3	490.0	414.7	375.7	518.7	420.3	379.3	513.3	**444.6**	**61.6**	**100.7**
202.1	497.3	488.7	439.3	404.3	518.3	573.3	422.0	**477.6**	**59.5**	**108.2**
405.0	516.3	529.3	439.0	480.0	583.3	492.0	519.0	**508.4**	**44.9**	**115.2**

Table KCP 10.6.2-34: Cabbage (*Brassica oleracea var. capitata*) – plant damage

Application rate [mL of the test item/ha]	Replicate	Phytotoxic ef								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		

nc – no changes

Pea (*Pisum sativum*)

After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the pea shoot length was between 96.8 – 107.9% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the pea shoot dry weight was between 90.0 – 110.4% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage were not observed.

Table KCP 10.6.2-35: Pea (*Pisum sativum*) – plant number at the end of the experiment

Application rate [mL of the test item/ha]	Total number of seeds	Number of plants in particular replicates							Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4	5	6	7			
control	21	3	3	3	3	3	3	3	21	100.0	-
25.4	21	3	3	3	3	3	3	3	21	100.0	100.0
50.7	21	3	3	3	3	3	3	3	21	100.0	100.0
101.3	21	3	3	3	3	3	3	3	21	100.0	100.0
202.1	21	3	3	3	3	3	3	3	21	100.0	100.0
405.0	21	3	3	3	3	3	3	3	21	100.0	100.0

Table KCP 10.6.2-36: Pea (*Pisum sativum*) – shoot length

Application rate [mL of the test item/ha]	Mean shoot length in particular replicates [mm]							Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4	5	6	7			
control	340.3	245.3	201.0	284.0	274.7	266.0	296.7	272.6	43.2	-
25.4	242.3	252.0	275.0	251.3	270.0	267.7	289.3	264.0	16.3	96.8
50.7	272.7	261.0	260.0	284.0	316.7	293.3	254.3	277.4	22.2	101.8
101.3	280.0	327.3	233.7	320.0	267.3	267.3	330.0	289.4	37.0	106.2
202.1	267.3	278.0	306.0	295.0	272.0	294.0	265.7	282.6	15.7	103.7
405.0	292.0	295.3	305.7	315.7	272.7	310.7	267.7	294.2	18.4	107.9

Table KCP 10.6.2-37: Pea (*Pisum sativum*) – plant weight

Application rate [mL of the test item/ha]	Mean shoot weight in particular replicates [mg]							Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4	5	6	7			
control	350.0	260.3	359.3	346.7	306.7	320.7	354.0	**328.2**	**35.5**	**-**
25.4	267.0	225.0	429.0	311.3	265.3	290.0	279.3	**295.3**	**64.6**	**90.0**
50.7	267.7	300.0	388.3	275.7	347.7	320.0	287.0	**312.3**	**43.2**	**95.2**
101.3	356.0	347.7	208.7	301.3	274.7	301.0	358.3	**306.8**	**54.0**	**93.5**
202.1	326.0	374.7	391.3	384.3	355.0	397.3	308.3	**362.4**	**34.1**	**110.4**
405.0	316.0	380.3	290.7	377.3	352.7	377.0	325.3	**345.6**	**35.5**	**105.3**

Table KCP 10.6.2-38: Pea (*Pisum sativum*) – plant damage

Application rate [mL of the test item /ha]	Replicate	Phytotoxic effects								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
	5	0			0			0		
	6	0			0			0		
	7	0			0			0		

nc – no changes

Carrot (*Daucus carota*)

After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the carrot shoot length was between 92.9 – 100.9% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the carrot shoot dry weight was between 90.1 – 110.9% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage were not observed.

Table KCP 10.6.2-39: Carrot (*Daucus carota*) – plant number at the end of the experiment

Application rate [mL of the test item /ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	**20**	**100.0**	-
25.4	20	5	5	5	5	**20**	**100.0**	**100.0**
50.7	20	5	5	5	5	**20**	**100.0**	**100.0**
101.3	20	5	5	5	5	**20**	**100.0**	**100.0**
202.1	20	5	5	5	5	**20**	**100.0**	**100.0**
405.0	20	5	5	5	5	**20**	**100.0**	**100.0**

Table KCP 10.6.2-40: Carrot (*Daucus carota*) – shoot length

Application rate [mL of the test item /ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	205.4	219.6	186.6	195.0	201.7	14.2	-
25.4	216.4	206.6	204.4	186.2	203.4	12.6	100.9
50.7	197.6	211.0	210.6	184.4	200.9	12.6	99.6
101.3	184.6	193.8	204.4	184.2	191.8	9.5	95.1
202.1	197.6	189.4	182.4	179.8	187.3	8.0	92.9
405.0	213.0	193.2	194.0	199.6	200.0	9.2	99.2

Table KCP 10.6.2-41: Carrot (*Daucus carota*) – plant weight

Application rate [mL of the test item /ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	168.6	141.4	141.4	155.2	**151.7**	**13.0**	-
25.4	160.2	148.0	163.6	159.2	**157.8**	**6.8**	**104.0**
50.7	154.2	157.0	122.0	113.4	**136.7**	**22.2**	**90.1**
101.3	157.0	127.8	126.2	137.2	**137.1**	**14.2**	**90.4**
202.1	167.4	182.8	162.0	156.6	**167.2**	**11.3**	**110.3**
405.0	167.6	164.2	180.8	160.4	**168.3**	**8.9**	**110.9**

Table KCP 10.6.2-42: Carrot (*Daucus carota*) – plant damage

Application rate [mL of the test item /ha]	Replicate	Phytotoxic effects								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		

nc – no changes

Perennial ryegrass (*Lolium perenne*)
After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the perennial ryegrass shoot length was between 90.1 – 103.6% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the perennial ryegrass shoot dry weight was between 93.2 – 111.3% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage was not observed.

Table KCP 10.6.2-43: Perennial ryegrass (*Lolium perenne*) – plant number at the end of the experiment

Application rate [mL of the test item /ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	-
25.4	20	5	5	5	5	20	100.0	100.0
50.7	20	5	5	5	5	20	100.0	100.0
101.3	20	5	5	5	5	20	100.0	100.0
202.1	20	5	5	5	5	20	100.0	100.0
405.0	20	5	5	5	5	20	100.0	100.0

Table KCP 10.6.2-44: Perennial ryegrass (*Lolium perenne*) – shoot length

Application rate [mL of the test item /ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	461.6	471.0	474.4	494.4	**475.4**	**13.8**	-
25.4	419.6	452.6	425.4	442.2	**435.0+**	**15.2**	**91.5**
50.7	451.0	419.4	415.0	430.6	**429.0+**	**16.1**	**90.2**
101.3	421.6	427.4	450.4	413.6	**428.3+**	**15.8**	**90.1**
202.1	455.8	532.6	510.2	471.0	**492.4**	**35.3**	**103.6**
405.0	467.6	481.6	432.8	459.4	**460.4**	**20.5**	**96.8**

Table KCP 10.6.2-45: Perennial ryegrass (*Lolium perenne*) – plant weight

Application rate [mL of the test item /ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	128.8	164.6	150.6	137.8	**145.5**	**15.6**	-
25.4	149.6	134.0	128.8	131.2	**135.9**	**9.4**	**93.4**
50.7	150.4	135.6	125.0	133.0	**136.0**	**10.6**	**93.5**
101.3	150.2	162.4	163.6	106.4	**145.7**	**26.9**	**100.1**
202.1	148.4	177.0	158.0	164.2	**161.9**	**12.0**	**111.3**
405.0	143.4	129.4	136.8	132.4	**135.5**	**6.1**	**93.2**

Table KCP 10.6.2-46: Perennial ryegrass (*Lolium perenne*) – plant damage

Application rate [mL of the test item /ha]	Replicate	Phytotoxic effects								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		

nc – no changes

Oats (*Avena sativa*)

After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, plant mortality was not observed. After the application of the test item at the rates between 25.4 to 405.0 mL of the test item/ha, the oats shoot length was between 105.6 – 117.5% of the control shoot length. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the oats shoot dry weight was between 101.1 – 136.1% of the control shoot weight. After the application of the test item at the rates ranging from 25.4 to 405.0 mL of the test item/ha, the plant damage was not observed.

Table KCP 10.6.2-47: Oats (*Avena sativa*) – plant number at the end of the experiment

Application rate [mL of the test item /ha]	Total number of seeds	Number of plants in particular replicates				Total number of plants [no.]	Total number of plants [%]	Number of plants in comparison to the control [%]
		1	2	3	4			
control	20	5	5	5	5	20	100.0	-
25.4	20	5	5	5	5	20	100.0	100.0
50.7	20	5	5	5	5	20	100.0	100.0
101.3	20	5	5	5	5	20	100.0	100.0
202.1	20	5	5	5	5	20	100.0	100.0
405.0	20	5	5	5	5	20	100.0	100.0

Table KCP 10.6.2-48: Oats (*Avena sativa*) – shoot length

Application rate [mL of the test item /ha]	Mean shoot length in particular replicates [mm]				Mean shoot length [mm]	SD	Shoot length in comparison to the control [%]
	1	2	3	4			
control	379.6	381.8	387.4	382.6	382.9	3.3	-
25.4	423.6	434.8	358.6	400.0	404.3	33.7	105.6
50.7	417.4	407.8	407.2	400.4	408.2	7.0	106.6
101.3	430.0	462.2	406.4	483.0	445.4	33.9	116.3
202.1	423.2	451.0	481.0	444.0	449.8	23.9	117.5
405.0	439.0	445.6	427.6	467.4	444.9	16.7	116.2

Table KCP 10.6.2-49: Oats (*Avena sativa*) – plant weight

Application rate [mL of the test item /ha]	Mean shoot weight in particular replicates [mg]				Mean shoot weight [mg]	SD	Shoot weight in comparison to the control [%]
	1	2	3	4			
control	125.0	176.8	169.6	160.0	**157.9**	**23.0**	-
25.4	176.2	158.2	154.8	149.0	**159.6**	**11.7**	**101.1**
50.7	163.0	172.8	169.0	151.4	**164.1**	**9.3**	**103.9**
101.3	181.0	192.0	172.8	184.8	**182.7**	**8.0**	**115.7**
202.1	177.0	211.2	200.4	209.2	**199.5**	**15.7**	**126.4**
405.0	215.6	222.6	195.4	225.6	**214.8**	**13.6**	**136.1**

Table KCP 10.6.2-50: Oats (*Avena sativa*) – plant damage

Application rate [mL of the test item /ha]	Replicate	Phytotoxic effects								
		Day 7			Day 14			Day 21		
		Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms	Mean effects/ replicate [%]	Mean effects/ application rate [%]	Symptoms
0 (control)	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
25.4	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
50.7	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
101.3	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
202.1	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		
405.0	1	0	0.0	nc	0	0.0	nc	0	0.0	nc
	2	0			0			0		
	3	0			0			0		
	4	0			0			0		

nc – no changes

CONCLUSION

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

Table KCP 10.6.2-51: Vegetative Vigour Test – final results (g of test item/ha)

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0
NOER	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0
Shoot length (plants without roots)						
ER_{50}	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0
NOER	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0
Plant dry weight (plants without roots)						
ER_{50}	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0	> 405.0
NOER	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0	≥ 405.0

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

Table KCP 10.6.2-52: Vegetative Vigour Test – final results (g of active substance/ha)

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
Shoot length (plants without roots)						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5
Plant dry weight (plants without roots)						
ER_{50}	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5	> 121.5
NOER	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5	≥ 121.5

The test item ASA-01 applied at rates ranging from 25.4 to 405.0 mL of the test item/ha had no impact on vegetative vigour of all analyzed species. The test item did not cause mortality of all analyzed species. On the basis of NOER, ER_{25} and ER_{50} values determined from the shoot length shoot dry weight it was proved that the test item did not inhibit the process of growth of all analyzed species. No phytotoxic symptoms were observed in all analyzed species.

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

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.8 KCP 10.8 Monitoring data

Not relevant. No studies submitted.