REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **AMINO 30 SL**

Product name(s): El Camino 30 SL, Ranchero 30 SL

Chemical active substance:

Aminopyralid, 30 g/L

Central Zone

Zonal Rapporteur Member State: PL

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 01/2025

zRMS Assessment: 18/04/2025

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Version history

When	What
April 2025	zRMS Assessment
July 2025	Following commenting period Verification of reference list

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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 organ-	Bees	Non-target ar-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter oilseed rape (BRSNW)	F	Dicotyledonous weeds	Spray, medium spray	Autumn BBCH 10-18	a) 1 b) 1	n/a	a) 0.2-0.267 L/ha b) 0.2-0.267 L/ha	a) 6-8.01 g a.s/ha b) 6-8.01 g a.s/ha	200-300	-	-							
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
Minor uses according to Article 51 (field uses)																				
Minor uses according to Article 51 (interzonal uses)																				

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

* 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), All TER values exceed the relevant trigger values indicating that AMINO 30 SL does not pose an unacceptable acute and long-term risk to mammals following applications according to recommended use pattern.

AMINO 30 SL presents no unacceptable risk to mammals resulting from exposure via drinking water. Since the log Pow value of aminopyralid is below the trigger of 3, the risk assessment for secondary poisoning is not triggered.

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

AMINO 30 SL poses no unacceptable risk to birds and mammals used according to the label.

9.1.1.3 Effects on aquatic organisms (KCP 10.2)

AMINO 30 SL poses no unacceptable risk to aquatic organisms used according to the label. The product AMINO 30 SL is classified as **Aquatic Chronic 2, H411.**
Based on the predicted rates of AMINO 30 SL, the ~~TER values~~ PEC/RAC ratios describing the risk for aquatic species following exposure to AMINO 30 SL according to the GAP of the formulation achieve the acceptability criteria with no need for risk mitigation measures.

9.1.1.4 Effects on bees (KCP 10.3.1)

AMINO 30 SL poses no unacceptable risk to bees used according to the label.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

AMINO 30 SL poses no unacceptable risk for in-field and off-field habitats to NTA used according to the label with no need for risk mitigation measures.

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

AMINO 30 SL poses no unacceptable risk to non-target soil meso- and macrofauna and microbial activity used according to the label.

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

AMINO 30 SL poses no unacceptable risk to non-target terrestrial plants used according to the label with appropriate buffer zone and drift reducing techniques.
For winter oilseed rape:

~~- 1 m and use of 50% drift reducing nozzles or,~~
~~- 5 m with no drift reducing technology to non-agricultural land.~~
- 1 m and 90% drift reducing nozzles or,
- 5 m and 50% drift reducing nozzles or,
- 10 m with no drift reducing technology to non-agricultural land is applied.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of AMINO 30 SL grouped according to crop, application rate, number of applications, timing, etc.

Grouping according to crop, application rate, number of applications, timing criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Birds	Winter oilseed rape BBCH 10-18 8.01 g [a.s]/ha	Crop, application rate, number of applications, timing criterion	Crop, application rate, number of applications, timing criterion
Terrestrial vertebrates other than birds			
Aquatic organisms			
Bees			
Arthropods other than bees			
Non-target soil meso- and macrofauna			
Soil microbial activity			
Non-target terrestrial plants			

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of AMINO 30 SL is indicated in the table.

Table 9.1-3 Metabolites of aminopyralid

No major metabolites were found in environmental compartments.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid.

However, the provision of further data on the AMINO 30 SL is not considered essential, because studies from Annex I inclusion can be used in the risk assessment.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail *Colinus virginianus*	aminopyralid	Acute	LD_{50} >2250 mg/kg bw per day	EFSA Journal 2013;11(9):3352, [redacted] 2001, *XDE-750: An Acute Oral Toxicity Study with the Nothern Bobwhite*, [redacted]
Bobwhite quail *Colinus virginianus*	aminopyralid	Short-term	Dietary LD_{50} >1457 mg/kg bw per day LC_{50}>5500 mg/kg feed	EFSA Journal 2013;11(9):3352, [redacted], J.B., Martin, K.H., 2001a, *XDE-750: A Dietary LC50 Study with the Nothern Bobwhite* [redacted];
Bobwhite quail *Colinus virginianus*	aminopyralid	Long-term	NOEL = 190.23 mg/kg bw per day NOEC = 2700 mg/kg feed	EFSA Journal 2013;11(9):3352, [redacted]., 2003a, *Avian Reproduction Study with XDE-750 in Nothern Bobwhite Quail (Colinus virginianus)* [redacted]

Species	Substance	Exposure System	Results	Reference
				[redacted]

9.2.1.1 Justification for new endpoints

Not relevant.

9.2.2 Risk assessment for spray applications

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

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

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of AMINO 30 SL in winter oilseed rape-aminopyralid

Intended use	Winter oilseed rape				
Active substance/product	Aminopyralid				
Application rate (g/ha)	1 × 8.01 g/ha				
Acute toxicity (mg/kg bw)	2250				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	1.0	1.27	1768.9
Reprod. toxicity (mg/kg bw/d)	190.2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like	Small omnivorous bird	64.8	0.53	0.28	691.5

crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower					

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.

9.2.2.2 Higher-tier risk assessment

The calculated TER value for the active substance is above the trigger value of 10 for acute risk assessment and above the trigger value of 5 for the chronic risk assessment, indicating that AMINO 30 SL does not pose an unacceptable acute and long-term risk to birds.
No further risk refinement is needed.

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 AMINO 30 SL 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 5.1427, aminopyralid belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	8.01		
Acute toxicity (mg/kg bw)	=	2250	quotient =	0.0036
Reprod. toxicity (mg/kg bw/d)	=	190.2	quotient =	0.042

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of aminopyralid amounts to -1.76 at 19 °C (pH 5) (EFSA Journal 2013;11(9):3352) 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 TER values exceed the relevant trigger values indicating that AMINO 30 SL does not pose an unacceptable acute and long-term risk to birds following applications according to recommended use pattern. AMINO 30 SL does not pose an unacceptable risk to birds resulting from exposure via drinking water. Since the log P_{ow} value of aminopyralid is below the trigger of 3, the risk assessment for secondary poisoning is not triggered.

zRMS comments:

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

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substance following use of AMINO 30 SL in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of AMINO 30 SL according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating birds are expected following application of AMINO 30 SL (El Camino 30 SL, Ranchero 30 SL) according to the proposed use pattern..

No risk mitigation measures are required.

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

9.3.1 Toxicity data

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

Effects on mammals of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. 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	aminopyralid	Acute oral	LD_{50} > 5000 mg/kg bw per day	EFSA Journal 2013;11(9):3352, [redacted] 2001a, *XDE-750: Acute Oral Toxicity Study in Fischer 344 Rats* [redacted]
Screening endpoint Rabbit	aminopyralid	Long-term	NOAEL = 26 mg/kg bw per day	EFSA Journal 2013;11(9):3352, [redacted]., 2004b, *Suplemental report for GF-871: Oral Gavage Developmental Toxicity Study in New Zealand white rabbits* [redacted]
Tier 1 endpoint Rabbit	aminopyralid	Long-term	NOAEL 256 mg/kg bw per day (acid equivalent)	EFSA Journal 2013;11(9):3352, [redacted]., 2004a, *GF-871: Oral Gavage Developmental Toxicity Study in New Zealand white rabbits* [redacted]

9.3.1.1 Justification for new endpoints

Not required.

9.3.2 Risk assessment for spray applications

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

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

The results of the acute and reproductive screening risk assessments are summarised in the following

tables.

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape				
Active substance/product	Aminopyralid				
Application rate (g/ha)	1 × 8.01 g a.s/ha				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.0	0.95	5272.1
Reprod. toxicity (mg/kg bw/d)	26				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	48.3	0.53	0.21	126.80

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

9.3.2.2 Higher-tier risk assessment

The calculated TER value for active substance is above the trigger value of 10 for acute risk assessment and above the trigger value of 5 for chronic risk assessment, indicating that AMINO 30 SL does not possess an unacceptable acute and long-term risk to mammals.
No further risk refinement is needed.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc of 5.1427, aminopyralid belongs to the group of less sorptive substances.

Effective application rate (g/ha)	= 8.01		
Acute toxicity (mg/kg bw)	= 5000	quotient	= 0.0016
Reprod. toxicity (mg/kg bw/d)	= 26	quotient	= 0.31

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of aminopyralid amounts to -1.76 at 19 °C (pH 5) (EFSA Journal 2013;11(9):3352) 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 TER values exceed the relevant trigger values indicating that AMINO 30 SL does not pose an unacceptable acute and long-term risk to mammals following applications according to recommended use pattern.

AMINO 30 SL presents no unacceptable risk to mammals resulting from exposure via drinking water. Since the log P_{ow} value of aminopyralid is below the trigger of 3, the risk assessment for secondary poisoning is not triggered.

zRMS comments:

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

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the highest predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for mammals resulting from acute and long-term exposure to active substance following use of AMINO 30 SL (El Camino 30 SL, Ranchero 30

SL) in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of AMINO 30 SL according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating mammals are expected following application of AMINO 30 SL according to the proposed use pattern..

No risk mitigation measures are required.

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

Not required.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Aminopyralid	96 hr (flow-through)	LC_{50} >100 (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, [redacted]., 2001, *XDE-750 Herbicide: An Acute Toxicity Study with the Rainbow Trout Oncothynchus mykiss Walbaum*, [redacted]
Lepomis macrochirus	Aminopyralid	96 hr (flow-through)	LC_{50} >100 (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, [redacted] 2002a, *Revised report*

Species	Substance	Exposure System	Results	Reference
				for XDE-750- Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Static Conditions [redacted]
Cyprinodon variegatus	Aminopyralid	96 hr (flow-through)	LC_{50} >100 (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, [redacted] 2002b, *XDE-750 – Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Static Acute Conditions* [redacted]
Cyprinodon variegatus	Aminopyralid	ELS study 28 d (flow through)	NOEC = 0.10 (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, [redacted] 2002b, *XDE-750 – Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Static Acute Conditions* [redacted]
Aquatic invertebrates				
Daphnia magna	Aminopyralid	48 h (static)	EC_{50} > 100 (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, Marino, T.S., Hales-McClymont, C.A., Yaroch, A.M., 2001, *XDE-750 Herbicide: an Acute Toxicity Study with the Daphnid, Daphnia magna Straus* Report No.: 011079 DR-0293-9028-042
Crassostrea virginica	Aminopyralid	48 h (static)	EC_{50} > 89 (mm) mg a.s./L	EFSA Journal 2013;11(9):3352, Cafarella, M.A., 2002, *XDE-750 – Acute Toxicity to Eastern Oysters (Crassostrea virginica) under Flow-Through*

Species	Substance	Exposure System	Results	Reference
				Conditions, Report No.: 011268
Daphnia magna	Aminopyralid	21 d (semi-static)	NOEC = 100 (highest concentration tested) (nom) mg a.s./L	EFSA Journal 2013;11(9):3352, Henry, K.S., Marino, T.A., Staley, J.L., McClymont, E.L., 2003, *XDE-750: 21-Day Chronic Toxicity with the Daphnid, Daphnia magna Straus* Report No.: 021085 DR-0293-9028-074
Sediment-dwelling organisms				
Chironomus riparius	Aminopyralid	28 d (static water spiked)	NOEC = 130 mg/L in aqueous phase (46.7 mg/kg TWA in sediment) (nom)	EFSA Journal 2013;11(9):3352, Putt, A. E., 2002, *XDE-750 – the Full Life-Cycle Toxicity to Midge (Chironomus riparius) Under Static Conditions* Springborn Smithers Inc, Wareham, USA Report No.: U09304 GLP/GEP (Y/N): Y Published (Y/N): N
Algae				
Navicula pelliculosa (diatom)	Aminopyralid	72 h (static)	EC_{50}: 21 mg/L (mm) E_rC_{50}: 21 mg/L (mm) E_bC_{50} : 18 mg/L (mm)	EFSA Journal 2013;11(9):3352, Hoberg, J.R., 2002b, *XDE-750 – Acute Toxicity to the Freshwater Diatom, Naviculla pelliculosa* Report No.: 12550.6199 011278 DR-0293-9028-065
Higher plant				
Lemna gibba (duckweed)	Aminopyralid	7 and 14 d	EC_{50} > 88 (mm) (frond count, growth rate and frond dry weight)	EFSA Journal 2013;11(9):3352, Hoberg, J.R., 2002e, *XDE-750 – Toxicity to Duckweed, Lemna gibba* Report No.: 011223R 12550.6160 DR-0293-9028-058R
Myriophyllum spicatum (rooted in sediment)	Aminopyralid	14 d	EC_{50} = 0.418 (mm) total shoot length growth rate	EFSA Journal 2013;11(9):3352, Wenzel, A., 2012,

Species	Substance	Exposure System	Results	Reference
			EC_{50} = 0.3231 (mm) total shoot length yield EC_{50} = 0.363 (mm) fresh weight growth yield EC_{50} = 0.188 (mm) fresh weight yield EC_{50} > 0.987 (mm) dry weight growth rate EC_{50} > 0.199 (mm) dry weight yield	*Effect of aminopyralid on the growth of Myriophyllum spicatum in the presence of sediment with exposure via the water phase,* Study ID: 120759

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 – AMINO 30 SL

Species	Substance	Exposure System	Results	Reference
Daphnia magna	AMINO 30 SL	48 h	The EC_{50}/48 h > 100 mg formulation/L (2.9 mg a.s/L) (nom)	Maga, D., 2024, *AMINO 30 SL Daphnia magna, Acute Immobilisation Test* Study code: W-27-24
Anabaena flos-aquae	AMINO 30 SL	72 h	Formulation (nom): E_rC_{50}/72 h = 162.57 mg/L, E_yC_{50}/72 h = 69.80 mg/L, LOEC/72 h for growth rate = 66.67 mg/L, NOEC/72 h for growth rate = 22.22 mg/L LOEC//72 h for yield = 22.22 mg/L NOEC/72h for yield = 7.41 mg/L Aminopyralid (nom): E_rC_{50}/72h = 4.714 mg/L, E_yC_{50}/72h = 2.024 mg/L, LOEC/72h for growth rate = 1.933 mg/L, NOEC/72h for growth rate = 0.644 mg/L,	Maga, D., 2024, *AMINO 30 SL Anabaena flos-aquae UTEX B 1444, Growth inhibition test* Study code: W-30-24

Species	Substance	Exposure System	Results	Reference
			LOEC//72h for yield = 0.644 mg/L, NOEC/72h for yield= 0.215 mg/L	
Raphidocelis subcapitata	AMINO 30 SL	72 h	Formulation (nom): E_rC_{50}/72h > 100 mg/L, E_yC_{50}/72h > 100 mg/L, LOEC/72h for growth rate > 100 mg/L, NOEC/72h for growth rate ≥ 100 mg/L, LOEC/72h for yield > 100 mg/L NOEC/72h for yield ≥ 100 mg/L Aminopyralid (nom): E_rC_{50}/72h > 2.9 mg/L, E_yC_{50}/72h > 2.9 mg/L, LOEC/72h for growth rate > 2.9 mg/L, NOEC/72h for growth rate ≥ 2.9 mg/L, LOEC/72h for yield > 2.9 mg/L NOEC/72h for yield ≥ 2.9 mg/L	Maga, D., 2024, *AMINO 30 SL Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test* Study code: W-28-24
Lemna gibba (duckweed)	AMINO 30 SL	7 d	Formulation based on the frond number and on the dry weight: E_rC_{50}/7 d > 100 mg/L, E_yC_{50}/7 d > 100 mg/L, LOEC/72 h for growth rate > 100 mg/L, NOEC/72 h for growth rate ≥ 100 mg/L LOEC/72 h for yield > 100 mg/L, NOEC/72 h for yield ≥ 100 mg/L Aminopyralid (nom) based on the frond number and on the dry weight:	Maga, D., 2024, *AMINO 30 SL Lemna gibba CPCC 310, Growth inhibition test* Study code: W-29-24

Species	Substance	Exposure System	Results	Reference
			E_rC_{50}/7 d > 2.9 mg/L, E_yC_{50}/7 d > 2.9 mg/L, LOEC/72 h for growth rate > 2.9 mg/L, NOEC/72 h for growth rate ≥ 2.9 mg/L LOEC/72 h for yield > 2.9 mg/L, NOEC/72 h for yield ≥ 2.9 mg/L	
Myriophyllum spicatum	AMINO 30 SL	14 d	Formulation (nom) based on the total shoot length: E_rC_{50}/14d = 63.74 mg/L (95% confidence limit: 47.61 - 93.14), NOEC/14d = 0.95 mg/L, LOEC/14d = 3.05 mg/L, E_yC_{50}/14 d = 67.72 mg/L (95% confidence limit: 54.18 – 89.49), NOEC/14d = 9.77 mg/L, LOEC/14d = 31.25 mg/L. Formulation (nom) based on the fresh weight: E_rC_{50}/14d = 56.48 mg/L (95% confidence limit: 30.97 – 165.81), NOEC/14d = 3.05 mg/L, LOEC/14d = 9.77 mg/L, E_yC_{50}/14d = 34.68 mg/L (95% confident limit: 18.92 – 81.14), NOEC/14d = 3.05 mg/L, LOEC/14d = 9.77 mg/L. Formulation (nom) based on the dry weight: E_rC_{50}/14d = 20.15 mg/L (95%	Czarnecka M., 2024 *Water-Sediment Myriophyllum Spicatum Toxicity Tes*t, Study code: W-26-24

Species	Substance	Exposure System	Results	Reference
			confidence limit: 14.34 – 28.39), NOEC/14d = 9.77 mg/L, LOEC/14d = 31.25 mg/L, E_yC_{50}/14d = 15.19 mg/L (95% confidence limit: 10.08 – 22.93), NOEC/14d = 9.77 mg/L, LOEC/14d = 31.25 mg/L. Aminopyralid (nom) based on the total shoot length: E_rC_{50}/14d = 1.85 mg/L (95% confidence limit: 1.38 – 2.70), NOEC/14d = 0.0276 mg/L, LOEC/14d = 0.0885 mg/L, E_yC_{50}/14d = 1.96 mg/L (95% confidence limit: 1.57 – 2.60), NOEC/14d = 0.283 mg/L, LOEC/14d = 0.906 mg/L. Aminopyralid (nom) based on the fresh weight: E_rC_{50}/14d = 1.64 mg/L (95% confidence limit: 1.38 – 2.70), NOEC/14d = ~~0.0276~~ 0.0885 mg/L, LOEC/14d = ~~0.0885~~ 0.283 mg/L, E_yC_{50}/14d = ~~1.96~~ 1.01 mg/L (95% confidence limit: 1.57 – 2.60), NOEC/14d = ~~0.283~~ 0.0855 mg/L, LOEC/14d = ~~0.906~~ 0.283 mg/L. Aminopyralid (nom) based on the dry	

Species	Substance	Exposure System	Results	Reference
			weight: E_rC_{50}/14d = 0.58 mg/L (95% confidence limit: 0.42 – 0.82), NOEC/14d = 0.283 mg/L, LOEC/14d = 0.906 mg/L, E_yC_{50}/14d = 0.44 mg/L (95% confidence limit: 0.29 – 0.66), NOEC/14d = 0.283 mg/L, LOEC/14d = 0.906 mg/L.	
Higher-tier studies (micro- or mesocosm studies)				
Not required.				

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

9.5.1.1 Justification for new endpoints

Not required.

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 aminopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of AMINO 30 SL in winter oilseed rape

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**		**Inverteb. prolonged**	**Algae**			**Sed. dwell. prolonged**	**Aquatic plants**	
Test species		***Oncorhynchus mykiss***	***Cyprinodon variegatus***	***Crassostrea virginica***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa* (diatom)**	***Raphidocelis subcapitata***	***Anabaena flos-aquae***	***Chironomus riparius***	***Myriophyllum spicatum* (rooted in sediment)**	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} 100000 (Aminopyralid based on EU level)	NOEC 100 (Aminopyralid based on EU level)	EC_{50} 89000 (Aminopyralid based on EU level)	EC_{50} 2900 (Aminopyralid based on AMINO 30 SL)	NOEC 100000 (Aminopyralid based on EU level)	E_bC_{50} / E_rC_{50} 18000/21000 (Aminopyralid based on EU level)	E_rC_{50} 2900 (Aminopyralid based on AMINO 30 SL)	E_yC_{50} / E_rC_{50} 2024/4714 (Aminopyralid based on AMINO 30 SL)	NOEC ~~46700~~ 130000 (Aminopyralid based on ~~AMINO 30 SL~~ EU agreed endpoint)	EC_{50}/ E_rC_{50} 188/363 (Aminopyralid based on EU level)	E_rC_{50} 580 (Aminopyralid based on AMINO 30 SL)
AF		100	10	100	100	10	10~~0~~	10~~0~~	10~~0~~	10	10	10
RAC (µg/L)		1000	10	890	29	10000	~~180~~ 1800 / 2100	~~29~~ 290	~~20.24~~ 202.4 / 471.4	~~4670~~ 13000	18.8 / 36.3	58
FOCUS Scenario	**PEC_{gl-max} (µg/L)**											
Step 1												
	2.73	0.00273	0.273	0.0031	0.094	0.000273	~~0.015~~ 0.002 / 0.001	~~0.094~~ 0.009	~~0.13~~ 0.013 / 0.006	~~0.00058~~ 0.0002	0.15 / 0.08	0.047

Group		Fish acute	Fish pro-longed	Inverteb. acute		Inverteb. prolonged	Algae			Sed. dwell. prolonged	Aquatic plants	
~~Step 2~~												
~~N-Europe June-Sep.~~	~~0.33~~	~~0.00033~~	~~0.033~~	~~0.00037~~	~~0.011~~	~~0.000033~~	~~0.0018~~	~~0.011~~	~~0.016~~	~~0.000071~~	~~0.018~~	~~0.0057~~
~~N-Europe Oct.-Feb.~~	~~0.73~~	~~0.00073~~	~~0.073~~	~~0.00082~~	~~0.025~~	~~0.000073~~	~~0.0041~~	~~0.025~~	~~0.036~~	~~0.00016~~	~~0.039~~	~~0.013~~

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

For the intended uses, calculated PEC/RAC ratios did indicate an unacceptable risk for the most sensitive group of aquatic organisms (risk for fish as characterised by a NOEC for *Cyprinodon variegatus* of 100 µg/L in connection with an assessment factor of 10) in FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

zRMS comments:

Since acceptable risk could be concluded with Step 1 PEC_{SW} values, calculations based on Step 2 were struck through in Table 9.5 3above.

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AMINO 30 SL for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape	
Formulation	AMINO 30 SL	
Application rate (g[prod]/ha)	1 x 272.87	
Entry into surface water via spray drift (Drift Calculator from SWASH)		
Buffer zone (m)	**PECsw [µg prod/L]**	
~~1~~ **no buffer zone no drift reduction***	Ditch*	~~1.7531~~ 1.7526
	Pond*	0.0598
	Stream*	1.3006
Entry into surface water via spray drift (Drift Calculator from SWASH)		
Buffer zone (m)	PEC/RAC ratio *Daphnia magna* =EC_{50} > 100 mg/L RAC=1 000 µg/L (AF=100)	
~~1~~ **no buffer zone no drift reduction***	Ditch*	~~0.0017~~ 0.0018
	Pond*	0.0001
	Stream*	0.0013
Buffer zone (m)	PEC/RAC ratio *Anabaena flow-aquae* E_yC_{50}=69.80 mg/L/ E_rC_{50} = 162.587 mg/L ~~RAC=698 µg/L (AF=100)~~ RAC=6980 µg/L / 16558 µg/L (AF=10)	
~~1~~ **no buffer zone no drift reduction***	Ditch*	~~0.0025~~ 0.00025 / 0.0001
	Pond*	0.00001 / 0.000004
	Stream*	0.0001 / 0.0001

Buffer zone (m)	PEC/RAC ratio *Raphidocelis subcapitata* E_rC_{50}/72h > 100 mg/L, ~~RAC=1000 µg/L (AF=100)~~ RAC=10 000 µg/L (AF=10)	
~~1~~ no buffer zone no drift reduction*	Ditch*	~~0.0018~~ 0.00018
	Pond*	0.00001
	Stream*	0.00013
Buffer zone (m)	PEC/RAC ratio *Lemna gibba* (duckweed) E_rC_{50}/7 d > 100 mg/L, RAC=10000 µg/L (AF=10)	
~~1~~ no buffer zone no drift reduction*	Ditch*	0.00018
	Pond*	0.00001
	Stream*	0.00013
Buffer zone (m)	PEC/RAC ratio *Myriophyllum spicatum* E_rC_{50}/14 d = 20.15 mg/L (95% confidence limit: 14.34 – 28.39), RAC=2015 µg/L (AF=10)	
~~1~~ no buffer zone no drift reduction*	Ditch*	0.00087
	Pond*	0.00003
	Stream*	0.00065

*According to Part B8

The drift exposure was performed in Fate and Behaviour section (Section 8) using the Drift Calculator in SWASH model. All the ratios PEC/RAC were below 1 for all aquatic organisms.
As a result, acceptable risk for aquatic organisms has been demonstrated with no need for mitigation measures for the formulation when used according to critical GAP.
Thus, for the formulated product, no potential risks are identified for aquatic organisms following application of AMINO 30 SL to winter oilseed rape.

9.5.3 Overall conclusions

A low risk to aquatic organisms was concluded for aminopyralid based on the maximum predicted exposure already at FOCUS Step 1 and Step 2 calculations for the use of AMINO 30 SL in winter oilseed rape. All the PEC/RAC ratios are < 1 and provided acceptable risk to aquatic organisms.
For the formulated product, no potential risks are identified for aquatic organisms following application of AMINO 30 SL to winter oilseed rape.

zRMS comments:

For the risk assessment in general the UE agreed endpoints for the active substance were used. *Myriophyllum spicatum* is the most sensitive aquatic organisms.

According to the "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290) growth rate is the preferred endpoint to be used in the risk assessment for algae and aquatic plants. Therefore for completeness, when an endpoint based on growth rate is available, this is added for PEC/RAC calculations by zRMS.

Following the recommendations of the EFSA "Outcome of the pesticides peer review meeting on general

recurring issues in ecotoxicology" (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic.
After comparison of measured toxicity data available for the formulation and the active substance aminopyralid can be concluded, that in general the formulation is more toxic to aquatic organisms than the active substance. Therefore for the risk assessment the endpoints of the formulation, expressed in terms of active substance, were used for the risk assessment by the Applicant.

For aquatic plants PEC/RAC calculations were performed for the most sensitive aquatic organisms *Myriophyllum spicatum*. However for the active substance and formulation the endpoints for *Lemna gibba* are available, therefore for completeness PEC/RAC calculations were added below.

Group		**Aquatic plants**	
Test species		***Lemna gibba***	***Lemna gibba***
Endpoint (µg/L)		E_rC_{50} 88000 (Aminopyralid based on EU level)	E_rC_{50} 2900 (Aminopyralid based on AMINO 30 SL)
AF		10	10
RAC (µg/L)		8800	290
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1	2.73	0.00031	0.0094

Additionally, the risk assessment for formulation was performed based on the comparison of the formulation toxicity values and PEC_{drift} values of the formulation, calculated using the Drift Swash Calculator.

All PEC/RAC ratios are below the trigger value of 1.

Conclusion
According to the performed risk assessment there is no potential of risk for aquatic organisms resulting from acute and long-term exposure to active substance following use of AMINO 30 SL in compliance with proposed GAP. No risk mitigation measures are required.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and

summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	aminopyralid	Acute oral	LD_{50} > 120 µg/bee	EFSA Journal 2013;11(9):3352, Aufderheide, J., 2001b, *XDE-750: Acute Oral Toxicity Test with the Honeybee (Apis mellifera)*, Report No.: 46596 011045 DR-0293-9028-028
	AMINO 30 SL	Acute oral	LD_{50} > 200 µg of formulation/bee (>5.8 µg of a.s./bee)	Dybek, M., 2024, *AMINO 30 SL Honeybees (Apis mellifera L.), Acute Oral Toxicity Test*, Study Code: B-95-24
Apis mellifera	aminopyralid	Acute contact	LD_{50} > 100 µg/bee	EFSA Journal 2013;11(9):3352, Aufderheide, J., 2001a, *XDE-750: Acute Contact Toxicity Test with the Honeybee (Apis mellifera)*, Report No.: 46595 011044 DR-0293-9028-028
	AMINO 30 SL	Acute contact	LD_{50} > 200 µg of formulation/bee (>5.8 µg of a.s./bee)	Dybek, M., 2024, *AMINO 30 SL Honeybees (Apis mellifera L.), Acute Contact Toxicity Test*, Study Code: B-96-24
Apis mellifera	AMINO 30 SL	Chronic Oral	LC_{50} > 3000 mg of test item/kg of diet, LDD_{50} for nominal dose > 90 µg of test item/bee/day (>2.6 µg a.s/bee/day), LDD_{50} for ingested dose > 114.9 µg of test item/bee/day (3.3 µg a.s/bee/day), NOEC ≥ 3000 mg/kg, NOEDD for nominal dose ≥ 90 µg if test item/bee/day (≥ 2.6 µg a.s/bee/day), NOEDD for ingested dose ≥ 114.9 µg of test item/bee/day (≥ 3.3	Dybek, M., 2024, *AMINO 30 SL Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test*, Study Code: B-94-24

Species	Substance	Exposure System	Results	Reference
			µg a.s/bee/day)	
Apis mellifera L.	AMINO 30 SL	Larval toxicity 22d	larval LOEC (8d) > 650 mg of test item/kg of diet, larval NOEC (8d) ≥ 650 mg of test item/kg of diet, pupal LOEC = 650 mg of test item/kg of diet, pupal NOEC = 216.67 mg of test item/kg of diet, Emergence results: LOED (22d) >100 µg of test item/larva, NOED = 33.33 µg of test item/larva, LOEC >50 mg of test item/kg of diet, NOEC = 216.67 mg of test item/kg of diet, EC_{10} = 2.90 mg of test item/kg of diet, EC_{20} = 34.59 mg of test item/kg of diet, EC_{50} n.d. ED_{10} = 0.45 µg of test item/larva, ED_{20} = 5.32 µg of test item/larva, ED_{50} n.d.	Niśkiewicz, M., 2024, *Honey bee larval toxicity test following repeated exposure of the test item AMINO 30 SL according to OECD GD 239 ENV/JM/MONO(2016)34*, Study code: 0038/0215/E
Bombus spp.	AMINO 30 SL	Acute oral	LD_{50} > 400 µg of test item/bumblebee (>11.6 µg a.s/bumblebee) NOED ≥ 400 µg of test item/bumblebee (≥11.6 µg a.s/bumblebee)	Dybek, M., 2024, *AMINO 30 SL Bumblebees (Bombus spp.), Acute Oral Toxicity Test*, Study Code: B-88-24
Bombus spp.	AMINO 30 SL	Acute contact	LD_{50} > 400 µg of test item/bumblebee (>11.6 µg a.s/bumblebee) NOED ≥ 400 µg of test item/bumblebee (≥11.6 a.s/bumblebee)	Dybek, M., 2024, *AMINO 30 SL Bumblebees (Bombus spp.), Acute Contact Toxicity Test*, Study Code: B-89-24
Higher-tier studies (tunnel test, field studies)				
Not required.				

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).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: Screening assessment of the risk for bees due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape		
Active substance	Aminopyralid		
Application rate (g/ha)	1 × 8.01 g a.s/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	~~5.8~~ >120	8.01	~~1.38~~ <0.07
Contact toxicity	~~5.8~~ >100		~~1.38~~ <0.08
Product	AMINO 30 SL		
Application rate (g/ha)	1 x 272.87 g product/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	272.87	1.36
Contact toxicity	200		1.36

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

An additional risk assessment for bees was performed using calculations in Bee-Tool v.3:

For AMINO 30 SL (application rate: 272.87 g a.s./ha):

Toxicity endpoints in µg/bee, µg/bee/day or µg/larva/developmental period for oral assessment:
Acute oral - LD_{50} honey bee - 200
Adult chronic - LDD_{50} honey bee - 90
Larva- NOEL honey bee - 33.33

	"Calculation factor" (Ef x SV)	ETR	Trigger	Risk indicator
Honey Bee - acute	7.6	0.01	0.2	OK
Honey Bee - chronic	7.6	0.023	0.03	OK
Honey Bee - larvae	4.4	0.04	0.2	OK

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

Not relevant.

9.6.3 Effects on bumble bees

Table 9.6-3: Screening assessment of the risk for bees due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape		
Active substance	Aminopyralid		
Application rate (g/ha)	1 × 8.01 g a.s/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Acute oral toxicity	11.6	8.01	0.69
Acute contact toxicity	11.6		0.69
Product	AMINO 30 SL		
Application rate (g/ha)	1 x 272.87 g product/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Acute oral toxicity	400	272.87	0.68
Acute contact toxicity	400		0.68

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

9.6.4 Effects on solitary bee

Not relevant.

9.6.5 Overall conclusions

All hazard quotients calculated are lower than 50, indicating that the acute oral and contact risk to bees and bumble bees is acceptable following the use according to the proposed use pattern of AMINO 30 SL.

The long-term risk for bees and evaluation of effects on honey bee development with either formulated product or active substance were assessed with the use of Bee-Tool v.3.

AMINO 30 SL poses no unacceptable risk to bees used according to the label.

zRMS Comments:

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

The EU agreed endpoints for active substance were used in risk assessment.
In addition to that, the Applicant submitted studies on chronic toxicity of formulation AMINO 30 SL to adult bees and larvae. New studies were accepted. Therefore, the requirements set out in Regulation 284/2013 are considered fulfilled.

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

There is currently no EU agreed chronic risk assessment scheme for bees. However, as agreed in the

Central Zone a risk assessment based on the EFSA bee GD is presented below for illustrative purposes.

The ETR values are less than the Tier 1 trigger values for downward sprays indicating that the chronic risk to honeybee larvae and bees is acceptable following use of AMINO 30 SL (El Camino 30 SL, Ranchero 30 SL) according to the proposed use pattern.

An acceptable risk to bees of the formulation AMINO 30 SL (El Camino 30 SL, Ranchero 30 SL) can be concluded, based on the risk assessment scheme of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2).

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea	AMINO 30 SL	Extended laboratory test	LR_{50} > 0.300 L formulation/ha, which equals >9 g a.s/ha $NOER_{mortality} \geq 0.300$ L formulation/ha	Dybek, M., 2024, *An extended laboratory test for evaluating the effects of AMINO 30 SL on the green lacewing, Chrysoperla carnea*, Study Code: B-93-24
Coccinella septempunctata	AMINO 30 SL	Extended laboratory test	LR_{50} > 0.300 L formulation/ha, which equals >9 g a.s/ha $NOER_{mortality} \geq 0.300$ L formulation/ha	Dybek, M., 2024, *An extended laboratory test for evaluating the effects of AMINO 30 SL on the ladybird beetle, Coccinella septempunctata (L.)* Study Code: B-90-24
Aphidius rhopalosiphi	AMINO 30 SL	Extended laboratory test	LR_{50} > 0.300 L formulation/ha, which equals >9 g a.s/ha ER_{50} > 0.300 L formulation/ha, $NOER_{mortality} \geq 0.300$ L formulation/ha, $NOER_{fecundity}$ < 0.075 L formulation/ha	Dybek, M., 2024, *An extended laboratory test for evaluating the effects of AMINO 30 SL on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez)*

Species	Substance	Exposure System	Results	Reference
				Study Code: B-92-24
Typhlodromus pyri	AMINO 30 SL	Extended laboratory test	LR_{50} > 0.300 L formulation/ha, which equals >9 g a.s/ha ER_{50} = 0.569 L formulation/ha, $NOER_{mortality} \geq$ 0.300 L formulation/ha, $NOER_{reproduction}$ < 0.075 L formulation/ha	Dybek, M., 2024, *An extended laboratory test for evaluating the effects of AMINO 30 SL on the predatory mite, Typhlodromus pyri (Sch.)* Study Code: B-91-24
Field or semi-field tests				
Not required				

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: Higher-tier assessment of the in-field risk for non-target arthropods due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape
Active substance/product	Aminopyralid
Application rate (g/ha)	1 × 8.01 g a.s/ha
MAF	1.0

Test species Higher-tier	**LR_{50} (g [a.s.]/ha)**	**$PER_{in\text{-}field}$ (g [a.s.]/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	>9	8.01	0.89
Aphidius rhopalosiphi	>9		0.89
Coccinella septempunctata	>9		0.89
Chrysoperla carnea	>9		0.89

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

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

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: Higher-tier assessment of the off-field risk for non-target arthropods due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape
Active substance/product	Aminopyralid
Application rate (g/ha)	1 × 8.01 g a.s/ha
MAF	1.0
vdf	1 (higher-tier)

Test species Higher-tier	**LR_{50} (g [a.s.]/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	LR_{50}>9	0.0277	0.22	5	~~0.0049~~ <0.12
Aphidius rhopalosiphi	LR_{50}>9				~~0.0049~~ <0.12
Coccinella septempunctata	LR_{50}>9				~~0.0049~~ <0.12
Chrysoperla carnea	LR_{50}>9				~~0.0049~~ <0.12

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

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

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 higher-tier risk assessment indicates that AMINO 30 SL applied at the maximum use rate in winter oilseed rape poses no risk to non-target arthropods. No risk mitigation needed.

zRMS Comments:
The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology" (2002) was accepted.

Acceptable risk may be concluded for in-field and off-field populations of non-target arthropods from the intended uses of AMINO 30 SL (El Camino 30 SL, Ranchero 30 SL) .

Conclusion
The risk to arthropods other than bees is acceptable if the AMINO 30 SL (El Camino 30 SL, Ranchero 30 SL) is applied in accordance with proposed use pattern.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Earthworms	aminopyralid	Chronic 8 weeks	NOEC = 3.2 mg a.s./kg d.w. soil (mg a.s/ha)	EFSA Journal 2013;11(9):3352, Davies, N., 2004, *XDE-750: Effects on Reproduction and Growth in the Earthworm, Eisenia foetida*, Report No. 040285
Earthworms	AMINO 30 SL	Reproduction test 8 weeks	NOEC (reproduction) = 171.5 mg of the test item/kg dry weight of the artificial soil (4.98 mg of a.s./kg dry weight of the artificial soil) EC_{10} = 262.0 mg of the test item/kg dry weight of the artificial soil (7.61 mg of a.s./kg dry weight of the artificial soil)	Gierbuszewska, A., 2024, *AMINO 30 SL Earthworm (Eisenia andrei) reproduction test,* Study code: G-54-24
Hypoaspis (Geolaelaps) aculeifer	AMINO 30 SL	reproduction test in soil 14 days	NOEC (reproduction) ≥ 1000 mg of the test item/kg dry weight of the artificial soil (≥29.03 mg of a.s./kg dry weight of the artificial soil) EC_{10} > 1000 mg of the test item/kg dry weight of the artificial soil (>29.03 mg of a.s./kg dry weight of the	Czarnynoga, M., 2024, *AMINO 30 SL Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil,* Study code: G-56-24

Species	Substance	Exposure System	Results	Reference
			artificial soil)	
Folsomia candida	AMINO 30 SL	reproduction test 28 days	NOEC (reproduction) = 555.60 mg of the test item/kg dry weight of the artificial soil (16.13 mg of a.s./kg dry weight of the artificial soil) EC_{10} = 664.94 mg of the test item/kg dry weight of the artificial soil (19.30 mg of a.s./kg dry weight of the artificial soil)	Czarnynoga, M., 2024, *AMINO 30 SL Collembolan (Folsomia candida) Reproduction Test,* Study code: G-55-24

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

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

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 AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape 1 x 272.87 g product/ha		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Aminopyralid based on EU level	3.2	0.0064	500
Aminopyralid based on AMINO 30 SL	4.98	0.0064	778.13
AMINO 30 SL	171.5	0.218	786.70
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Hypoaspis (Geolaelaps) aculeifer			

Aminopyralid based on AMINO 30 SL	>29.03	0.0064	4535.94
AMINO 30 SL	≥1000	0.218	4587.16
Folsomia candida			
Aminopyralid based on AMINO 30 SL	16.13	0.0064	2520.31
AMINO 30 SL	555.60	0.218	2548.62

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The TER values calculated for AMINO 30 SL and active substance were above the triggers indicating acceptable long-term risk to earthworms and other soil macro-organisms.
The risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable due to the use of AMINO 30 SL in winter oilseed rape.

zRMS comments:

PEC_{soil} values were calculated considering GAP of AMINO 30 SL. The highest predicted environmental concentrations (PEC_{soil}) of the active substance and formulation were taken into account for the risk assessment.

For the active substance the EU agreed endpoint was used for the risk assessment. For formulation the risk assessment was performed based on the endpoints from the formulation studies.

The lower of NOEC and EC_{10} value was used in the risk assessment.

All TER values are above trigger value of 5.

Conclusion:
According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substance following use of AMINO 30 SL in compliance with proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Ap-

pendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	aminopyralid	28 d	No effects at aminopyralid rates up to 100 times (8.4 mg a.s./kg dry soil (6000 g a.s./ha) the treatment rate for grasslands. Effects <25% on nitrogen mineralisation (nitrogen transformation and nitrate production) at 28 days	EFSA Journal 2013;11(9):3352, McMurray, A., 2002, *A Laboratory Assessment of the Effects of XDE-750 on Soil Microflora Respiration and Nitrogen Transformation According to OECD Guidelines*, Report No.: GHE-T-1180
N-mineralisation	AMINO 30 SL	28 d	AMINO 30 SL at the concentrations corresponding to the PEC: 0.41 mg test item/kg dry weight of soil and 5 x PEC: 2.05 mg test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	Wróbel, A., 2024, *AMINO 30 SL Soil Microorganisms: Nitrogen Transformation Test*, Study Code: G-57-24

9.9.1.1 Justification for new endpoints

Not required.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape 1 x 8.01 g a.s/ha		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Aminopyralid based on EU level	8.4	0.0064	yes
AMINO 30 SL	2.05	0.218	yes

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation AMINO 30 SL and its active substance aminopyralid in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 28 days or more of exposure, indicating that the proposed use of AMINO 30 SL poses an acceptable risk to soil microorganisms.

zRMS comments:

For the risk assessment the EU agreed endpoint for active substance was used. For AMINO 30 SL the endpoint from the formulation study was used.

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

Conclusion:
Since no effects (> 25%) were seen at application rates far higher than the values of PECsoil for active substance and formulation it can be concluded that application of AMINO 30 SL, according to the GAP, will not cause any detrimental effect to soil micro-organisms.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of AMINO 30 SL were not evaluated as part of the EU assessment of aminopyralid. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

Species	**Substance**	**Exposure System**	**Results [mL formulation/ha]**	**Reference**
tomato $_d$	AMINO 30 SL	21 d, Vegetative Vigour Test	ER_{50} = 23.80 mL/ha plant dry weight, which equals 24.32	Czarnynoga, M., 2024, *AMINO 30 SL Terrestial Plant Test:*

Species	Substance	Exposure System	Results [mL formulation/ha]	Reference
			g/ha ER_{50} = 6.92 mL/ha plant damage,which equals 7.07 g/ha	*Vegetative Vigour Test,* Study Code: G-58-24
pea [d]			ER_{50} = 29.87 mL/ha plant dry weight, which equals 30.53 g/ha ER_{50} = 24.66 mL/ha plant damage, which equals 25.20 g/ha	
carrot [d]			ER_{50} = 172.50 mL/ha plant dry weight, which equals 176.30 g/ha ER_{50} = 185.73 mL/ha plant damage, which equals 189.82 g/ha	
onion [m]			ER_{50} > 300 mL/ha plant dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.6 g/ha	
winter wheat [m]			ER_{50} > 300 mL/ha plant dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.6 g/ha	
oats [m]			ER_{50} > 300 mL/ha plant dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.6 g/ha	
tomato [d]	AMINO 30 SL	14 d, Seedling emergence and Seedling Growth Test 14 d	ER_{50} = 40.7 mL/ha shoot dry weight, which equals 41.60 g/ha ER_{50} = 36.0 mL/ha plant damage, which equals 36.79 g/ha	Wróbel, A., 2024, *AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test,* Study Code: G-59-24
pea [d]			ER_{50} = 141.1 mL/ha shoot dry weight, which equals 144.20 g/ha ER_{50} = 58.0 mL/ha plant damage, which equals 59.23 g/ha	

Species	Substance	Exposure System	Results [mL formulation/ha]	Reference
carrot $_d$			ER_{50} = 258.1 mL/ha shoot dry weight, which equals 263.78 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.3 g/ha	
onion $_m$			ER_{50} = 199.3 mL/ha shoot dry weight, which equals 203.68 g/ha ER_{50} = 225.6 mL/ha plant damage, which equals 230.56.3 g/ha	
oats $_m$			ER_{50} > 300 mL/ha shoot dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.3 g/ha	
winter wheat $_m$			ER_{50} > 300 mL/ha shoot dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.3 g/ha	
Sugar beet *Beta vulgaris*			ER_{50} = 104.9 mL/ha shoot dry weight, which equals 107.21 g/ha ER_{50} = 111.4 mL/ha plant damage, which equals 113.85 g/ha	Wróbel, A., 2024, *AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test* Study code: G-93-24
Corn *Zea mays*			ER_{50} > 300 mL/ha shoot dry weight, which equals 306.6 g/ha ER_{50} > 300 mL/ha plant damage, which equals 306.3 g/ha	

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Not required.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of AMINO 30 SL in winter oilseed rape

Intended use	Winter oilseed rape
Active substance/product	Aminopyralid/AMINO 30 SL
Application rate (g/ha)	1 x 272.87 g formulation/ha
MAF	1

Test species	**ER_{50} (g formulation/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**	
Tomato *(Solanum lycopersicon)*	24.32 7.07*	0.0277	7.56	**3.22** **0.94**	21d, Vegetative Vigour Test
	41.60 36.79*	0.0277	7.56	5.50 **4.87**	14 d, Seedling emergence and Seedling Growth Test
Pea *(Pisum sativum)*	30.53 25.20*	0.0277	7.56	**4.04** **3.33**	21d, Vegetative Vigour Test;
	144.20 59.23*	0.0277	7.56	19.07 7.83	14 d, Seedling emergence and Seedling Growth Test
Carrot *(Daucus carota)*	176.30	0.0277	7.56	23.32	21d, Vegetative Vigour Test; 14 d
	263.78	0.0277	7.56	34.89	Seedling emergence and Seedling Growth Test
Onion *(Allium cepa)*	306.6	0.0277	7.56	40.56	21d, Vegetative Vigour Test; 14 d
	203.68	0.0277	7.56	26.94	Seedling emergence and Seedling Growth Test

Winter wheat *(Triticum aestivum)*	306.6	0.0277	7.56	40.56	21d, Vegetative Vigour Test; 14 d
	306.6	0.0277	7.56	40.56	Seedling emergence and Seedling Growth Test
Oats *(Avena sativa)*	306.6	0.0277	7.56	40.56	21d, Vegetative Vigour Test; 14 d
	306.6	0.0277	7.56	40.56	Seedling emergence and Seedling Growth Test
Sugar beet (*Beta vulgaris*)	107.21	0.0277	7.56	14.18	Seedling emergence and Seedling Growth Test
Corn (*Zea mays*)	306.6	0.0277	7.56	40.56	Seedling emergence and Seedling Growth Test

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*worst-case endpoint taking into account phytotoxic effect

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of AMINO 30 SL in winter oilseed rape considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter oilseed rape			
Active substance/product		Aminopyralid/AMINO 30 SL			
Application rate (g/ha)		1 x 272.87 g formulation/ha			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	0.0277	7.56	3.78	1.89	0.76

5	0.0057	1.56	0.78	0.39	0.16
10	0.0029	0.79	0.40	0.20	0.08
Toxicity value ER_{50} = 24.32 g/ha ER_{50} =7.07 g/ha*		**TER** **criterion: TER ≥ 5**			
1		**3.22** **0.94**	6.43 **1.87**	12.87 **3.74**	32 9.30
5		15.59 **4.53**	31.18 9.06	62.36 18.13	152 44.19
10		8.95	17.68	35.35	88.38

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.
*worst-case endpoint taking into account phytotoxic effect

9.10.3 Overall conclusions

Based on the predicted rates of AMINO 30 SL in off-field areas, the TER values describing the risk for non-target plants following exposure to AMINO 30 SL according to the GAP achieve the acceptability criteria TER ≥ 5. with applying:
For winter oilseed rape:
- 1 m and use of ~~50~~ 90% drift reducing nozzles or,
- 5 m and use of 50% drift reducing nozzles or,
- ~~5~~ 10 m with no drift reducing technology to non-agricultural land.

zRMS comments:

The risk assessment was based on the results of studies for formulation presented in Appendix 2 (vegetative vigour and on seedling emergence). Tomato *(Solanum lycopersicon)* was found to be the most sensitive species in the vegetative vigour test, and in the seedling emergence test.

According to Working document on Risk Assessment of Plant Protection Products in the Central Zone Ecotoxicology, Version 3.0, December 2024, point 3.8.1 Endpoint based on phytotoxicity (EFSA PPR Meeting on general recurring issues, (EFSA, 2019)): "*The majority of the MSs agreed that phytotoxicity endpoint should be considered in the risk assessment, in line with EFSA Technical Report (2019), i.e. all effects and endpoints will be reported in the study summary and the lowest endpoint should be used by the zRMS ensuring an harmonized risk assessment at zonal level*." Therefore, where an endpoint based on phytotoxicity effects was relevant it was used in the risk assessment by zRMS.

The TER is above the trigger value when:
- 1 m and 90% drift reducing nozzles or,
- 5 m and 50% drift reducing nozzles or,
- 10 m with no drift reducing technology to non-agricultural land was applied.

Conclusion:
According to the performed risk assessment there is low risk to non-target terrestrial plants resulting from exposure to active substances following use of AMINO 30 SL in compliance with proposed GAP when:
- 1 m and 90% drift reducing nozzles or,
- 5 m and 50% drift reducing nozzles or,
- 10 m with no drift reducing nozzles to non-agricultural land is applied.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Please refer to the point 9.5 (KCP 10.2)

9.13 Classification and Labelling

In accordance with CLP Regulation, based on calculation method taking into consideration hazards of constituent substances product AMINO 30 SL is classified as **Aquatic Chronic 2, H411 .**

Classification:
Aquatic Chronic 2, H411

Hazard statement:
H411 - Toxic to aquatic life with long lasting effects.

Labelling:
H411 - Toxic to aquatic life with long lasting effects.

Pictogram:
GHS09

Signal word:
No signal word is used

Precautionary statement:
P273: Avoid release to the environment.
P391: Collect spillage.

EUH401: To avoid risks to man and the environment, comply with the instructions for use.

zRMS comments:

zRMS agrees with the classification proposal. For details see 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 10.2/01	Maga, D.	2024	AMINO 30 SL *Lemna gibba* CPCC 310, Growth inhibition test Study Code: W-29-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.2/02	Maga, D.	2024	AMINO 30 SL *Daphnia magna,* Acute Immobilisation Test Study Code: W-27-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.2/03	Maga, D.	2024	AMINO 30 SL *Raphidocelis subcapitata* SAG 61.81(formerly *Pseudokirchneriella subcapitata*), Growth inhibition test Study Code: W-28-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP	Maga, D.	2024	AMINO 30 SL *Anabaena flos-aquae* UTEX B 1444, Growth inhibition test	N	PUH Chemirol

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
10.2/04			Study Code: W-30-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N		sp. z o.o.
KCP 10.2/05	Czarnecka, M.	2024	AMINO 30 SL Water-Sediment Myriophyllum Spicatum Toxicity Test Study Code: W-26-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.1/01	Dybek, M.	2024	AMINO 30 SL Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test Study Code: B-96-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.1/02	Dybek, M.	2024	AMINO 30 SL Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Study Code: B-95-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.1/03	Dybek, M.	2024	AMINO 30 SL Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test Study Code: B-94-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.1/04	Niśkiewicz, M.	2024	Honey bee larval toxicity test following repeated exposure of the test item AMINO 30 SL according to OECD GD 239 ENV/JM/MONO(2016)34 Study code: 0038/0215/E SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland	N	PUH Chemirol sp. z o.o.

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/GEP (Y/N): Y Published (Y/N): N		
KCP 10.3.1/05	Dybek, M.	2024	AMINO 30 SL Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test Study Code: B-88-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.1/06	Dybek, M.	2024	AMINO 30 SL Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Study Code: B-89-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.2/01	Dybek, M.	2024	An extended laboratory test for evaluating the effects of AMINO 30 SL on the green lacewing, *Chrysoperla carnea* Study Code: B-93-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.2/02	Dybek, M.	2024	An extended laboratory test for evaluating the effects of AMINO 30 SL on the ladybird beetle, *Coccinella septempunctata* (L.) Study Code: B-90-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.3.2/03	Dybek, M.	2024	An extended laboratory test for evaluating the effects of AMINO 30 SL on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) Study Code: B-92-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2/04	Dybek, M.	2024	An extended laboratory test for evaluating the effects of AMINO 30 SL on the predatory mite, *Typhlodromus pyri* (Sch.) Study Code: B-91-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.4/01	Gierbuszewska, A.	2024	AMINO 30 SL Earthworm (*Eisenia andrei*) reproduction test Study Code: G-54-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.4/02	Czarnynoga, M.	2024	AMINO 30 SL Collembolan (*Folsomia candida*) Reproduction Test Study Code: G-55-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.4/03	Czarnynoga, M.	2024	AMINO 30 SL Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil Study Code: G-56-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.5/01	Wróbel. A.	2024	AMINO 30 SL Soil Microorganisms: Nitrogen Transformation Test Study Code: G-57-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.6/01	Czarnynoga, M.	2024	AMINO 30 SL Terrestial Plant Test: Vegetative Vigour Test Study Code: G-58-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y	N	PUH Chemirol sp. z o.o.

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
			Published (Y/N): N		
KCP 10.6/02	Wróbel, A.	2024	AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study Code: G-59-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 10.6/03	Wróbel, A.	2024	AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study Code: G-93-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1/01	[redacted]	2001	XDE-750: An Acute Oral Toxicity Study with the Nothern Bobwhite [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.1.1/02	[redacted]	2001a	XDE-750: A Dietary LC50 Study with the Nothern Bobwhite [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

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 10.1.1/03	[redacted]	2003a	Avian Reproduction Study with XDE-750 in Nothern Bobwhite Quail (Colinus virginianus) [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.1.1/04	Madsen, S.	2003	Determination of the n-octanol/water partition coefficient (shake flask method) of XDE-750 technical Dow AgroSciences, Indiana, USA Report No: FOR01009 (Masterfile Number) N/A GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.1.2/01	[redacted]	2001a	XDE-750: Acute Oral Toxicity Study in Fischer 344 Rats [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.1.2/02	[redacted]	2004b	Supplemental report for GF-871: Oral Gavage Developmental Toxicity Study in New Zealand white rabbits [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.1.2/03	[redacted]	2004a	GF-871: Oral Gavage Developmental Toxicity Study in New Zealand white rabbits [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.2/05	[redacted]	2001	XDE-750 Herbicide: An Acute Toxicity Study with the Rainbow Trout Oncorhynchus mykiss Walbaum [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.2/06	[redacted]	2002a	Revised report for XDE-750- Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Static Conditions	Y	DAS

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
			[redacted] GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 10.2/07	[redacted]	2002b	XDE-750 – Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Acute Conditions [redacted] GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 10.2/08	Marino, T.S., Hales-McClymont, C.A., Yaroch, A.M.	2001	XDE-750 Herbicide: an Acute Toxicity Study with the Daphnid, *Daphnia magna Straus* The Dow Chemical Company, Midland, USA Report No.: 011079 DR-0293-9028-042 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.2/09	Henry, K.S., Marino, T.A., Staley, J.L., McClymont, E.L.	2003	XDE-750: 21-Day Chronic Toxicity with the Daphnid, Daphnia magna Straus The Dow Chemical Company, Midland, USA Report No.: 021085 DR-0293-9028-074 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.2/10	Cafarella, M.A.	2002	XDE-750 – Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) under Flow-Through Conditions, Springborn Smithers Inc, Wareham, USA Report No.: 011268 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.2/11	Putt, A.E.	2002	XDE-750 - the Full Life-Cycle Toxicity to Midge (Chironomus riparius) Under Static Conditions Springborn Smithers Inc, Wareham, USA Report No.: U09304 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP	Hoberg, J.R.	2002b	XDE-750 – Acute Toxicity to the Freshwater Diatom, *Navicula pelliculosa*.	N	DAS

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
10.2/12			Springborn Smithers Inc. Wareham, USA Report No.: 12550.6199 011278 DR-0293-9028-065 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 10.2/13	Hoberg, J.R.	2002e	XDE-750 - Toxicity to Duckweed, Lemna gibba Springborn Smithers Inc, Wareham, USA Report No.: 011223R 12550.6160 DR-0293-9028-058R GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.2/14	Wenzel, A.	2012	Effect of aminopyralid on the growth of *Myriophyllum spicatum* in the presence of sediment with exposure via the water phase. Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) Study ID: 120759 GLP: Yes Published: No	N	DAS
KCP 10.3.1/06	Aufderheide, J.	2001a	XDE-750: Acute Contact Toxicity Test with the Honeybee, Apis mellifera ABC Laboratories Inc, Missouri, USA Report No.: 46595 011044 DR-0293-9028-028 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 10.3.1/07	Aufderheide, J.	2001b	XDE-750: Acute Oral Toxicity Test with the Honeybee (Apis mellifera) ABC Laboratories Inc, Missouri USA Report No.: 46596 011045 DR-0293-9028-028 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP	Davies, N.	2004	XDE-750: Effects on Reproduction and Growth in the Earthworm, *Eisenia foetida*.	N	DAS

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
10.4/04			CEM Analytical Services Limited, UK Report No.: 040285 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 10.5/02	McMurray, A.	2002	A Laboratory Assessment of the Effects of XDE-750 on Soil Microflora Respiration and Nitrogen Transformation According to OECD Guidelines Chemex Environmental International Ltd, Cambridge, UK Report No.: GHE-T-1180 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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

No additional studies were performed.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional studies were performed.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No additional studies were performed.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional studies were performed.

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

No additional studies were performed.

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 2.2.1.1.1 Study 1

Comments of zRMS:	The study was performed according to OECD TG 202 and principles of GLP. The validity criteria are met: - the percentage of immobilization of *Daphnia magna* in the control was 0% (criterion: not more than 10%), - the dissolved oxygen concentrations at exposure termination in the test vessels were within the range of 7.4 – 7.5 mg/L (criterion: not less than 3 mg/L). No deviations to the study plan were noted. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.2/02
Report	AMINO 30 SL *Daphnia magna*, Acute Immobilisation Test; 2024; Maga Damian; Study Code: W-27-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 202 (2004)/EU method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

Immobilisation of *Daphnia magna* exposed to the test item, Amino 30 SL was investigated during a 48-hour 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 with a single test item concentration of 100.0 mg/L as a limit test plus the control.

The *Daphnia magna* were observed for immobilisation after 24 and 48 hours 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.

During the exposure in the test item concentration of 100.0 mg/L and the control, no immobilisation of *Daphnia magna* was observed. No abnormal behavior of *Daphnia magna* was observed during exposure.

The concentration of aminopyralid in the test item concentration was determined using a validated high performance liquid chromatographic method with Diode Array detection.

Samples for chemical determination were collected from the test item concentration of 100.0 mg/L and the control at exposure initiation and at exposure termination.

In the sample collected at exposure initiation, the determined aminopyralid concentration was 103% of nominal concentration. Therefore, the test item concentration was prepared correctly.

In the sample collected at exposure termination, the determined aminopyralid concentration was 107% of nominal concentration. Therefore, the aminopyralid concentration was stable under test conditions.

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

Materials and methods

Test item:
Amino 30 SL; batch no.: 1/24; the content of aminopyralid: 29.67 g/L; density (20°C): 1.0220 g/cm^3; manufacturing date: July 01, 2024, expiry date: not specified by the sponsor.

Test system:
Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms of Toxicology.

Test design:
Static test (48 h of exposure); 4 replicates per test item concentration and the control; 5 Daphnia magna in each replicate.

Nominal test item concentration:
100.0 mg/L plus the control.

Nominal concentration of aminopyralid:
2.9 mg/L plus the control

Test conditions:
Temperature: 20.9 – 21.0°C; pH of the control: 7.42 – 7.85; dissolved oxygen concentration in the control: 7.50 – 7.70 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.

Chemical determinations:
The concentration of aminopyralid in the test item concentration was determined using a validated high performance liquid chromatographic method with Diode Array Detection

Endpoint value:
EC_{50}/48 h

Results and discussions

The effect of the test item on immobilisation of *Daphnia magna* was assessed. The test item concentration used in the definitive test was determined on the basis of the preliminary range finding and stability test results. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

Preliminary range-finding test (non-GLP)
The preliminary test was performed using the following test item concentrations: 100.0, 10.0, 1.0, 0.1 mg/L plus the control. The test was performed in a semi-static design with one renewal after 24 h.
The recorded temperature was in the ranges of 20.6 – 21.2°C. The pH values and dissolved oxygen concentrations were measured in all test item concentrations and the control before splitting up into replicates at exposure initiation, during the renewal in fresh and spent vessels and at exposure termination in pooled replicates [SOP/W/36].
The pH values measured in the test item concentrations and the control were in the range of 7.44 – 7.50 at exposure initiation, during the renewal in the range of 7.33 – 7.40 in spent vessels and in the range of 7.41 – 7.53 in fresh vessels and in the range of 7.38 – 7.48 at exposure termination. The measured dissolved oxygen concentrations in the test item concentrations and the control were in the range of 8.60 – 8.70 mg/L at exposure initiation, during the renewal in the range of 7.50 – 7.90 in spent vessels and in the range of 8.30 – 8.60 in fresh vessels and in the range of 7.80 – 8.10 mg/L at exposure termination.
In the preliminary test, no immobilization of Daphnia magna was observed during the exposure in the control and in the test item concentration of 0.1, 1.0 and 10.0 mg/L. At exposure termination, in the test

item concentration of 100.0 mg/L, the immobilisation of Daphnia magna was 5%.

Stability test

After the validation of the analytical method, the stability test was performed in a single test item concentration of 100.0 mg/L and the control (Elendt M7 Medium) in order to check if the active substance is stable under the test conditions.

The stability test was conducted under the test conditions with the test system. The recorded temperature was in range of 20.7 – 21.3°C during the exposure.

Results of chemical determinations in stability test

In the stability test, the concentration of aminopyralid was chemically analyzed using a validated high performance liquid chromatography with Diode Array Detection [10], [SOP/C/328, SOP/C/592]. Samples of the test item concentration of 100.0 mg/L plus the control were chemically analyzed immediately after the preparation, after 24 h and at exposure termination [SOP/W/83].

At exposure initiation, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 95.1% of nominal concentration. The results confirm that the test item concentration were prepared correctly.

After 24 hours of exposure, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 96.1% of nominal concentration.

At exposure termination, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 96.7% of the nominal concentration. Therefore, the concentration of aminopyralid was stable under test conditions.

Definitive test

The definitive test was performed using a single test item concentration of 100.0 mg/L plus the control in a static design.

In the definitive test, the recorded temperature during exposure was in the range of 20.9– 21.0°C and constant within 0.1ºC.

The pH value, in the control and the test item concentration of 100.0 mg/L, were in the ranges 7.42 - 7.75 at exposure initiation and 7.68 - 7.85 at exposure termination.

The dissolved oxygen concentration, in the control and the test item concentration of 100.0 mg/L, were in the ranges 7.70 – 7.80 mg/L at exposure initiation and 7.40 – 7.50 mg/L at exposure termination [SOP/W/36]. During the exposure in the test item concentration of 100.0 mg/L and the control, no immobilization of *Daphnia magna* was observed (Table 5). No abnormal behavior of *Daphnia magna* was observed during exposure.

Table 5. 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 (0.0)	20	0	0	0	0	0	0	0	0	0	0
100.0	20	0	0	0	0	0	0	0	0	0	0

Time of exposure: 27.08.2024 – 29.08.2024

Results of chemical determination

Samples for chemical determination were collected from the test item concentration of 100.0 mg/L and the control at exposure initiation and at exposure termination.

In the sample collected at exposure initiation, the determined aminopyralid concentration was 103% of nominal concentration. Therefore, the test item concentration was prepared correctly.

In the sample collected at exposure termination, the determined aminopyralid concentration was 107% of

nominal concentration. Therefore, the aminopyralid concentration was stable under test conditions.

Endpoints values
The endpoint values were determined based on the nominal test item concentration and on the nominal concentration of aminopyralid. In the test item concentration of 100.0 mg/L and in the control, no immobilization of *Daphnia magna* was observed during exposure. Since the immobilization of *Daphnia magna* was less than 10%, no statistical analysis was needed. The EC_{50}/48 h value is higher than 100.0 mg/L. The endpoint values are presented in Table 6 and 7.

Table 6. Endpoint values based on the nominal test item concentration, definitive test

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	>100	>100

Table 7. Endpoint values based on the nominal concentration of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	>2.9	>2.9

Conclusion

The endpoint values based on nominal test item concentrations are given below:
the EC_{50}/48 h is higher than 100.0 mg/L;

The endpoint values based on the nominal concentration of aminopyralid:
the EC_{50}/48 h is higher than 2.9 mg/L;

The validity criteria:

In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004) and EU Method C.2.:
- the percentage of immobilization of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations at exposure termination in the test vessels were within the range of 7.4 – 7.5 mg/L (criterion: not less than 3 mg/L).

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

A 2.2.2.1.1 Study 2

Comments of zRMS:	The study was performed according to OECD TG 221 and principles of GLP. The validity criteria are met: - the doubling time of frond number in the control was 2.0 days, criterion: less

	than 2.5 days (the factor of frond number in the control between 0 and 7 day was 11.5), - the average specific growth rate in the control between day 0 and day 7 was 0.348 d-1 (minimum requirement: higher than 0.275 d-1). No deviations to the study plan were noted. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.2/01
Report	AMINO 30 SL Lemna gibba CPCC 310, Growth inhibition test; 2024; Maga Damian; Study Code: W-29-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 221 (2006)/ EU Method C.26.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

The growth of *Lemna gibba* exposed to the test item, Amino 30 SL, was investigated in a 7 day static test design. The test was performed in a glass beakers of 600 mL capacity containing 400 mL of either the test item concentration or the control. The initial frond number in test item concentration and the control was nine. The definitive test was conducted with a single test item concentration of 100.0 mg/L and the control as a limit test.

The total number of fronds in each test vessel was counted twice during exposure (day 3 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time.

During the exposure no distinctive changes from the normal development of plants in the control were observed.

The concentration of aminopyralid was chemically determined. The concentration of active substance was chemically analyzed using the validated high performance liquid chromatographic method with Diode Array Detection.

Samples for chemical determination were collected from the test item concentration of 100.0 mg/L and the control at exposure initiation and at exposure termination.

In fresh samples at exposure initiation, the determined concentrations of aminopyralid was 109% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

In samples collected at exposure termination, the determined concentration of aminopyralid was 109% of the nominal concentration. Therefore, the concentration of aminopyralid was stable under test conditions. The endpoints value was determined based on the nominal test item concentration as well as on the nominal concentration of aminopyralid.

Materials and methods

Test item:
Amino 30 SL; batch no.: 1/24; the content of aminopyralid: 29.67 g/L; density (20°C): 1.0220 g/cm3; manufacturing date: July 01, 2024, expiry date: Not specified by the sponsor.

Test system:
Freshwater aquatic plant Lemna gibba L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.

Test design:
Static system (7 days of exposure); six replicates for test item concentration and the control.
Nominal test item concentrations:
100.0 mg/L plus the control
Nominal concentration of aminopyralid:
2.9 mg/L plus the control.

Test conditions:
Temperature: 22.6 – 22.9ºC; pH of the control: 7.41 – 8.75; light intensity: 7790 – 8097.5 lux; constant illumination; test vessels: glass beakers containing 400 mL of treatment or the control; initial frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP.

Statistics:
Analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), STUDENT-t test for Homogeneous Variances.
Endpoint value:
E_rC_{50}, E_yC_{50}, NOEC/72 h and LOEC/72 h values based on frond number and dry weight.

Results and discussions

The effect of the test item on growth of *Lemna gibba* was estimated. The test item concentration used in the definitive test and the test design were determined on the basis of the preliminary range-finding test and stability test (both non-GLP).

Preliminary range-finding test (non-GLP)
The test was performed using the following test item concentrations: 100.0, 10.0, 1.0, and 0.1 mg/L plus the control in a semi-static test design with daily renewals.
In the test, the recorded temperature was in the range of 22.7 – 23.2°C, whereas the mean light intensity was in the range of 7317.5 – 7470 lux.
The pH values measured in fresh test item concentrations and the control at exposure initiation and during renewals were in the range of 7.36 – 7.78 and in the range of 8.03 – 8.94 in spent test item concentrations and the control during renewals and at exposure termination.
Number of fronds distinctly visible in each test vessel was counted and recorded as well as changes in plant development were observed on days 3, 5 and after 7 days of exposure.
At exposure termination, the growth rate inhibition and yield inhibition based on the frond number were in the ranges of 2.3 – 4.0% and 7.0 – 11.8% in the test item concentrations, respectively.
No growth rate and yield inhibition were observed in the test item concentration of 100.0 mg/L based on the dry weight.
The growth rate inhibition and yield inhibition based on the dry weight were in the ranges of 1.5 – 2.1% and 5.8 – 7.1% respectively in the test item concentrations of 0.1, 1.0 and 10.0.

Stability test (non-GLP)
After the validation of analytical method, the stability test was performed in order to check if the active substance of the test item is stable under the test conditions. For this purpose, the test item concentration

of 100.0 mg/L and the control (20X AAP Medium) were used.
The stability test was conducted under the test conditions with the test system. The recorded temperature was in range of 22.6 – 23.0°C during the exposure.

Results of chemical determinations in stability test
In the stability test, the determination of aminopyralid in 20X AAP medium was accomplished by the high performance liquid chromatography (HPLC) with Diode Array Detection [SOP/C/328, SOP/C/592]. At exposure initiation, in the test item concentration of 100.0 mg/L, the determined concentrations of aminopyralid was 107% of nominal concentration. The result confirms that the test item concentration was prepared correctly. After the 48 h of exposure in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 107% of nominal concentration. After the 72 h of exposure, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 108% of nominal concentration. At test termination, in the test item concentration of 100 mg/L, the determined concentration of aminopyralid was 104% of nominal concentration. Therefore, the concentration of aminopyralid was stable under test conditions.

Definitive test
The definitive test was performed using a single test item concentration of 100.0 mg/L plus the control in a static test design. The exposure was for 7 days.
In the definitive test the recorded temperature was in the range of 22.6 – 22.9ºC with a variation of up to 0.3°C, whereas the average light intensity was in the range of 7790 – 8097.5 lux.
The pH values measured in fresh test item concentration and the control at exposure initiation were in the range of 7.41 – 7.55 and at exposure termination were in the range of 8.69 - 8.75.
The number of fronds distinctly visible in each test vessel was counted and recorded as well as changes in plant development on days 3, 5 and after 7 days of exposure. The frond number and the dry weight are given in Table 5 [SOP/OG/7].

Table 5. Frond number and dry weight – definitive test

Nominal test item concentration [mg/L]	Frond number			Dry weight [mg]
	day 3	day 5	day 7	day 7
Control	39	67	93	23.8
	37	68	100	27.2
	39	71	95	27.2
	45	80	108	29.4
	47	81	110	27.8
	43	78	113	28.5
mean	**41.7**	**74.2**	**103.2**	**27.3**
standard deviation	*3.93*	*6.24*	*8.33*	*1.92*
100.0	43	83	104	28.8
	44	93	120	31.6
	39	73	97	27.8
	45	85	117	29.4
	50	93	121	29.4
	48	92	109	25.7
mean	**44.8**	**86.5**	**111.3**	**28.8**
standard deviation	*3.87*	*7.89*	*9.65*	*1.96*
Inoculum	Day 0		9	1.6
			9	*1.0*
			9	*1.0*
mean			**9**	**1.2**

Dates of exposure: 07-14.10.2024

The section-by-section growth rates and yield calculated for the whole exposure are provided in Table 6.

Table 6. Section-by-section growth rate, growth rate and yield based on frond number – definitive test

Nominal test item concentration [mg/L]	Section-by-section growth rate *			Mean growth rate	Yield**		
	0 – 3 d	**3 – 5 d**	**5 – 7 d**	**0 – 7 d**	**3 d**	**5 d**	**7 d**
Control	0.489	0.271	0.164	0.334	30	58	84
	0.471	0.304	0.193	0.344	28	59	91
	0.489	0.300	0.146	0.337	30	62	86
	0.536	0.288	0.150	0.355	36	71	99
	0.551	0.272	0.153	0.358	38	72	101
	0.521	0.298	0.185	0.361	34	69	104
mean	**0.510**	**0.289**	**0.165**	**0.348**	**32.7**	**65.2**	**94.2**
standard deviation	*0.0313*	*0.0145*	*0.0197*	*0.0116*	*3.93*	*6.24*	*8.33*
n	6						
CV	6.2	5.0	11.9	3.3	12.0	9.6	8.8
100.0	0.521	0.329	0.113	0.350	34	74	95
	0.529	0.374	0.127	0.370	35	84	111
	0.489	0.313	0.142	0.340	30	64	88
	0.536	0.318	0.160	0.366	36	76	108
	0.572	0.310	0.132	0.371	41	84	112
	0.558	0.325	0.085	0.356	39	83	100
mean	**0.534**	**0.328**	**0.126**	**0.359**	**35.8**	**77.5**	**102.3**
standard deviation	*0.0291*	*0.0235*	*0.0257*	*0.0126*	*3.87*	*7.89*	*9.65*
n	6						
CV	5.4	7.2	20.3	3.5	10.8	10.2	9.4

Example calculations: *growth rate = [ln (frond number on day 3) - ln (frond number on day 0)]/3 days,
** yield = (frond number on day 7) – (frond number on day 0)
n: number of replicates
CV: coefficient of variation

The morphology of plants was observed in the test item concentrations and the control after 3, 5 days and at exposure termination. The morphological effects were compared with appearance of colonies in the control.
During the exposure, in the test item concentration of 100.0 mg/L, no distinctive changes from the normal development of plants in the control were observed.
The effect of the test item on the growth rate and the yield of *Lemna gibba* after 7 days of exposure based on frond number is presented in Table 8.
The effect of the test item on the growth rate and the yield of *Lemna gibba* after 7 days of exposure based on dry weight is presented in Table 8.

Table 8. Results inhibition of growth rate and yield – definitive test

Nominal test item concentration [mg/L]	Based on frond number		Based on dry weight	
	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield
Control	0.0	0.0	0.0	0.0
100.0	-3.1*	-8.7*	-1.7*	-5.6*

Dates of exposure: 07-14.10.2024

*inhibition is lower than 0% what means that growth rate and yield based on frond number and dry weight in the test item concentration at exposure termination was higher than in the control.

Results of the chemical determinations

The concentration of aminopyralid was chemically analyzed using the validated high performance liquid chromatographic method with Diode Array detection [SOP/C/328, SOP/C/592]. Samples of the test item concentration and the control collected at exposure initiation and at exposure termination were chemically determined.

In fresh samples at exposure initiation, the determined concentration of aminopyralid was 109% of the nominal concentration. The results confirm that the test item concentrations was prepared correctly.

In pooled spent samples at exposure termination, the determined concentration of aminopyralid was 109% of the nominal concentration. Therefore, the concentration of aminopyralid was stable under test conditions. The results are presented in the Final Report PART II.

Endpoints values

The endpoint values were determined on the basis of the nominal test item concentration and the nominal concentration of aminopyralid. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyses and on the expert analysis. To conduct statistical analyses, the ToxRat Professional commercial software Version 3.3.0 was used (Appendix 1), [8], [SOP/W/68]. The endpoint values are presented in Tables 9 and 10.

Table 9. Growth rate and yield endpoint values based on the nominal test item concentration [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E_rC_{50}	> 100	> 100	> 100	> 100
E_yC_{50}	> 100	> 100	> 100	> 100
LOEC	> 100	> 100	> 100	> 100
NOEC	≥ 100	≥ 100	≥ 100	≥ 100

Calculations according to [8], [SOP/W/68]

Table 10. Growth rate and yield endpoint values based on the nominal concentration of aminopyralid [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-3 d	0-5 d	0-7 d	0-7 d
E_rC_{50}	> 2.9	> 2.9	> 2.9	> 2.9
E_yC_{50}	> 2.9	> 2.9	> 2.9	> 2.9
LOEC	> 2.9	> 2.9	> 2.9	> 2.9
NOEC	≥ 2.9	≥ 2.9	≥ 2.9	≥ 2.9

Calculations according to [8], [SOP/W/68]

Conclusion

The endpoint values based on the nominal test item concentration:
Endpoints based on the frond number:
The E_rC_{50}/7 d and The E_yC_{50}/7 d values are higher than 100.0 mg/L.
The LOEC/72 h value for growth rate is higher than 100.0 mg/L.
The NOEC/72 h value for growth rate is higher or equal 100.0 mg/L.
The LOEC/72 h value for yield is higher than 100.0 mg/L.
The NOEC/72 h value for yield is higher or equal 100.0 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d and the EyC50/7 d values are higher than 100.0 mg/L.
The LOEC/72 h value for growth rate is higher than 100.0 mg/L.
The NOEC/72 h value for growth rate is higher or equal 100.0 mg/L.
The LOEC/72 h value for yield is higher than 100.0 mg/L.
The NOEC/72 h value for yield is higher or equal 100.0 mg/L.

The endpoint values based on the nominal concentration of aminopyralid:
Endpoints based on the frond number:
The E_rC_{50}/7 d and the EyC50/7 d values are higher than 2.9 mg/L.
The LOEC/72 h value for growth rate is than higher 2.9 mg/L.
The NOEC/72 h value for growth rate is higher or equal 2.9 mg/L.
The LOEC/72 h value for yield is higher than 2.9 mg/L.
The NOEC/72 h value for yield is higher or equal 2.9 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d and the EyC50/7 d values are higher than 2.9 mg/L.
The LOEC/72 h value for growth rate is higher than 2.9 mg/L.
The NOEC/72 h value for growth rate is higher or equal 2.9 mg/L.
The LOEC/72 h value for yield is higher than 2.9 mg/L.
The NOEC/72 h value for yield is higher or equal 2.9 mg/L.

Test validity criteria

In the definitive test, the following validity criteria specified in the OECD Guideline No. 221 and the EU Method C.26. were met:

- the doubling time of frond number in the control was 2.0 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 11.5),
- the average specific growth rate in the control between day 0 and day 7 was 0.348 d-1 (minimum requirement: higher than 0.275 d-1).

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Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met: - the biomass in the control increased by a factor of 230.5 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.7% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 19.3% (criterion: it must not exceed 35%). No deviations from the study plan were noted: The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.2/03
Report	AMINO 30 SL *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*), Growth inhibition test 2024; Maga Damian; Study Code: W-28-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

The growth of the green algae Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) exposed to the test item, Amino 30 SL, was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the algae was 1 x 104 cells/mL. The definitive test was performed with a single test item concentration of 100.0 mg/L and the control as a limit test.

The number of algal cells was determined with an indirect method, which involves a spectrophotometric measurement of the absorbance of algal suspension at 670 nm and converting its value into the number of cells using a standard curve. The absorbance for each replicate of each test item concentration and the control were measured after 24, 48, and 72 hours of exposure.

Calculated inhibition of growth rate for the test item concentration of 100.0 mg/L after 72 h of exposure was 0.74% when compared to the control. Inhibition of yield for the test item concentration of 100.0 mg/L after 72 h of exposure was 4.10% when compared to the control.

Morphology observations of the algae cells were performed at exposure termination. In the test item concentration of 100.0 mg/L no differences in shape, size and color of algal cells were reported as compared to the algal cells in the control.

The concentration of aminopyralid was chemically determined. The concentration of active substance was chemically analyzed using the validated high performance liquid chromatographic method with Diode Array detection.

Samples for chemical determination were collected from the test item concentration of 100.0 mg/L and the control at exposure initiation and at exposure termination.

In the sample collected at exposure initiation, the determined concentration of aminopyralid was 105% of nominal concentration. Therefore, the test item concentration was prepared correctly.

In the sample collected at exposure termination, the determined concentration of aminopyralid was 98.2% of nominal concentration. Therefore, the concentration of aminopyralid was stable under the test conditions.

The endpoint value was determined based on the nominal test item concentration as well as on the nominal concentration of aminopyralid.

Materials and methods

Test item:
Amino 30 SL; batch no.: 1/24; the content of aminopyralid: 29.67 g/L; density (20°C): 1.0220 g/cm3; manufacturing date: July 01, 2024, expiry date: not specified by the sponsor.

Test system:
The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.

Test design:
72 hours of exposure; six replicates per test item concentration and the control; a background for test item concentration and the control; initial algal cell density: 1 x 104 cells/mL.

Nominal test item concentration:
100.0 mg/L plus the control

Nominal concentration of aminopyralid:
2.9 mg/L plus the control

Test conditions:
Temperature: 22.2 – 22.8 °C; pH of the control: 7.45 – 8.32; mean light intensity: 7147.5 – 7555 lux; constant illumination and shaking; medium: AAP.

Statistics:
Non-linear regression and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), STUDENT-t test for Homogeneous Variances.

Endpoint values:
E_rC_{50}/72 h, E_yC_{50}/72 h, NOEC72/ h and LOEC/72 h values

Results and discussions

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

Preliminary range-finding and stability test (non-GLP)
The preliminary test was performed using the following test item concentrations: 100.0, 10.0, 1.0 and 0.1 mg/L plus the control.
The recorded temperature was in the range of 22.8 – 23.4°C, whereas the mean light intensity at the beginning of the exposure was 6742.5 lux and at the exposure termination was 6815 lux [SOP/G/85].
The pH values in each test item concentration and control were measured at exposure initiation, i.e. before the division into replicates and at exposure termination in pooled replicates [SOP/W/36]. The pH values at exposure initiation were in the range of 7.47 – 7.51 and at exposure termination in the range of 8.12 – 8.81.
The average transmittance values were between 99.8 – 100.0% at exposure initiation and 95.5 – 100.0% at exposure termination when compared to the control. [SOP/W/12].
After 72 hours of exposure in the test item concentration of 0.1 mg/L no growth rate and yield inhibition were observed when compared to the control.
The growth rate inhibition after 72 hours of exposure was 0.73, 1.29 and 0.19% in the test item concentrations of 1.0, 10.0, and 100.0 mg/L, respectively.
The yield inhibition after 72 hours of exposure was 3.82, 6.89, and 0.79% in the test item concentrations of 1.0, 10.0, and 100.0 mg/L, respectively.
Morphology observations of the algae cells were performed at exposure termination. In all test item concentrations no differences in shape, size and color of algal cells were reported as compared to the algal cells in the control.

Stability test
After the validation of the analytical method, the stability test was performed with the single test item concentration of 100.0 mg/L and the control (AAP medium) in order to check if the active substance is stable under the test conditions.
The stability test was conducted under the test conditions with the test system. The recorded temperature was in range of 23.2 – 23.6°C during the exposure.

Results of chemical determinations in stability test:
In the stability test, the determination of aminopyralid in AAP medium was accomplished by the high performance liquid chromatography (HPLC) with Diode Array Detection [SOP/C/328]. Samples of the test item concentration of 100.0 mg/L plus the control collected at exposure initiation and at exposure termination were chemically determined [10], [SOP/C/592, SOP/W/83].
At exposure initiation, in the test item concentration of 100 mg/L, the concentration of aminopyralid was 108.0% of nominal concentration. The results confirmed that the test item concentration was prepared correctly. At exposure termination, in the test item concentration of 100 mg/L, the concentration of aminopyralid was 98.3% of nominal concentration. Therefore, the concentration of aminopyralid was stable under test conditions.

Definitive test

The definitive test was performed using a single test item concentration of 100.0 mg/L plus the control.

The recorded temperature was in the range of 22.2 – 22.8°C and constant within 0.6°C. The mean light intensity was in the range of 7147.5 – 7555 lux [SOP/W/39]. The pH values measured at exposure initiation were in the range of 7.45 – 7.46 and at exposure termination were in the range of 7.90 – 8.32.

Morphology observations of the algae cells were performed at exposure termination. In the test item concentration of 100.0 mg/L no differences in shape, size and color of algal cells were reported as compared to the algal cells in the control.

The transmittance was measured at 670 nm in replicates without the algae at exposure initiation and at exposure termination. The average transmittance values was 100.0% at exposure initiation and 97.6 % at exposure termination when compared with the control. Hence, the indirect method was adequate to determine the number of algal cells.

The average specific growth rates and yield for the whole exposure were calculated using algal cells densities determined after 24, 48, and 72 h of exposure. Algal cell density for the test item concentrations and the control is presented in Table 7. Section-by-section growth rate, mean specific growth rates, and yield calculated for the whole exposure are provided in Table 8.

Table 7. Average absorbance values converted into the algal cell density, definitive test

Nominal test item concentration [mg/L]	Average absorbance* obtained for each replicate at each time			Algal cell density [10^6 cells per mL] calculated according to the standard curve formula*		
	24 h	48 h	72 h**	24 h	48 h	72 h
Control	0.038	0.332	1.384	0.066	0.580	2.420
	0.040	0.327	1.378	0.070	0.572	2.409
	0.040	0.348	1.346	0.070	0.608	2.353
	0.036	0.301	1.206	0.063	0.526	2.108
	0.040	0.315	1.452	0.070	0.551	2.538
	0.039	0.277	1.146	0.068	0.484	2.003
mean	**0.039**	**0.317**	**1.319**	**0.068**	**0.554**	**2.305**
standard deviation	*0.002*	*0.025*	*0.117*	*0.003*	*0.044*	*0.205*
100.0	0.039	0.350	1.204	0.068	0.612	2.105
	0.040	0.334	1.318	0.070	0.584	2.304
	0.039	0.312	1.236	0.068	0.545	2.161
	0.038	*0.298*	*1.260*	0.066	0.521	2.203
	0.041	*0.317*	*1.370*	0.072	0.554	2.395
	0.039	*0.295*	*1.200*	0.068	0.516	2.098
mean	**0.039**	**0.318**	**1.265**	**0.069**	**0.555**	**2.211**
standard deviation	*0.001*	*0.021*	*0.067*	0.002	0.037	0.118

** Calculated final mean values of absorbance for diluted samples for the control and the test item concentration of 100.0 mg/L

*- Average absorbance read at λ 670 nm in a glass cuvette with a lengh of 5 cm was calculated according to the following standard curve fomula: ΔA670 = 0.572x [10^6 cells per mL], R^2 = 0.9965

Time of exposure: 20.09.2024 – 23.09.2024

Table 8. Growth rate and yield, definitive test

Nominal test item concentration [mg/L]	Growth rate* [10^6 cells/mL]				Yield** [10^6 cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.887	2.173	1.428	1.830	2.410
	1.946	2.101	1.438	1.828	2.399
	1.946	2.162	1.353	1.820	2.343
	1.841	2.122	1.388	1.784	2.098
	1.946	2.063	1.527	1.846	2.528
	1.917	1.963	1.420	1.767	1.993
mean	**1.914**	**2.097**	**1.426**	**1.812**	**2.295**
standard deviation	0.043	0.077	0.059	0.030	0.205
100.0	1.917	2.197	1.235	1.783	2.095
	1.946	2.121	1.373	1.813	2.294
	1.917	2.081	1.378	1.792	2.151
	1.887	2.066	1.442	1.798	2.193
	1.974	2.040	1.464	1.826	2.385
	1.917	2.027	1.403	1.782	2.088
mean	**1.926**	**2.089**	**1.382**	**1.799**	**2.201**
standard deviation	0.030	0.063	0.080	0.018	0.118

n.d. – not determined due mathematical reasons

* - Growth rate [10^6 cells/mL] was calculated according to the following formula:

$$\text{Growth rate (0 – 72 h)} = \frac{[\ln(\text{cell density at 72 h})] - [\ln(\text{cell density at 0 h})]}{3 \text{ days}}$$

** - Yield was calculated according to the following below formula:

$$\text{Yield } [10^6\text{cells /mL}] = (\text{cell density at 72 h}) - (\text{cell density at 0 h})$$

The relationship between the inhibition of growth rate and the nominal test item concentration at 72 h is provided in Table 9.
The relationship between the inhibition of yield and the nominal test item concentrations at 72 h is provided in Table 9.

Table 9. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
100.0	0.74	4.10

Time of exposure: 20.092024 – 23.09.2024

Endpoint values

The endpoint values were determined on the basis of the nominal test item concentration and the nominal concentration of aminopyralid. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyses and on the expert evaluation. To conduct statistical analyses, the ToxRat Professional commercial software was used (Appendix

1) [9], [SOP/W/68]. The endpoint values are presented in Tables 10 - 13.

Table 10. Growth rate endpoint values based on the nominal test item concentration, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>100	>100	>100
E_rC_{20}	>100	>100	>100
E_rC_{10}	>100	>100	>100
LOEC	> 100	> 100	> 100
NOEC	≥ 100	≥ 100	≥ 100

Calculations were made according to [9], [SOP/W/68]

Table 11. Yield endpoint values based on the nominal test item concentration, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	>100	>100	>100
E_yC_{20}	>100	>100	>100
E_yC_{10}	>100	>100	>100
LOEC	> 100	> 100	> 100
NOEC	≥ 100	≥ 100	≥ 100

Calculations were made according to [9], [SOP/W/68]

Table 12. Growth rate endpoints values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>2.9	>2.9	>2.9
E_rC_{20}	>2.9	>2.9	>2.9
E_rC_{10}	>2.9	>2.9	>2.9
LOEC	> 2.9	> 2.9	> 2.9
NOEC	≥ 2.9	≥ 2.9	≥ 2.9

Calculations were made according to [9], [SOP/W/68]

Table 13. Yield endpoint values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	>2.9	>2.9	>2.9
E_yC_{20}	>2.9	>2.9	>2.9
E_yC_{10}	>2.9	>2.9	>2.9
LOEC	> 2.9	> 2.9	> 2.9
NOEC	≥ 2.9	≥ 2.9	≥ 2.9

Calculations were made according to [9], [SOP/W/68]

Conclusion

The endpoint values based on the nominal test item concentrations:
The E_rC_{50}/72 h value is higher than 100.0 mg/L
The LOEC/72 h value for growth rate is higher than 100.0 mg/L.
The NOEC/72 h value for growth rate is higher or equal 100.0 mg/L.
The EyC50/72 h value is higher than 100.0 mg/L
The LOEC/72 h value for yield is higher than 100.0 mg/L.
The NOEC/72 h value for yield is higher or equal 100.0 mg/L.

The endpoint values based on the nominal concentration of aminopyralid:
The E_rC_{50}/72 h value is higher than 2.9 mg/L
The LOEC/72 h value for growth rate is higher than 2.9 mg/L.
The NOEC/72 h value for growth rate is higher or equal 2.9 mg/L
The EyC50/72 h value is higher than 2.9 mg/L
The LOEC/72 h value for yield is higher than 2.9 mg/L.
The NOEC/72 h value for yield is higher or equal 2.9 mg/L.

Test validity criteria

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:
-the biomass in the control increased by a factor of 230.5 within the 72-hour test period (criterion: at least a 16-fold growth),
-the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.7% (criterion: it must not exceed 7%),
-the mean coefficient of variation for the section-by-section growth rate in the control culture was 19.3% (criterion: it must not exceed 35%).

A 2.2.2.1.3 Study 4

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met: - the biomass in the control increased by a factor of 54.0 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 1.7% (criterion: it must not exceed 10%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.1% (criterion: it must not exceed 35%). No deviations from the study plan were noted: The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.2/04
Report	AMINO 30 SL *Anabaena flos-aquae* UTEX B 1444, Growth inhibition test, 2024; Maga Damian; Study Code: W-30-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

The growth of the cyanobacteria, Anabaena flos-aquae UTEX B 1444 exposed to the test item, Amino 30 SL was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration or the control, per replicate. The initial density of the cyanobacteria was 1 x 104 cells/mL. The definitive test was performed with the following test item concentrations: 600.0, 200.0, 66.67, 22.22, 7.41 and 2.47 mg/L plus the control.

The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope. In case of each replicate, the number of cells was determined after 24, 48, and 72 h of exposure.

No growth rate and yield inhibitions were observed in the test item concentration of 2.47 mg/L. Calculated inhibition of growth rate for the test item concentrations ranging from 7.41 to 600 mg/L after 72 h of exposure was in the range of 2.14 – 91.70% when compared to the control. Inhibition of yield for the test item concentrations ranging from 7.41 to 600 mg/L after 72 h of exposure was in the range of 8.52 – 99.25% when compared to the control.

Morphology observations of the cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control.

The concentrations of aminopyralid was chemically determined by validated high performance liquid chromatographic method with Diode Array detection. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.

At exposure initiation, the determined concentrations of aminopyralid were in range of 86.1 – 110% of nominal concentrations. Therefore, the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of aminopyralid were in the range of 87.5 – 111% of nominal concentrations. The results confirm, that the concentrations of test item were stable under test conditions.

The endpoints value were determined based on the nominal test item concentrations as well as on the nominal concentrations of aminopyralid.

Materials and methods

Test item:
Amino 30 SL; batch no.: 1/24; the content of aminopyralid [150114-71-9]: 29.67 g/L, density (20°C): 1.0220 g/cm3; manufacturing date: July 01, 2024; expiry date: not specified by the Sponsor.
Test system:
The freshwater cyanobacteria, Anabaena flos-aquae (Lyng.) Bréb UTEX B 1444 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.

Test design:
72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial cyanobacterial cell density: 1 x 10^4 cells/mL.

Nominal test item concentrations:
600.00, 200.00, 66.67, 22.22, 7.41 and 2.47 mg/L plus the control.

Nominal concentrations of aminopyralid:
17.400, 5.800, 1.933, 0.644, 0.215, 0.072 mg/L plus the control.

Test conditions:
Temperature: 22.7 – 23.1°C; pH of the control: 7.59 – 7.63; mean light intensity: 3180 – 3365 lux; con-

stant illumination and shaking; medium: AAP.

Statistics:
Linear regression and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Concentration/Response), Williams Multiple Sequential t-test Procedure.

Endpoint values:
E_rC_{50}/72 h, E_yC_{50}/72 h, NOEC/72 h, LOEC/72 h.

Results and discussions

The effect of the test item on the cyanobacterial growth was assessed. The range of the test item concentrations used in the definitive test was determined on the basis of the preliminary range-finding and stability tests results. The growth inhibition was estimated on the basis of the density of the cyanobacteria cells determined in the definitive test.

Preliminary range-finding and stability test (non-GLP)
The preliminary range-finding test was performed using the following test item concentrations: 100.0, 10.0, 1.0, and 0.1 mg/L plus the control.
The recorded temperature was in the range of 22.9 – 23.5°C, whereas the mean light intensity was in the range of 3522,5 – 3960 lux [SOP/W/39, SOP/G/85].
The pH values at exposure initiation were in the range of 7.62 – 7.69 and at exposure termination in the range of 7.70 – 7.94.
In the test item concentration of 1.0 mg/L, no growth rate and yield inhibition were observed when compared to the control.
The growth rate inhibition after 72 hours of exposure was 0.20, 4.16 and 41.20% in the test item concentrations of 0.1, 10.0 and 100.0 mg/L, respectively.
The yield inhibition after 72 hours of exposure was 0.77, 14.73 and 80.04% in the test item concentrations of 0.1, 10.0 and 100.0 mg/L, respectively.

The stability test
After the validation of analytical method, the stability test was performed in order to check if the active substance of the test item is stable under the test conditions.
For this purpose, the test item concentrations of 600.0 and 2.47 mg/L and the control (AAP Medium) were used.
The stability test was conducted under the test conditions with the test system. The recorded temperature was in range of 22.7 – 23.2°C during the exposure.
Results of chemical determinations in stability test:
In the stability test, the concentrations of aminopyralid was chemically analyzed using a validated high performance liquid chromatography (HPLC) with Diode Array Detection [SOP/C/328]. Samples of the test item concentrations of 600.0 and 2.47 mg/L plus the control collected at exposure initiation and at exposure termination were chemically determined [SOP/C/592, SPO/W/83].
At exposure initiation, in the test item concentration of 600.0 mg/L, the determined concentration of aminopyralid was 107% of nominal concentration. In the test item concentration of 2.47 mg/L, the determined concentration of aminopyralid was 99.6% of nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, in the test item concentration of 600.0 mg/L, the determined concentration of aminopyralid was 109% of nominal concentration. In the test item concentration of 2.47 mg/L, the determined concentration of aminopyralid was 103% of nominal concentration. Therefore, concentration of aminopyralid was stable under test conditions.

Definitive test

The definitive test was performed using the test item concentrations: 600.0, 200.0, 66.67, 22.22, 7.41 and 2.47 mg/L (with a spacing factor 3.0) plus the control.

The recorded temperature was in the range of 22.7 – 23.1°C and constant within 0.4°C. The mean light intensity was in the range of 3180 – 3365 lux. The pH values in the test item concentrations and the control measured at exposure initiation were in the range of 7.43 – 7.59 and at exposure termination were in the range of 7.39 – 7.63.

Morphology observations of cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control.

Cyanobacterial cell density for the test item concentrations and the control is presented in Table 6. Section-by-section growth rate, mean specific growth rates, and yield calculated for the whole exposure are provided in Table 7 [SOP/OG/7].

Table 6. Cyanobacterial cell density – definitive test

Nominal test item concentration [mg/L]	Cyanobacterial cell density [x10^6 cells/mL]		
	24 h	48 h	72 h
Control	0.059	0.228	0.553
	0.044	0.191	0.475
	0.050	0.203	0.563
	0.063	0.250	0.556
	0.056	0.219	0.522
	0.053	0.228	0.572
mean	**0.054**	**0.220**	**0.540**
standard deviation	0.007	0.021	0.036
2.47	0.059	0.269	0.619
	0.072	0.203	0.519
	0.056	0.275	0.500
mean	**0.062**	**0.249**	**0.546**
standard deviation	0.009	0.040	0.064
7.41	0.056	0.200	0.494
	0.063	0.241	0.516
	0.053	0.213	0.475
mean	**0.057**	**0.218**	**0.495**
standard deviation	0.005	0.021	0.021
22.22	0.041	0.153	0.450
	0.038	0.163	0.419
	0.047	0.209	0.506
mean	**0.042**	**0.175**	**0.458**
standard deviation	0.005	0.030	0.044
66.67	0.028	0.122	0.238
	0.034	0.122	0.372
	0.022	0.131	0.334
mean	**0.028**	**0.125**	**0.315**
standard deviation	0.006	0.005	0.069
200.0	0.025	0.044	0.059
	0.022	0.044	0.050
	0.028	0.031	0.038
mean	**0.025**	**0.040**	**0.049**
standard deviation	0.003	0.008	0.011
600.0	0.019	0.016	0.016
	0.016	0.022	0.013
	0.019	0.016	0.013
mean	**0.018**	**0.018**	**0.014**
standard deviation	0.002	0.003	0.002

Time of exposure: 30.09.2024 – 03.10.2024

Table 7. Growth rate and yield, definitive test

Nominal test item concentration [mg/L]	Growth rate* [10^6 cells/mL*day^{-1}]				Yield** [10^6 cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.775	1.352	0.886	1.338	0.543
	1.482	1.468	0.911	1.287	0.465
	1.609	1.401	1.020	1.344	0.553
	1.841	1.378	0.799	1.339	0.546
	1.723	1.364	0.869	1.318	0.512
	1.668	1.459	0.920	1.349	0.562
mean	**1.683**	**1.404**	**0.901**	**1.329**	**0.530**
standard deviation	0.127	0.049	0.072	0.023	0.036
2.47	1.775	1.517	0.833	1.375	0.609
	1.974	1.037	0.939	1.316	0.509
	1.723	1.591	0.598	1.304	0.490
mean	**1.824**	**1.382**	**0.790**	**1.332**	**0.536**
standard deviation	0.133	0.301	0.175	0.038	0.064
7.41	1.723	1.273	0.904	1.300	0.484
	1.841	1.342	0.761	1.315	0.506
	1.668	1.391	0.802	1.287	0.465
mean	**1.744**	**1.335**	**0.823**	**1.300**	**0.485**
standard deviation	0.088	0.059	0.074	0.014	0.021
22.22	1.411	1.317	1.079	1.269	0.440
	1.335	1.456	0.944	1.245	0.409
	1.548	1.492	0.884	1.308	0.496
mean	**1.431**	**1.422**	**0.969**	**1.274**	**0.448**
standard deviation	0.108	0.093	0.100	0.032	0.044
66.67	1.030	1.472	0.668	1.057	0.228
	1.224	1.278	1.115	1.205	0.362
	0.788	1.784	0.936	1.170	0.324
mean	**1.014**	**1.511**	**0.906**	**1.144**	**0.305**
standard deviation	0.218	0.256	0.225	0.078	0.069
200.0	0.916	0.565	0.293	0.592	0.049
	0.788	0.693	0.128	0.536	0.040
	1.030	0.102	0.204	0.445	0.028
mean	**0.911**	**0.453**	**0.208**	**0.524**	**0.039**
standard deviation	0.121	0.311	0.083	0.074	0.011
600.0	0.642	n.d#	0.000	0.157	0.006
	0.470	0.318	n.d#	0.087	0.003
	0.642	n.d#	n.d#	0.087	0.003
mean	**0.585**	**n.d#**	**n.d#**	**0.111**	**0.004**
standard deviation	0.099	n.d#	n.d#	0.040	0.002

e.g.* - Growth rate [day^{-1}] was calculated according to the following formula:

n.d# – not determined due mathematical reasons

$$\text{Growth rate (0 – 72 h)} = \frac{[\ln(\text{cell density at 72 h})] - [\ln(\text{cell density at 0 h})]}{3 \text{ days}}$$

** - Yield was calculated according to the following below formula:

$$\text{Yield } [10^6\text{cells /mL}] = (\text{cell density at 72 h}) - (\text{cell density at 0 h})$$

The relationship between the inhibition of yield and the nominal test item concentrations at 72 h is provided in Table 8.

Table 8. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
2.47	-0.19*	-1.10*
7.41	2.14	8.52
22.22	4.15	15.44
66.67	13.93	42.53
200.0	60.55	92.64
600.0	91.70	99.25

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

Time of exposure: 30.09.2024 – 03.10.2024

Results of chemical determinations

The concentrations of aminopyralid, were chemically analyzed using a validated high performance liquid chromatography with Diode Array Detection [SOP/C/328, SOP/C/592]. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined [SOP/W/83].

At exposure initiation, the determined concentrations of aminopyralid was in the range of 86.1 – 110% of nominal concentration. Therefore, the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of aminopyralid was in the range of 87.5 – 111% nominal concentration.

The results are presented in the Final Report Part II.

Endpoint values

The endpoint values are based on the nominal test item concentrations and on nominal concentrations of aminopyralid. The ECx values were calculated with a linear regression. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyses. To conduct statistical analyses, the ToxRat Professional commercial software was used (Appendix 1) [9], [SOP/W/68]. The endpoint values are presented in Tables 9 - 12.

Table 9. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_rC_{50}	*215.07 (155.27 – 318.65)*	181.09 *(160.37 – 204.86)*	**162.57** *(149.20 – 177.10)*
E_rC_{20}	*31.18 (16.70 – 47.40)*	62.44 *(50.38 – 74.14)*	**77.58** *(66.76 – 87.56)*
E_rC_{10}	*11.36 (4.48 – 20.33)*	35.79 *(26.66 – 44.96)*	**52.70** *(43.01 – 61.78)*
LOEC	22.22	22.22	66.67
NOEC	7.41	7.41	22.22

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

Table 10. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_yC_{50}	72.59 *(51.85 - 102.50)*	66.54 *(55.56 – 79.69)*	**69.80** *(57.55 – 84.68)*
E_yC_{20}	15.38 *(7.94 - 23.72)*	24.02 *(17.28 – 30.50)*	**31.88** *(21.83 – 40.56)*
E_yC_{10}	6.84 *(2.73 - 12.06)*	14.10 *(9.00 – 19.25)*	**21.16** *(12.56 – 28.90)*
LOEC	22.22	22.22	22.22
NOEC	7.41	7.41	7.41

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

Table 11. Growth rate endpoint values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	6.237 *(4.502 – 9.241)*	5.251 *(4.650 – 5.941)*	**4.714** *(4.327 – 5.136)*
E_rC_{20}	0.904 *(0.484 – 1.375)*	1.810 *(1.461 – 2.150)*	**2.249** *(1.936 – 2.539)*
E_rC_{10}	0.330 *(0.130 – 0.590)*	1.038 *(0.773 – 1.304)*	**1.528** *(1.247 – 1.791)*
LOEC	0.644	0.644	1.933
NOEC	0.215	0.215	0.644

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

Table 12. Yield endpoint values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	2.105 *(1.504 – 2.973)*	1.929 *(1.611 – 2.311)*	**2.024** *(1.668 – 2.455)*
E_yC_{20}	0.446 *(0.230 – 0.688)*	0.696 *(0.501 – 0.884)*	**0.924** *(0.633 – 1.176)*
E_yC_{10}	0.198 *(0.079 – 0.350)*	0.409 *(0.261 – 0.558)*	**0.613** *(0.364 – 0.837)*
LOEC	0.644	0.644	0.644
NOEC	0.215	0.215	0.215

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

Conclusion

The endpoint values based on the nominal test item concentrations:
The E_rC_{50}/72 h value is 162.57 mg/L (95% confidence interval: 149.20 – 177.10).
The E_yC_{50}/72 h value is 69.80 mg/L (95% confidence interval: 57.55 – 84.68).
The LOEC/72 h value for growth rate is 66.67 mg/L.
The NOEC/72 h value for growth rate is 22.22 mg/L.
The LOEC/72 h value for yield is 22.22 mg/L.
The NOEC/72 h value for yield is 7.41 mg/L.

The endpoint values based on the nominal concentration of aminopyralid:
The E_rC_{50}/72 h value is 4.714 mg/L (95% confidence interval: 4.327 – 5.136).
The E_yC_{50}/72 h value is 2.024 mg/L (95% confidence interval: 1.668 – 2.455).
The LOEC/72 h value for growth rate is 1.933 mg/L.
The NOEC/72 h value for growth rate is 0.644 mg/L.

The LOEC/72 h value for yield is 0.644 mg/L.
The NOEC/72 h value for yield is 0.215 mg/L.

The validity criteria

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:
-the biomass in the control increased by a factor of 54.0 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 1.7% (criterion: it must not exceed 10%),
-the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.1% (criterion: it must not exceed 35%).

A 2.2.2.1.4 Study 5

Comments of zRMS:	The study was performed according to OECD TG 239 and principles of GLP. Since the study results and discussion, validity criteria, deviations to the study plan and test conditions do not apply to this study, they have been crossed out. The correct summary and study results from study report have been added by zRMS and are marked in grey. The validity criteria are met: - the mean total shoot length in the control in comparison with the mean total shoot length at exposure initiation increased 2.2-fold. The criterion of at least doubling the total shoot length was met; -t he mean fresh weight in the control in comparison with the mean fresh weight for representative group at exposure initiation increased 2.0-fold. The criterion of at least doubling the fresh weight was met; - the plants in the control were without visual symptoms of chlorosis and during the exposure phase no contamination with algae, fungi or bacteria on the plants, on the sediment surface or in the test medium was observed; - the mean coefficient of variation for yield based on fresh weight in replicates of the control in a period from exposure initiation to termination was 30.1%; did not exceed 35%. The following deviations from the study plan were noted: In the study two deviations occurred. The first deviation concerned the OECD Test Guidelines No. 239 (2014) 'Water-sediment *Myriophyllum spicatum* Toxicity Test', standard operating procedure SOP/W/87 and study plan. The degree of hydration of the reagent used to prepare the Smart and Barko medium was different than the one specified in the study plan, OECD guideline and standard operating procedure. The CaCl2 × 6H2O was used instead of CaCl2 × 2H2O. The second deviation concerned only the study plan. The study was finished in January 2025, not in December 2024 as initially planned. These deviations did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.2/05
Report	AMINO 30 SL Water-Sediment Myriophyllum Spicatum Toxicity Test, 2024; Czarnecka Małgorzata, Study Code: W-26-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 239
Deviations:	~~No~~ In the study two deviations occurred. The first deviation concerned the OECD Test Guidelines No. 239 (2014) 'Water-sediment Myriophyllum spicatum Toxicity Test', standard operating procedure SOP/W/87 and study plan. The degree of hydration of the reagent used to prepare the Smart and Barko medium was different than the one specified in the study plan, OECD guideline and standard operating procedure. The CaCl2 × 6H2O was used instead of CaCl2 × 2H2O. The second deviation concerned only the study plan. The study was finished in January 2025, not in December 2024 as initially planned.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The growth of watermilfoil *Myriophyllum spicatum* exposed to the test item AMINO 30 SL, for 14 days was studied in a water-sediment system using spiking water, in static test design, in conditions required for the vegetative growth. The toxicity test consisted of a rooting phase (7 days) and an exposure phase (14 days).
The plants (representative group) of the mean total shoot length 8.40 cm and mean fresh weight 228.25 mg were exposed in a set of the nominal test item concentrations: 100, 31.25, 9.77, 3.05, and 0.95 mg/L plus control. Three plants rooted in a pot with sediment were placed in a beaker and overlaid with test medium. The test item was applied into aqueous phase of water-sediment system. For each nominal test item concentration four replicates (i.e. 12 plants) and for the control six replicates (i.e. 18 plants) were used for exposure.
Mean total shoot length in the control in comparison to the mean total shoot length at exposure initiation increased 2.2-fold. Mean fresh weight in the control in comparison to the mean fresh weight for representative group at exposure initiation increased 2.0-fold.
The impact of the test item on the plants growth was assessed based on total shoot length (i.e. sum of each side shoot length and main shoot length), fresh weight and dry weight of plants. In the tested range of the test item concentrations the inhibition of growth rate for total shoot length ranged from -6.2 to 57.8%, for fresh weight ranged from -22.2 to 58.5%, for dry weight ranged from -20.4 to 94.0% in comparison with plants in the control. The inhibition of yield for total shoot length ranged from -29.3 to 58.6%, for fresh weight ranged from -30.8 to 67.1%, for dry weight ranged from -31.3 to 95.9% in comparison with plants in the control.
At exposure termination in the control the plants were healthy, with green leaves and stems, without discolorations with very good developed roots, without deformations with correct morphological shape, anchored in sediment. In the test item concentrations of 0.95 and 3.05 mg/L no visible changes in upper parts of plants and very good developed roots, without deformations with correct morphological shape, anchored in sediment were observed in comparison with plants in the control. In the test item concentration of 9.77 mg/L, leaves in whorls laid down to the stem, distorted shoot tips and moderate root development were observed. In the test item concentration of 31.25 mg/L, loss of turgor, distorted shoot tips, leaves in whorls laid down to the stem and moderate root development were observed. In the test item concentration of 100 mg/L, loss of turgor, increased length of internodal leaves, distorted shoot tips, leaves in whorls laid down to the stem and few, short roots were observed.
The concentrations of aminopyralid in aqueous phase were determined using the validated high performance liquid chromatographic method (HPLC) with Diode Array Detection.

In samples collected from all test item concentrations at exposure initiation, the determined concentration of aminopyralid in aqueous phase were in the range of 93.8 – 111.0% of nominal concentration. The results confirm that the test item concentrations were prepared correctly.
In samples collected from all test item concentrations at exposure termination, the determined concentrations of aminopyralid in aqueous phase were in the range of 93.6 – 109.0% of nominal concentration. The results confirm, that the concentrations of aminopyralid in aqueous phase were stable under test conditions. The results concerning chemical analysis are presented in the Final Report Part II (Analytical Phase).

Materials and methods

Test item:
Test item:
AMINO 30 SL; batch no.: 1/24; the content of aminopyralid: result of the test 29.67 g/L, density at 20°C: 1.0220 g/cm3; manufacturing date: July 01, 2024; expiry date: not provided by Sponsor.
Test system:
Watermilfoil Myriophyllum spicatum Linné, dicotyledonous freshwater submerged plant, macrophyte, maintained in culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Toxicology.
Test design:
Rooting phase of 7 days; during the rooting four plants per replicate. Exposure phase of 14 days; exposure with application of the test item into aqueous phase of water-sediment system, exposure in a static design; three plants per replicate; four replicates for each test item concentration and six replicates for the control.
Nominal test item concentrations:
100, 31.25, 9.77, 3.05, and 0.95 mg/L plus the control.
Nimonal concentrations of aminopyralid:
2.9, 0.906, 0.283, 0.0885 and 0.0276 mg/L plus the control
Test conditions:
Temperature: ~~22.7 – 23.1°C~~ 19.3 – 20.6ºC~~,~~ ; pH of the control: ~~7.59 – 7.63~~ 6.80 – 7.98, dissolved oxygen concentration in the control: 69.3 – 100.8% ASV;; mean light intensity: ~~3180 – 3365 lux~~ 10.91 – 11.03 klux in a daily cycle of 16 h day and 8 h night, aerated test medium Smart and Barko and a conditioned sediment; ~~constant illumination and shaking; medium: AAP~~.

~~Statistics:~~
~~Linear regression and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Concentration/Response), Williams Multiple Sequential t-test Procedure.~~

~~Endpoint values:~~
~~E_rC_{50}/72 h, E_yC_{50}/72 h, NOEC/72 h, LOEC/72 h.~~

Chemical determinations:	The concentrations of aminopyralid were determined with a validated high performance liquid chromatographic method (HPLC) with Diode Array Detection.
Statistics:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure.
Endpoint values:	E_rC_x (growth rate), E_yC_x (yield), LOEC and NOEC calculated on the basis of shoot length, fresh and dry weight.

Results and discussions

The effect of the test item on the cyanobacterial growth was assessed. The range of the test item concentrations used in the definitive test was determined on the basis of the preliminary range-finding and stability tests results. The growth inhibition was estimated on the basis of the density of the cyanobacteria cells determined in the definitive test.

Preliminary range-finding and stability test (non-GLP)
The recorded temperature was in the range of 22.9 – 23.5°C, whereas the mean light intensity was in the range of 3522,5 – 3960 lux [SOP/W/39, SOP/G/85].
The pH values at exposure initiation were in the range of 7.62 – 7.69 and at exposure termination in the range of 7.70 – 7.94.
In the test item concentration of 1.0 mg/L, no growth rate and yield inhibition were observed when compared to the control.
The growth rate inhibition after 72 hours of exposure was 0.20, 4.16 and 41.20% in the test item concentrations of 0.1, 10.0 and 100.0 mg/L, respectively.
The yield inhibition after 72 hours of exposure was 0.77, 14.73 and 80.04% in the test item concentrations of 0.1, 10.0 and 100.0 mg/L, respectively.

The stability test
The stability test was conducted under the test conditions with the test system. The recorded temperature was in range of 22.7 – 23.2°C during the exposure.
Results of chemical determinations in stability test:
In the stability test, the concentrations of aminopyralid was chemically analyzed using a validated high performance liquid chromatography (HPLC) with Diode Array Detection [SOP/C/328]. Samples of the test item concentrations of 600.0 and 2.47 mg/L plus the control collected at exposure initiation and at exposure termination were chemically determined [SOP/C/592, SPO/W/83].
At exposure initiation, in the test item concentration of 600.0 mg/L, the determined concentration of aminopyralid was 107% of nominal concentration. In the test item concentration of 2.47 mg/L, the determined concentration of aminopyralid was 99.6% of nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, in the test item concentration of 600.0 mg/L, the determined concentration of aminopyralid was 109% of nominal concentration. In the test item concentration of 2.47 mg/L, the determined concentration of aminopyralid was 103% of nominal concentration. Therefore, concentration of aminopyralid was stable under test conditions.

Definitive test
The recorded temperature was in the range of 22.7 – 23.1°C and constant within 0.4°C. The mean light intensity was in the range of 3180 – 3365 lux. The pH values in the test item concentrations and the control measured at exposure initiation were in the range of 7.43 – 7.59 and at exposure termination were in the range of 7.39 – 7.63.
Morphology observations of cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control.
Cyanobacterial cell density for the test item concentrations and the control is presented in Table 6. Section by section growth rate, mean specific growth rates, and yield calculated for the whole exposure are provided in Table 7 [SOP/OG/7].

Table 6. Cyanobacterial cell density – definitive test

Nominal test item concentration [mg/L]	Cyanobacterial cell density [x10^6 cells/mL]		
	24 h	48 h	72 h
Control	0.059	0.228	0.553
	0.044	0.191	0.475
	0.050	0.203	0.563
	0.063	0.250	0.556
	0.056	0.219	0.522
	0.053	0.228	0.572
mean	**0.054**	**0.220**	**0.540**
standard deviation	0.007	0.021	0.036
2.47	0.059	0.269	0.619
	0.072	0.203	0.519
	0.056	0.275	0.500
mean	**0.062**	**0.249**	**0.546**
standard deviation	0.009	0.040	0.064
7.41	0.056	0.200	0.494
	0.063	0.241	0.516
	0.053	0.213	0.475
mean	**0.057**	**0.218**	**0.495**
standard deviation	0.005	0.021	0.021
22.22	0.041	0.153	0.450
	0.038	0.163	0.419
	0.047	0.209	0.506
mean	**0.042**	**0.175**	**0.458**
standard deviation	0.005	0.030	0.044
66.67	0.028	0.122	0.238
	0.034	0.122	0.372
	0.022	0.131	0.334
mean	**0.028**	**0.125**	**0.315**
standard deviation	0.006	0.005	0.069
200.0	0.025	0.044	0.059
	0.022	0.044	0.050
	0.028	0.031	0.038
mean	**0.025**	**0.040**	**0.049**
standard deviation	0.003	0.008	0.011
600.0	0.019	0.016	0.016
	0.016	0.022	0.013
	0.019	0.016	0.013
mean	**0.018**	**0.018**	**0.014**
standard deviation	0.002	0.003	0.002

Time of exposure: 30.09.2024 – 03.10.2024

Table 7. Growth rate and yield, definitive test

Nominal test item concentration [mg/L]	Growth rate* [10^6 cells/mL*day^{-1}]				Yield** [10^6 cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.775	1.352	0.886	1.338	0.543
	1.482	1.468	0.911	1.287	0.465
	1.609	1.401	1.020	1.344	0.553
	1.841	1.378	0.799	1.339	0.546
	1.723	1.364	0.869	1.318	0.512
	1.668	1.459	0.920	1.349	0.562
mean	**1.683**	**1.404**	**0.901**	**1.329**	**0.530**
standard deviation	0.127	0.049	0.072	0.023	0.036
2.47	1.775	1.517	0.833	1.375	0.609
	1.974	1.037	0.939	1.316	0.509
	1.723	1.591	0.598	1.304	0.490
mean	**1.824**	**1.382**	**0.790**	**1.332**	**0.536**
standard deviation	0.133	0.301	0.175	0.038	0.064
7.41	1.723	1.273	0.904	1.300	0.484
	1.841	1.342	0.761	1.315	0.506
	1.668	1.391	0.802	1.287	0.465
mean	**1.744**	**1.335**	**0.823**	**1.300**	**0.485**
standard deviation	0.088	0.059	0.074	0.014	0.021
22.22	1.411	1.317	1.079	1.269	0.440
	1.335	1.456	0.944	1.245	0.409
	1.548	1.492	0.884	1.308	0.496
mean	**1.431**	**1.422**	**0.969**	**1.274**	**0.448**
standard deviation	0.108	0.093	0.100	0.032	0.044
66.67	1.030	1.472	0.668	1.057	0.228
	1.224	1.278	1.115	1.205	0.362
	0.788	1.784	0.936	1.170	0.324
mean	**1.014**	**1.511**	**0.906**	**1.144**	**0.305**
standard deviation	0.218	0.256	0.225	0.078	0.069
200.0	0.916	0.565	0.293	0.592	0.049
	0.788	0.693	0.128	0.536	0.040
	1.030	0.102	0.204	0.445	0.028
mean	**0.911**	**0.453**	**0.208**	**0.524**	**0.039**
standard deviation	0.121	0.311	0.083	0.074	0.011
600.0	0.642	n.d#	0.000	0.157	0.006
	0.470	0.318	n.d#	0.087	0.003
	0.642	n.d#	n.d#	0.087	0.003
mean	**0.585**	**n.d#**	**n.d#**	**0.111**	**0.004**
standard deviation	0.099	n.d#	n.d#	0.040	0.002

e.g.* - Growth rate [day^{-1}] was calculated according to the following formula:

n.d# – not determined due mathematical reasons

$$\text{Growth rate (0 – 72 h)} = \frac{[\ln(\text{cell density at 72 h})] - [\ln(\text{cell density at 0 h})]}{3 \text{ days}}$$

** - Yield was calculated according to the following below formula:

$$\text{Yield } [10^6\text{cells /mL}] = (\text{cell density at 72 h}) - (\text{cell density at 0 h})$$

The relationship between the inhibition of yield and the nominal test item concentrations at 72 h is provided in Table 8.

Table 8. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
2.47	-0.19*	-1.10*
7.41	2.14	8.52
22.22	4.15	15.44
66.67	13.93	42.53
200.0	60.55	92.64
600.0	91.70	99.25

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

Time of exposure: 30.09.2024 – 03.10.2024

Results of chemical determinations

The concentrations of aminopyralid, were chemically analyzed using a validated high performance liquid chromatography with Diode Array Detection [SOP/C/328, SOP/C/592]. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined [SOP/W/83].

At exposure initiation, the determined concentrations of aminopyralid was in the range of 86.1 – 110% of nominal concentration. Therefore, the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of aminopyralid was in the range of 87.5 – 111% nominal concentration.

The results are presented in the Final Report Part II.

Endpoint values

The endpoint values are based on the nominal test item concentrations and on nominal concentrations of aminopyralid. The ECx values were calculated with a linear regression. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyses. To conduct statistical analyses, the ToxRat Professional commercial software was used (Appendix 1) [9], [SOP/W/68]. The endpoint values are presented in Tables 9 - 12.

Table 9. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_rC_{50}	*215.07 (155.27 – 318.65)*	181.09 *(160.37 – 204.86)*	**162.57** *(149.20 – 177.10)*
E_rC_{20}	*31.18 (16.70 – 47.40)*	62.44 *(50.38 – 74.14)*	**77.58** *(66.76 – 87.56)*
E_rC_{10}	*11.36 (4.48 – 20.33)*	35.79 *(26.66 – 44.96)*	**52.70** *(43.01 – 61.78)*
LOEC	22.22	22.22	66.67
NOEC	7.41	7.41	22.22

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

Table 10. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_yC_{50}	72.59 *(51.85 - 102.50)*	66.54 *(55.56 – 79.69)*	**69.80** *(57.55 – 84.68)*
E_yC_{20}	15.38 *(7.94 - 23.72)*	24.02 *(17.28 – 30.50)*	**31.88** *(21.83 – 40.56)*
E_yC_{10}	6.84 *(2.73 - 12.06)*	14.10 *(9.00 – 19.25)*	**21.16** *(12.56 – 28.90)*
LOEC	22.22	22.22	22.22
NOEC	7.41	7.41	7.41

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

Table 11. Growth rate endpoint values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	6.237 *(4.502 – 9.241)*	5.251 *(4.650 – 5.941)*	**4.714** *(4.327 – 5.136)*
E_rC_{20}	0.904 *(0.484 – 1.375)*	1.810 *(1.461 – 2.150)*	**2.249** *(1.936 – 2.539)*
E_rC_{10}	0.330 *(0.130 – 0.590)*	1.038 *(0.773 – 1.304)*	**1.528** *(1.247 – 1.791)*
LOEC	0.644	0.644	1.933
NOEC	0.215	0.215	0.644

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

Table 12. Yield endpoint values based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	2.105 *(1.504 – 2.973)*	1.929 *(1.611 – 2.311)*	**2.024** *(1.668 – 2.455)*
E_yC_{20}	0.446 *(0.230 – 0.688)*	0.696 *(0.501 – 0.884)*	**0.924** *(0.633 – 1.176)*
E_yC_{10}	0.198 *(0.079 – 0.350)*	0.409 *(0.261 – 0.558)*	**0.613** *(0.364 – 0.837)*
LOEC	0.644	0.644	0.644
NOEC	0.215	0.215	0.215

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

~~**Conclusion**~~

~~The endpoint values based on the nominal test item concentrations:~~
~~The E_rC_{50}/72 h value is 162.57 mg/L (95% confidence interval: 149.20 – 177.10).~~
~~The E_yC_{50}/72 h value is 69.80 mg/L (95% confidence interval: 57.55 – 84.68).~~
~~The LOEC/72 h value for growth rate is 66.67 mg/L.~~
~~The NOEC/72 h value for growth rate is 22.22 mg/L.~~
~~The LOEC/72 h value for yield is 22.22 mg/L.~~
~~The NOEC/72 h value for yield is 7.41 mg/L.~~

~~The endpoint values based on the nominal concentration of aminopyralid:~~
~~The E_rC_{50}/72 h value is 4.714 mg/L (95% confidence interval: 4.327 – 5.136).~~
~~The E_yC_{50}/72 h value is 2.024 mg/L (95% confidence interval: 1.668 – 2.455).~~
~~The LOEC/72 h value for growth rate is 1.933 mg/L.~~
~~The NOEC/72 h value for growth rate is 0.644 mg/L.~~

~~The LOEC/72 h value for yield is 0.644 mg/L.~~
~~The NOEC/72 h value for yield is 0.215 mg/L.~~

~~**The validity criteria**~~

~~In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:~~
~~- the biomass in the control increased by a factor of 54.0 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 1.7% (criterion: it must not exceed 10%),~~
~~- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.1% (criterion: it must not exceed 35%).~~

The aim of the study was to evaluate the toxic impact of the test item on the growth rate and yield of watermilfoil based on total shoot length and based on biomass (fresh weight and dry weight) after exposure.
The test item concentrations used in the definitive test were chosen based on the preliminary range-finding test and stability non-GLP tests results.
The inhibition of growth rate of watermilfoil *Myriophyllum spicatum* was evaluated based on the measured total shoot length (i.e. sum of every side shoot and main shoot length), the fresh weight and the dry weight of plants after exposure.

Preliminary range-finding test

In the rooting phase plants of a total shoot length 7.0 cm and with healthy shoot meristematic apex were potted; four plants per pot, and maintained in conditions needed for the vegetative growth to allow formation of roots. Plants of similar measured total shoot length were used. Once they were planted into pots the total shoot length above sediment was approximately 4.0 cm at test initiation of the rooting phase.
The rooting phase was 7 days. The temperature of the aqueous phase in the water-sediment system was in the range of 20.3 – 22.0ºC. The average light intensity was in the range of 9.59 – 9.65 klux. The pH value measured in the test medium was was in the range of 7.82 – 7.86. The dissolved oxygen concentration was in the range of 76.0 – 77.0% ASV. The pH value in the sediment was 6.71.
After 7 days of the rooting phase the root growth was visible. Each pot with three similar plants (one was removed) was transferred into a glass beaker. Among the removed plants the representative group of 15 plants were measured: mean total shoot length was 10.9 cm, mean fresh weight of plants was 384.97 mg, mean dry weight was 31.79 mg.
The exposure phase was 14 days. The test was conducted with the following nominal test item concentrations: 50, 5.0, 0.5, 0.05 mg/L plus the control.
After application of the test item to the aqueous phase in water-sediment system the exposure phase was started. Each pot with plants in a beaker with the overlaid aqueous phase was a replicate. In the exposure phase three replicates for each test item concentration (with three plants per replicate) and six replicates for the control (with three plants per replicate) were used.
In the exposure phase the temperature of the aqueous phase in water-sediment system was in the range of 19.4 – 21.4ºC.
In the exposure phase the average light intensity was in the range of 9.97 – 10.14 klux.
At initiation of the exposure phase (on day 0) the pH values in aqueous phase of all test item concentrations and the control were in the range of 7.45 – 7.67 and the dissolved oxygen concentration was in the range of 67.3 – 76.2% ASV.
On day 7 of exposure, the pH values in the aqueous phase of all test item concentrations and the control were in the range of 7.03 – 7.17 and the dissolved oxygen concentration was in the range of 50.4 – 69.6% ASV.
At termination of the exposure phase (on day 14) the pH values in the aqueous phase of all test item concentrations and the control were in the range of 7.03 – 7.21 and the dissolved oxygen concentration was in the range of 29.2 – 68.0% % ASV.
At exposure termination, the mean total shoot length in the control was 26.72 cm in comparison with the

mean total shoot length (above sediment) at exposure initiation 10.89 cm. At exposure termination the mean fresh weight in the control was 913.79 mg in comparison with the mean fresh weight for the representative group at exposure initiation 384.97 mg.
At exposure termination in the control the plants were healthy, with green leaves and stems, without discolorations with very good developed roots, anchored in sediment. In the test item concentrations of 0.05 and 0.5 mg/L no visible changes in plant appearance were observed in comparison with plants in the control. In the test item concentration of 5.0 mg/L, leaves in whorls laid down to the stem and distorted shoot tips was observed. In the test item concentrations of 0.05, 0.5 and 5.0 mg/L very good developed roots, anchored in sediment were observed in comparison with plants in the control. In the test item concentration of 50.0 mg/L, increased length of internodal leaves, distorted shoot tips, loss of turgor, leaves in whorls laid down, few and shortened roots was observed.
The impact of the test item on the plants growth was assessed based on total shoot length (i.e. sum of each side shoot length and main shoot length), fresh weight and dry weight of plants.

Results of chemical determinations
First stability non-GLP test
The concentrations of aminopyralid were determined in the test item concentrations, as well as in aqueous and sediment phases. At test initiation, the first replicates from each water-sediment system (100 mg/L, 100 mg/kg and control) were collected and transferred for chemical determinations. Moreover, each water-sediment system (systems using spiked water and sediment and control), i.e. the second and the third replicate was transferred for chemical determinations after 7 and 14 days of test initiation, respectively [SOP/W/83].
In the water-sediment system using spiking water at test initiation, the determined concentration of aminopyralid in aqueous phase was 106% of the nominal concentration. The result confirm that the test item concentration was prepared correctly. The concentration of aminpyralid determined I sediment was < LOD.
In the water-sediment system using spiking water after 7 days of test initiation, the determined concentration of aminopyralid in aqueous phase was 103% of the nominal concentration. In sediment, determined concentration of test item was 0.35 mg/kg.
In the water-sediment system using spiking water after 14 days of test initiation, the determined concentration of aminopyralid in aqueous phase was 96.2% of the nominal concentration. In sediment, determined concentration of aminopyralid was 0.54 mg/kg.
In the water-sediment system using spiking sediment at test initiation, the determined concentration of aminopyralid in sediment was 95.2% of the nominal concentration. The result confirm that the test item concentration was prepared correctly.
In the water-sediment system using spiking sediment after 7 days of test initiation, the determined concentration of aminopyralid in sediment was 14.5% of the nominal concentration. In aqueous phase, the determined concentration of aminopyralid was 0.339 mg/L.
In the water-sediment system using spiking sediment after 14 days of test initiation, the determined concentration of aminopyralid in sediment was 24.8% of the nominal concentration. In aqueous phase, the determined concentration of aminopyralid was 0.421 mg/L.
Based on the first stability test results, the definitive test was planned to be performed in spiking of water design.

Second stability non-GLP test
The concentrations of aminopyralid were determined in the test item concentrations of 100 and 0.95 mg/L plus control in aqueous phase. At test initiation, the first replicates from each water-sediment system were collected and transferred for chemical determinations. Moreover, the second and the third replicate from each water-sediment system was transferred for chemical determinations after 7 and 14 days of test initiation, respectively [SOP/W/83].
In the water-sediment system using spiking water at test initiation, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 108% of nominal concentration, whereas in the test item concentration of 0.95 mg/L, the determined concentration of aminopyralid was 107% of nominal concentration. The results confirmed that the test item concentrations were prepared correctly.
After 7 days of test initiation, in the test item concentration of 100.0 mg/L, the determined concentration

of aminopyralid was 102% of nominal concentration, whereas in the test item concentration of 0.95 mg/L, the determined concentration of aminopyralid was 109% of nominal concentration.
After 14 days of test initiation, in the test item concentration of 100.0 mg/L, the determined concentration of aminopyralid was 97% of nominal concentration, whereas in the test item concentration of 0.95 mg/L, the determined concentration of aminopyralid was 114% of nominal concentration.
Based on the second stability test results, definitive test was performed in a static design.

Definitive test
In the rooting phase plants of a total shoot length of 7.0 cm and with healthy shoot meristematic apex were potted; four plants per pot (exposure group) and three plants (representative group), and maintained in conditions needed for the vegetative growth to allow formation of roots. Plants of similar measured total shoot length were used. Once they were planted into pots the total shoot length above sediment was approximately 4.0 cm at initiation of the rooting phase.
The rooting phase was 7 days. The temperature of the aqueous phase in the water-sediment system was in the range of 19.0 – 20.8°C (Figure 1). The average light intensity was in the range of 9.86 – 10.75 klux [SOP/W/39]. The pH value measured in the test medium was in the range of 7.66 – 7.98. The dissolved oxygen concentration measured in the test medium was in the range of 87.1 – 90.2 % ASV. The pH value measured in the sediment was 6.98.
After 7 days of the rooting phase the root growth was visible. Each pot with three similar plants was transferred into a glass beaker. Among the removed plants the representative group of 15 plants were measured: mean total shoot length above sediment was 8.40 cm, mean fresh weight of plants was 228.25 mg, mean dry weight was 35.25 mg. The shoot length of every plant remaining in pots (used for exposure) was measured (without taking out of the sediment).
The exposure phase was 14 days. The test was conducted with the following nominal test item concentrations: 100, 31.25, 9.77, 3.05, and 0.95 mg/L plus the control with the spacing factor of 3.2 [1].
After application of the test item to the aqueous phase in water-sediment system the exposure phase was started. Each pot with plants in a beaker with the overlaid aqueous phase was a replicate. In the exposure phase four replicates for each test item concentration and six replicates for the control were used. Additionally, one extra replicate (fifth) for all test item concentrations and the control (seventh) was used for purpose of chemical analysis at exposure termination.
The ratio of wet sediment to overlaid aqueous phase was approximately 1 : 4.
In the exposure phase the temperature of the aqueous phase in water-sediment system was in the range of 19.3 – 20.6ºC (Figure 2). The temperature was in the range recommended by the OECD Test Guidelines No. 239 (2014) [1].
In the exposure phase the average light intensity was in the range of 10.91 – 11.03 klux. The optimal conditions for plant growth were maintained.
At initiation of the exposure phase, the pH values in aqueous phase of all test item concentrations and the control were in the range of 7.90 – 8.00 and the dissolved oxygen concentration was in the range of 98.1 – 103.0% ASV. On day 7 of exposure, the pH values in the aqueous phase of all test item concentrations and the control were in the range of 7.15 – 7.43 and the dissolved oxygen concentration was in the range of 92.0 – 100.1% ASV. At termination of the exposure phase (on day 14) the pH values in the aqueous phase of all test item concentrations and the control were in the range of 6.80 – 7.16 and the dissolved oxygen concentration was in the range of 61.3 – 80.0% ASV (Tables 7 – 8).
The pH values and dissolved oxygen concentrations in the aqueous phase depend on the plant growth intensity. Plants by photosynthesis process build their biomass using the energy supplied with the light and nutrients in the test medium and the sediment. Since the daily cycle of lighting was used, the intensity of photosynthesis changed periodically in daily cycle.
The mean total shoot length in the control was 7.97 cm at exposure initiation and 17.22 cm at exposure termination, therefore the increase was 2.2 -fold. At exposure termination, the mean fresh weight in the control was 460.49 mg in comparison with the mean fresh weight for the representative group at exposure initiation 228.25 mg, therefore the increase was 2.0-fold.
In the tested range of the test item concentrations the inhibition of growth rate for total shoot length ranged from -6.2 to 57.8%, for fresh weight ranged from -22.2 to 58.5%, for dry weight ranged from -20.4 to 94.0% in comparison with plants in the control (Table 12). The inhibition of yield for total shoot length ranged from -29.3 to 58.6%, for fresh weight ranged from -30.8 to 67.1%, for dry weight ranged

from -31.3 to 95.9% in comparison with plants in the control (Table 13). The observations for morphology changes in plant parts above sediment (i.e. stems and leaves) were performed on day 7 of exposure and at exposure termination and parts in sediment (roots) were performed at exposure termination (Table 14).
On day 7 of exposure, in the control the plants were healthy, with green leaves and stems, without discolorations. In the test item concentrations of 0.95 and 3.05 mg/L no visible changes were observed in comparison with plants in the control. In the test item concentrations of 9.77 mg/L leaves in whorls laid down to the stem and distorted shoot tips were observed. In the test item concentration of 31.25 mg/L, loss of turgor, leaves in whorls laid down to the stem and distorted shoot tips were observed. In the test item concentration of 100 mg/L loss of turgor, increased length of internodal leaves, distorted shoot tips and leaves in whorls laid down to the stem were observed.
At exposure termination in the control the plants were healthy, with green leaves and stems, without discolorations with very good developed roots, anchored in sediment. In the test item concentrations of 0.95 and 3.05 mg/L no visible changes in upper parts of plants and very good developed roots, anchored in sediment were observed in comparison with plants in the control. In the test item concentration of 9.77 mg/L, leaves in whorls laid down to the stem, distorted shoot tips and moderate root development were observed. In the test item concentration of 31.25 mg/L, loss of turgor, distorted shoot tips, leaves in whorls laid down to the stem and moderate root development were observed. In the test item concentration of 100 mg/L, loss of turgor, increased length of internodal leaves, distorted shoot tips, leaves in whorls laid down to the stem and few, short roots were observed.

Results of chemical determinations
Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined [SOP/C/592].
In samples collected from all test item concentrations at exposure initiation, the determined concentration of aminopyralid in aqueous phase were in the range of 93.8 – 111.0% of nominal concentration. The results confirm that the test item concentrations were prepared correctly.
In samples collected from all test item concentrations at exposure termination, the determined concentrations of aminopyralid in aqueous phase were in the range of 93.6 – 109.0% of nominal concentration. The results confirm, that the concentrations of aminopyralid in aqueous phase were stable under test conditions. Therefore, aminopyralid concentrations were stable under test conditions.

TEST VALIDITY CRITERIA
In the definitive test, the validity criteria according to the OECD Test Guidelines No. 239 (2014) were met:
- the mean total shoot length in the control in comparison with the mean total shoot length at exposure initiation increased 2.2-fold. The criterion of at least doubling the total shoot length was met;
- the mean fresh weight in the control in comparison with the mean fresh weight for representative group at exposure initiation increased 2.0-fold. The criterion of at least doubling the fresh weight was met;
- the plants in the control were without visual symptoms of chlorosis and during the exposure phase no contamination with algae, fungi or bacteria on the plants, on the sediment surface or in the test medium was observed;
- the mean coefficient of variation for yield based on fresh weight in replicates of the control in a period from exposure initiation to termination was 30.1%; did not exceed 35%.

DEVIATIONS IN THE STUDY
In the study two deviations occurred. The first deviation concerned the OECD Test Guidelines No. 239 (2014) 'Water-sediment Myriophyllum spicatum Toxicity Test', standard operating procedure SOP/W/87 and study plan. The degree of hydration of the reagent used to prepare the Smart and Barko medium was different than the one specified in the study plan, OECD guideline and standard operating procedure. The $CaCl2 \times 6H2O$ was used instead of $CaCl2 \times 2H2O$.
The second deviation concerned only the study plan. The study was finished in January 2025, not in December 2024 as initially planned.

Table 5. Inhibition of growth rate and yield, preliminary range-finding test (non-GLP)

Nominal test item concentration [mg/L]	Total shoot length		Fresh weight		Dry weight	
	Inhibition of growth rate [%]	Inhibition of yield [%]	Inhibition of growth rate [%]	Inhibition of yield [%]	Inhibition of growth rate [%]	Inhibition of yield [%]
Control	0.0	0.0	0.0	0.0	0.0	0.0
0.05	-5.2*	-22.1*	-22.7*	-36.2*	-17.1*	-34.4*
0.5	-7.5*	-17.9*	-11.8*	-18.3*	-6.2*	-12.6*
5.0	18.3	20.0	29.8	35.3	36.6	51.4
50.0	37.4	48.1	46.2	55.6	67.2	79.8

Exposure phase: 13.09.2024 – 27.09.2024
* Inhibitions are lower than 0.0%, which means that the growth rates and yield based on total shoot length, fresh weight and dry weight at exposure termination were higher than in the control.

Table 12. Inhibition of growth rate, definitive test

Nominal test item concentration [mg/L]	Inhibition of growth rate at exposure termination [%]		
	Total shoot length	Fresh weight	Dry weight
Control	0.0	0.0	0.0
0.95	-6.2*	-22.2*	-20.4*
3.05	12.2	-7.0*	-6.7*
9.77	18.8	31.1	17.2
31.25	38.3	38.0	64.7
100	57.8	58.5	94.0

* Inhibitions are lower than 0.0%, which means that the growth rates based on total shoot length, fresh weight and dry weight at exposure termination were higher than in the control.

Exposure phase: 19.11.2024 – 03.12.2024

Table 13. Inhibition of yield, definitive test

Nominal test item concentration [mg/L]	Inhibition of yield at exposure termination [%]		
	Total shoot length	Fresh weight	Dry weight
Control	0.0	0.0	0.0
0.95	-29.3*	-30.8*	-31.3*
3.05	-20.3*	-9.0*	-12.7*
9.77	11.7	38.1	20.6
31.25	32.9	45.7	73.9
100	58.6	67.1	95.9

* Inhibitions are lower than 0.0%, which means that the yield based on total shoot length, fresh weight and dry weight at exposure termination were higher than in the control.

Exposure phase: 19.11.2024 – 03.12.2024

Table 14. Observations of morphology at exposure termination, definitive test

Nominal test item concentration [mg/L]	Plant parts above sediment	Plant parts below sediment
Control	Normal shape of plants, green color of leaves and stems, no discoloration	Very good development of roots, anchored in sediment
0.95	No changes	No changes, i.e. very good root development, similar to control
3.05	No changes	No changes, i.e. very good root development, similar to control
9.77	leaves in whorls laid down, distorted shoot tips	Moderate root development
31.25	loss of turgor, distorted shoot tips, leaves in whorls laid down	Moderate root development
100	loss of turgor, increased length of internodal leaves, distorted shoot tips, leaves in whorls laid down	Few, short roots

Table 15. Endpoint values for growth rate based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_rC_{50}	>100	63.74 (47.61 – 93.14)	56.48 (30.97 – 165.81)	20.15 (14.34 – 28.39)
E_rC_{20}	31.59 (19.40 – 42.12)	9.16 (5.59 – 12.93)	8.16 (1.64 – 16.04)	7.40 (3.58 – 10.93)
E_rC_{10}	15.91 (6.95 – 24.24)	3.32 (1.54 – 5.47)	2.97 (0.22 – 7.49)	4.38 (1.59 –7.22)
LOEC	31.25	3.05	9.77	31.25
NOEC	9.77	0.95	3.05	9.77

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68]

Table 16. Endpoint values for yield based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_yC_{50}	>100	67.72 (54.18 – 89.49)	34.68 (18.92 – 81.14)	15.19 (10.08 – 22.93)
E_yC_{20}	59.46 (46.99 – 68.25)	16.46 (10.96 – 21.76)	5.79 (1.12 – 11.61)	5.55 (2.21 – 8.65)
E_yC_{10}	40.43 (27.03 – 50.20)	7.86 (4.24 – 11.66)	2.27 (0.19 – 5.75)	3.28 (0.90 – 5.75)
LOEC	100.0	31.25	9.77	31.25
NOEC	31.25	9.77	3.05	9.77

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68]

Table 17. Endpoint values for growth rate based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_rC_{50}	3.40 (2.56 – 5.52)	1.85 (1.38 – 2.70)	1.64 (0.90 – 4.81)	0.58 (0.42 – 0.82)
E_rC_{20}	0.92 (0.56 – 1.22)	0.27 (0.16 – 0.37)	0.24 (0.05 – 0.47)	0.21 (0.10 – 0.32)
E_rC_{10}	0.46 (0.20 – 0.70)	0.10 (0.04 – 0.16)	0.09 (0.01 – 0.22)	0.13 (0.05 – 0.21)
LOEC	0.906	0.0885	0.283	0.906
NOEC	0.283	0.0276	0.0885	0.283

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68]

Table 18. Endpoint values for yield based on the nominal concentrations of aminopyralid, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_yC_{50}	> 2.9	1.96 (1.57 – 2.60)	1.01 (0.55 – 2.35)	0.44 (0.29 – 0.66)
E_yC_{20}	1.72 (1.36 – 1.98)	0.48 (0.32 – 0.63)	0.17 (0.03 – 0.34)	0.16 (0.06 – 0.25)
E_yC_{10}	1.17 (0.78 – 1.46)	0.23 (0.12 – 0.34)	0.07 (0.01 – 0.17)	0.10 (0.03 – 0.17)
LOEC	2.9	0.906	0.283	0.906
NOEC	0.906	0.283	0.0885	0.283

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68]

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional studies were performed.

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

A 2.3.1.1.2 Study 5

Comments of zRMS:	The study was performed fully in line with OECD 213 with no deviations. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD_{50} > 200 µg product/bee 48h contact LD_{50} > 200 µg product/bee Validity criteria were met during the test.

Reference:	KCP 10.3.1/02
Report	AMINO 30 SL Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test 2024; Dybek Marcin; Study Code: B-95-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 213 (1998)
Deviations:	Yes Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses will not be carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
content: 29.67 g/L of aminopyralid (CAS No.: 150114-71-92)
batch no.: 1/24
manufacturing date: 01.07.2024
expiry date: not specified by Sponsor

Biological test system:
the honeybee, Apis mellifera L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna [SOP/B/14],

Test design:
the test item:
− exposure duration: 48 hours
−number of doses: 5 doses and a control
−number of replicates: 3 replicates
−number of bees: 10 bees/replicate

the reference item:
−exposure duration: 24 hours
−number of doses: 3 doses
−number of replicates: 3 replicates
−number of bees: 10 bees/replicate

Test item doses:
12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Reference item doses:
0.1, 0.2 and 0.4 µg a.i./bee

Test conditions:
– temperature: 24 – 25°C
– relative air humidity: 54 − 58%

Place: dark room

Statistical analysis:
Probit analysis using linear max. likelihood regression

Endpoints:
–honeybee mortality after 24 and 48 hours of the exposure,
–the oral LD_{50}/24 h of the reference item (dimethoate).

Results and discussions

Preliminary non-GLP range findings test
Mortality of the control group after 48 hours of exposure was 0.0%.
The percentages of mortality of the bees treated with the test item at all the doses, i.e. of 8.0, 40.0 and 200.0 µg/bee after 4, 24 and 48 hours were 0.0, 0.0 and 10.0 %, respectively.
No abnormal behavioural effects were observed during the test.

Definitive test
Mortality of the treated insects is presented in Tables 5 – 7.
Mortality of the control group after 48 hours of exposure was 0.0%.
The percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 4 hours was 0.0% for all doses.
The percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 24 hours were 0.0, 3.3, 0.0, 3.3 and 3.3%, respectively.
The percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 48 hours were 0.0, 3.3, 0.0, 6.7 and 6.7%, respectively.
The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than the highest test item dose used in the test, i.e. 200.0 µg/honeybee, i.e. > (5.8 µg of aminopyralid) (Tables 6 and 7).
No abnormal behavioural effects were observed during the test (Table 8).
Mortality of the bees treated with the reference item after 4 and 24 hours is presented in Tables 9 and 10.
The median lethal dose of dimethoate (LD_{50}/24 h) determined with the log-probit method is 0.149 µg/bee (95% confidence limit are not specified) (Table 10).

Table 5. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	**0.0**
12.5	30	0	0	0	0	**0.0**
25.0	30	0	0	0	0	**0.0**
50.0	30	0	0	0	0	**0.0**
100.0	30	0	0	0	0	**0.0**
200.0	30	0	0	0	0	**0.0**

Table 6. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50}	
		Number of dead bees [no.] replicates			Total		µg of test item/ bee	µg of a.i./bee
		I	II	III	[no.]	[%]		
0.0 (control)	30	0	0	0	0	**0.0**	**> 200.0**	**> 5.8**
12.5	30	0	0	0	0	**0.0**		
25.0	30	0	1	0	1	**3.3**		
50.0	30	0	0	0	0	**0.0**		
100.0	30	0	1	0	1	**3.3**		
200.0	30	0	0	1	1	**3.3**		

Table 7. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50}	
		Number of dead bees [no.] replicates			Total		µg of test item/ bee	µg of a.i./bee
		I	II	III	[no.]	[%]		
0.0 (control)	30	0	0	0	0	0.0	> 200.0	> 5.8
12.5	30	0	0	0	0	0.0		
25.0	30	0	1	0	1	3.3		
50.0	30	0	0	0	0	0.0		
100.0	30	0	1	1	2	6.7		
200.0	30	0	1	1	2	6.7		

The definitive test was conducted between 19 – 21.08.2024

Table 8. Behavioural effects – definitive test

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h
		Number of bees showing adverse behaviour/number of living bees		
0.0 (control)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
12.5	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
25.0	I	0/10	0/10	0/10
	II	0/10	0/9	0/9
	III	0/10	0/10	0/10
50.0	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
100.0	I	0/10	0/10	0/10
	II	0/10	0/9	0/9
	III	0/10	0/10	0/9
200.0	I	0/10	0/10	0/10
	II	0/10	0/10	0/9
	III	0/10	0/9	0/9

Table 9. Honeybee mortality after 4 hours of exposure – dimethoate

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	**0.0**
0.1	30	1	0	0	1	**3.3**
0.2	30	2	0	1	3	**10.0**
0.4	30	6	7	4	17	**56.7**

Table 10. Honeybee mortality and the LD_{50} after 24 hours of exposure – dimethoate

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	**0.0**	**0.149*** (n.d – n.d)
0.1	30	4	3	3	10	**33.3**	
0.2	30	6	5	5	16	**53.3**	
0.4	30	10	10	10	30	**100.0**	

*: contact LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software) [7], [SOP/B/67]

n.d.: not determined

Conclusion

The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee, i.e. > (5.8 µg of aminopyralid).

The validity criteria:

The following validity criteria were met during the test:
-the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
-the LD_{50}/24 h of the reference item (dimethoate) was 0.149 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.3 Study 6

Comments of zRMS:	The study was performed fully in line with OECD 247 with minor deviations. These deviations has no impact on the quality, integrity and final results of the study.

	Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD_{50} > 400 µg product/ bumblebee (i.e. ≥ 11.6 µg a.s/bumblebee). 48h contact LD_{50} > 400 µg product/ bumblebee (i.e. ≥ 11.6 µg a.s/bumblebee). Validity criteria were met during the test.

Reference:	KCP 10.3.1/05
Report	AMINO 30 SL Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test, 2024; Dybek Marcin; Study Code: B-88-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline for Testing of Chemicals No. 247 (2017)
Deviations:	Yes In the study following deviation occurred. According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration with consumption controlled by weight. In the experiment they were replaced by glass calibrated pipettes. The use of glass pipettes provided a more precise administration of the contaminated diet in a given volume and allowed for visual real-time control of consumption during exposure. This deviation has no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
29.67 g/L of aminopyralid (CAS No: 150114-71-92)
batch number: 1/24
production date: 07.2024
expiry date: not specified by the Sponsor

Biological test system:
species: bumblebee, Bombus spp.
source: Koppert Polska sp. z o.o. (a commercial supplier)
age: adult worker bumblebees

Experimental design:
−a control (50% (w/v) aqueous sucrose solution)
number of replicates: 50;
number of insects: 1 insect/replicate; 13 of 42
−test item:
number of doses: 1,
number of replicates: 50;
number of insects: 1 insect/replicate;

−the reference item:
number of doses: 1,
number of replicates: 30;
number of insects: 1 insect/replicate

Dose of the test item: 400.0 µg test item/bumblebee

Dose of the reference item: 4.0 µg/bumblebee

Exposure duration: 48 hours

Test conditions: temperature: 24 – 25°C (required: 25 ± 2°C)
relative air humidity: 59 – 61% (required: 60 ± 20%) (Appendix No. 6)
place: a dark climate room

Endpoints:
−bumblebee mortality after 48 hours of exposure
−LD_{50}/24 h and LD_{50}/48 h
−NOED after 48 h of exposure

Statistical method: Due to the lack of mortality in group treated of the test item, statistical analysis was not conducted

Results and discussions

Preliminary non-GLP test
After 4, 24 and 48 hours there was no dead bumblebees in the control group.
The percentages of mortality of bumblebees exposed to the test item at all doses after 4, 24 and 48 hours was 0.0%.
During the experiment no sublethal effects (toxic symptoms) were observed.

Definitive test
Mortality of the treated insects is presented in Tables 8 – 10.
After 4, 24 and 48 hours of exposure there were no dead bumblebees in the control group. The percentage of mortality after 4, 24 and 48 hours in the group exposed to the test item at the dose of 400.0 µg/bumblebee was 0.0%.
Due to the lack of mortality, dose-effect curves showing the influence of the test item on mortality are not provided.
During the experiment sublethal effects (toxic symptoms) were no observed (Table 11).
The percentage of mortality after 4, 24 and 48 hours of exposure to the reference item at the dose of 4.0 µg/bumblebee were 86.7, 100.0 and 100.0%, respectively (Tables 8 and 10).
In group treated with the reference item, sublethal effects (toxic symptoms) were no observed (Table 11).
Summary of results for endpoints for the test item was presented in Table 13.
The mean weight of the bumblebees in the control group at test initiation was 0.248 g. The mean weight of the bumblebees in the groups exposed to the test item at dose of 400.0 µg/bumblebee was 0.252 g. The mean weight of the bumblebees in the group treated with the reference item was 0.245 g.

Table 8. Bumblebee mortality after 4 hours of exposure – definitive test

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control (0.0)	50	0	**0.0**
400.0	50	0	**0.0**
Reference item: dimethoate			
4.0	30	26	**86.7**

Table 9. Bumblebee mortality after 24 hours of exposure and LD_{50}/24 h – definitive test

Dose [µg/ bumblebee]	Number of tested bumble-bees [no.]	Mortality		LD_{50}	
		Number of dead bumblebees [no.]	[%]	[µg test item/ bumblebee]	[µg a.s. /bumblebee]
Control (0.0)	50	0	**0.0**	**> 400.0**	**> 11.6**
400.0	50	0	**0.0**		
NOED				**≥ 400.0**	**≥ 11.6**
Reference item: dimethoate					
4.0	30	30	**100.0**	–	

Table 10. Bumblebee mortality after 48 hours of exposure and LD_{50}/48 h – definitive test

Dose [µg/ bumblebee]	Number of tested bumble-bees [no.]	Mortality		LD_{50}	
		Number of dead bumblebees [no.]	[%]	[µg test item/ bumblebee]	[µg a.s. /bumblebee]
Control (0.0)	50	0	**0.0**	**> 400.0**	**> 11.6**
400.0	50	0	**0.0**		
NOED				**≥ 400.0**	**≥ 11.6**
Reference item: dimethoate					
4.0	30	30	**100.0**	–	

The exposure in the definitive test was performed between 09 – 11.10.2024.

Table 11. Sublethal effects – definitive test

Dose [µg test item/bumblebee]	Time of exposure [h]		
	4	24	48
	Number of bumblebees showing signs of toxicity/number of living bumblebees		
Control (0.0)	0/50	0/50	0/50
400.0	0/50	0/50	0/50
Reference item: dimethoate			
4.0	0/4	0/0	0/0

Table 13. Summary of results for endpoints for the test item

	Test item concentration [µg test item/bumblebee]	Aminopyralid concentration [µg a.s./bumblebee]
LD_{50} / 24	> 400.0	> 11.6
NOED	≥ 400.0	≥ 11.6
LD_{50} / 48	> 400.0	> 11.6
NOED	≥ 400.0	≥ 11.6

Conclusion

The median lethal doses (LD_{50}/24 h, LD_{50}/48 h) are higher than the dose used in the test, i.e. > 400.0 µg test item/bumblebee (i.e. > 11.6 µg a.s/bumblebee).
The NOED value after 48 hours is higher than or equal to 400.0 µg test item/bumblebee (i.e. ≥ 11.6 µg a.s/bumblebee).

Obtained results are summarized in Table 13.

Results obtained at the end of the definitive test (after 48 hours):

Test item dose [µg/bumblebee]	Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}	
		[no.]	[%]	[µg test item/ bumblebee]	[µg a.s./ bumblebee]
Control (0.0)	50	0	**0.0**	**> 400.0**	**> 11.6**
400.0	50	0	**0.0**		
NOED				**≥ 400.0**	**≥ 11.6**
Reference item: dimethoate					
Dose [4.0 µg/ bumblebee]	30	30	**100.0**	–	

Validity of the study

The following validity criteria were met:
-Mortality of 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%).

A 2.3.1.1.4 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.5 Study 7

Comments of zRMS:	The study was performed fully in line with OECD 214 with minor deviations. These deviations has no impact on the quality, integrity and final results of the study. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD50 > 200 µg product/ honeybee, (i.e. ≥ 5.8 µg a.s/honeybee,). 48h contact LD50 > 200 µg product/ honeybee, (i.e. ≥ 5.8 µg a.s/honeybee,). Validity criteria were met during the test.

Reference: KCP 10.3.1/01

Report: AMINO 30 SL Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test 2024; Dybek Marcin; Study Code: B-96-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished

Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	Yes 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. The mentioned deviation had no effect on the results of the study. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test material, such analyses were not carried out. The waiver of these analyses constitutes a deviation from the OECD Principles on Good Laboratory Practice Number 1.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
content: 29.67 g/L of aminopyralid (CAS No.: 150114-71-92)
batch no.: 1/24
manufacturing date: 01.07.2024
expiry date: not specified by Sponsor

Biological test system:
the honeybee, Apis mellifera L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,

Test design:
the test item:
−exposure duration: 48 hours
−number of doses: 5 doses with surfactant and two controls: water control and control with surfactant (0.1% Tergitol 15-S-9)
−number of replicates: 3 replicates
−number of bees: 10 bees/replicate

the reference item:
−exposure duration: 24 hours
−number of doses: 3 doses
−number of replicates: 3 replicates
−number of bees: 10 bees/replicate

Test item doses:
12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and water control (0.0 µg/bee), 0.1% Tergitol control (0.0 µg/bee)

Reference item doses:
0.1, 0.2 and 0.4 µg a.i./bee

Test conditions:
– temperature: 24 – 25°C
– relative air humidity: 54 − 58%

Place:
dark room

Statistical analysis:
Probit analysis using linear max. likelihood regression

Endpoints:
–honeybee mortality after 4, 24 and 48 hours of the exposure,
–the contact LD_{50}/24 h of the reference item (dimethoate).

Results and discussions

Preliminary range-finding test
Mortality of the both control groups after 48 hours of exposure was 0.0%.
The percentages of mortality of the bees treated with the test item at all the doses, i.e. of 8.0, 40.0 and 200.0 µg/bee after 24 and 48 hours were 0.0, 10.0 and 10.0 %, respectively.
No abnormal behavioural effects were observed during the test.

Definitive test
Mortality of the treated insects is presented in Tables 4 – 6.
Mortality of the both control groups after 48 hours of exposure was 0.0%.
The percentages of mortality of the bees treated with the test item (with surfactant) at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 4 hours were 0.0, 0.0, 0.0, 0.0 and 3.3%, respectively.
The percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 24 hours were 0.0, 3.3, 0.0, 0.0 and 3.3%, respectively.
The percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 48 hours were 0.0, 3.3, 0.0, 3.3 and 3.3%, respectively.
Graph showing the effect of AMINO 30 SL, at the doses mentioned above, on mortality after 4, 24 and 48 hours is given in Figures 1, 2 and 3.
The median lethal doses (LD_{50}/24 h and LD_{50}/48 h contact) are higher than 200.0 µg/honeybee i.e. (5.8 µg of aminopyralid/bee).
During the definitive test no abnormal behavioural effects were observed (Table 7).
Mortality of the bees treated with the reference item after 4 and 24 hours are presented in Tables 8 and 9.
The median lethal dose of dimethoate (LD_{50}/24 h) determined with the log-probit method is 0.246 µg a.i./bee (95% confidence limit are not specified). The LD_{50}/24 h is presented in Table 9.

Table 4. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (water control)	30	0	0	0	0	**0.0**
0.0 (0.1% *w/v* of Tergitol control)	30	0	0	0	0	**0.0**
12.5 (with surfactant)	30	0	0	0	0	**0.0**
25.0 (with surfactant)	30	0	0	0	0	**0.0**
50.0 (with surfactant)	30	0	0	0	0	**0.0**
100.0 (with surfactant)	30	0	0	0	0	**0.0**
200.0 (with surfactant)	30	0	1	0	1	**3.3**

Table 5. Honeybee mortality after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50}	
		Number of dead bees [no.] replicates			Total		µg of test item/ bee	µg of a.i./bee
		I	II	III	[no.]	[%]		
0.0 (water control)	30	0	0	0	0	**0.0**	**> 200.0**	**> 5.8**
0.0 (0.1% *w/v* of Tergitol control)	30	0	0	0	0	**0.0**		
12.5 (with surfactant)	30	0	0	0	0	**0.0**		
25.0 (with surfactant)	30	0	1	0	1	**3.3**		
50.0 (with surfactant)	30	0	0	0	0	**0.0**		
100.0 (with surfactant)	30	0	0	0	0	**0.0**		
200.0 (with surfactant)	30	0	1	0	1	**3.3**		

Table 6. Honeybee mortality after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50}	
		Number of dead bees [no.] replicates			Total		µg of test item/ bee	µg of a.i./bee
		I	II	III	[no.]	[%]		
0.0 (water control)	30	0	0	0	0	**0.0**	**> 200.0**	**> 5.8**
0.0 (0.1% *w/v* of Tergitol control)	30	0	0	0	0	**0.0**		
12.5 (with surfactant)	30	0	0	0	0	**0.0**		
25.0 (with surfactant)	30	0	1	0	1	**3.3**		
50.0 (with surfactant)	30	0	0	0	0	**0.0**		
100.0 (with surfactant)	30	1	0	0	1	**3.3**		
200.0 (with surfactant)	30	0	1	0	1	**3.3**		

The definitive test was conducted between 19 – 21.08.2024

Table 7. Behavioural effects – definitive test

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h
		Number of bees showing adverse behaviour/number of living bees		
0.0 (water control)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
0.0 (0.1% *w/v* of Tergitol control)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
12.5 (with surfactant)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
25.0 (with surfactant)	I	0/10	0/10	0/10
	II	0/10	0/9	0/9
	III	0/10	0/10	0/10
50.0 (with surfactant)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
100.0 (with surfactant)	I	0/10	0/10	0/9
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
200.0 (with surfactant)	I	0/10	0/10	0/10
	II	0/9	0/9	0/9
	III	0/10	0/10	0/10

Table 8. Honeybee mortality after 4 hours of exposure – dimethoate

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (water control)	30	0	0	0	0	**0.0**
0.0 (0.1% *w/v* of Tergitol control)	30	0	0	0	0	**0.0**
0.1 (with surfactant)	30	0	0	0	0	**0.0**
0.2 (with surfactant)	30	1	1	0	2	**6.7**
0.4 (with surfactant)	30	5	3	3	11	**36.7**

Table 9. Honeybee mortality after 24 hours of exposure – dimethoate

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (water control)	30	0	0	0	0	**0.0**	**0.246*** (n.d – n.d)
0.0 (0.1% *w/v* of Tergitol control)	30	0	0	0	0	**0.0**	
0.1 (with surfactant)	30	0	0	1	1	**3.3**	
0.2 (with surfactant)	30	1	2	2	5	**16.7**	
0.4 (with surfactant)	30	9	10	10	29	**96.7**	

*: contact LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software) [8], [SOP/B/67]

n.d.: not determined

Table 10. Active substance content in doses used in the preliminary non-GLP range-finding test and in the definitive test

Dose [µg/bee]	Content of aminopyralid [µg a.s./bee]
Preliminary non-GLP range-finding test	
8.0	0.2
40.0	1.2
200.0	5.8
Definitive test	
12.5	0.4
25.0	0.7
50.0	1.5
100.0	2.9
200.0	5.8

Conclusion

The median lethal doses LD_{50}/24 h and LD_{50}/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee, i.e. > (5.8 µg of aminopyralid/bee).

The acute contact toxicity study of the test item, AMINO 30 SL on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD_{50}	
		Total		µg of test item/bee	µg of a.s./bee
		[no.]	[%]		
0.0 (water control)	30	0	**0.0**	**> 200.0**	**> 5.8**
0.0 (0.1% *w/v* of Tergitol control)	30	0	**0.0**		
12.5 with 0.1% *w/v* of Tergitol	30	0	**0.0**		
25.0 with 0.1% *w/v* of Tergitol	30	1	**3.3**		
50.0 with 0.1% *w/v* of Tergitol	30	0	**0.0**		
100.0 with 0.1% *w/v* of Tergitol	30	1	**3.3**		
200.0 with 0.1% *w/v* of Tergitol	30	1	**3.3**		

Test validity criteria:

The following validity criteria were met during the test:

-the mortality for the controls was 0.0% after 48 h (criterion: it must not exceed 10.0%, ≤10.0%),
-the LD_{50}/24 h of the reference item (dimethoate) was 0.246 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

A 2.3.1.1.6 Study 8

Comments of zRMS:	The study was performed fully in line with OECD 246 with minor deviations. These deviations has no impact on the quality, integrity and final results of the study. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD50 > 400 µg product/ bumblebee, (i.e. ≥ 11.6 µg a.s/ bumblebee)). 48h contact LD50 > 400 µg product/ bumblebee, (i.e. ≥ 11.6 µg a.s/ bumblebee,). 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%).

Reference: KCP 10.3.1/05

Report: AMINO 30 SL Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test, 2024; Dybek Marcin; Study Code: B-89-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished

Guideline(s):	according to the OECD Guideline for Testing of Chemicals No. 246 (2017)
Deviations:	Yes According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation. This deviation has no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
29.67 g/L of aminopyralid (CAS No: 150114-71-92)
batch number: 1/24
production date: 07.2024
expiry date: not specified by the Sponsor

Biological test system:
species: bumblebee, Bombus spp.
source: Koppert Polska sp. z o. o. (a commercial supplier)
age: adult worker bumblebees

Experimental design:
−control (with surfactant):
number of replicates: 50,
number of insects: 1 insect/replicate;
−test item with surfactant:
number of doses: 1,
number of replicates: 50,
number of insects: 1 insect/replicate;
−the reference item with surfactant:
number of doses: 1,
number of replicates: 30,
number of insects: 1 insect/replicate;

Dose of the test item:
400.0 µg of test item/bumblebee
(i.e. 11.6 µg of a.s./bumblebee)

Dose of the reference item:
10.0 µg/bumblebee

Exposure duration:
48 hours

Test conditions: temperature: 24 - 25°C (required: 25 ± 2°C), relative air humidity: 59.0 – 61.0% (required: 60 ± 20%) (Appendix No. 6), place: a dark climate room.

Endpoints:
−bumblebee mortality after 48 hours of exposure,
−LD_{50} after 48 hours of exposure
−NOED after 48 hours of exposure

Statistical method: due to the lack of mortality statistical analysis was not performed

Results and discussions

Preliminary non-GLP test
After 4, 24 and 48 hours, there were no dead bumblebees in the control group.
The percentages of mortality of bumblebees exposed to the test item at the doses of 16.0, 80.0 and 400.0 µg/bumblebee after 4, 24 and 48 hours was 0.0% for all doses.
In the control group and all groups exposed to the test item (i.e. 16.0, 80.0 and 400.0 µg/bumblebee) no abnormal behavioral effects were observed during the preliminary non-GLP test.

Definitive test
Mortality of the treated bumblebees is presented in Tables 8 – 10.
After 4, 24 and 48 hours, there were no dead bumblebees in the control group (0.1% (*w/v*) water solution of surfactant, Tergitol 15-S-9).
The percentages of mortality after 4, 24 and 48 hours of exposure to the test item at the dose of 400.0 µg/bumblebee in 0.1% (*w/v*) surfactant Tergitol 15-S-9 was 0.0%. Due to the lack of mortality statistical analysis was not performed.
In the control group and the group exposed to the test item at the dose of 400.0 µg/bumblebee no abnormal behavioral effects were observed during the definitive test (Table 11).
The median lethal doses for the test item (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 400.0 µg of test item/bumblebee (i.e. > 11.6 µg of a.s./bumblebee).
The NOED value after 24 and 48 hours is higher than or equal to the dose used in the test, i.e. ≥ 400.0 µg of test item/ bumblebee (i.e. ≥ 11.6 µg of a.s./bumblebee).
Due to the lack of mortality during the definitive test after test item exposure, dose-effect curves showing the influence of the test item on mortality are not provided.
The percentages of mortality after 4, 24 and 48 hours of exposure to the reference item at the dose of 10.0 µg/bumblebee (with 0.1% (*w/v*) water solution of surfactant, Tergitol 15-S-9) were 70.0, 83.3 and 100.0%, respectively (Tables 8 - 10). In group treated with the reference item no sublethal effects (toxic symptoms) were observed (Table 11).
The mean weights of the bumblebees in each group were: 0.251 g for the control (0.1% (*w/v*) water solution of surfactant, Tergitol 15-S-9), 0.243 g for the group treated with the test item (with 0.1% (*w/v*) water solution of surfactant) and 0.241 g for the group treated with the reference item (with 0.1% (*w/v*) water solution of surfactant).
Summary of results for endpoints for the test item are presented in Table 13.

Table 8. Bumblebee mortality after 4 hours of exposure – definitive test

Dose [µg of test item/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control (0.0) (0.1% (*w/v*) surfactant)	50	0	**0.0**
400.0 (with 0.1% (*w/v*) surfactant)	50	0	**0.0**
Reference item: dimethoate			
10.0 (with 0.1% (*w/v*) surfactant)	30	21	**70.0**

Table 9. Bumblebee mortality after 24 hours of exposure and LD_{50}/24 h – definitive test

Dose [µg of test item/bumblebee]	Number of tested bumble-bees [no.]	Mortality		LD_{50}	
		Number of dead bumble-bees [no.]	[%]	[µg test item/ bumblebee]	[µg a.s./bumblebee]
Control (0.0) (0.1% (*w/v*) surfactant)	50	0	**0.0**	**> 400.0**	**> 11.6**
400.0 (with 0.1% (*w/v*) surfactant)	50	0	**0.0**		
NOED				**≥ 400.0**	**≥ 11.6**
Reference item: dimethoate					
10.0 (with 0.1% (*w/v*) surfactant)	30	25	**83.3**	–	

Table 10. Bumblebee mortality after 48 hours of exposure and LD_{50}/48 h – definitive test

Dose [µg of test item/bumblebee]	Number of tested bumble-bees [no.]	Mortality		LD_{50}	
		Number of dead bumble-bees [no.]	[%]	[µg test item/ bumblebee]	[µg a.s./bumblebee]
Control (0.0) (0.1% (*w/v*) surfactant)	50	0	**0.0**	**> 400.0**	**> 11.6**
400.0 (with 0.1% (*w/v*) surfactant)	50	0	**0.0**		
NOED				**≥ 400.0**	**≥ 11.6**
Reference item: dimethoate					
10.0 (with 0.1% (*w/v*) surfactant)	30	30	**100.0**	**–**	

The definitive test was performed between 09 – 11.10.2024

Table 11. Sublethal effects – definitive test

Dose [µg of test item/bumblebee]	Time of exposure [h]		
	4	24	48
	Number of bumblebees showing signs of toxicity */ number of living bumblebees		
Control (0.0) (0.1% (*w/v*) surfactant)	0/50	0/50	0/50
400.0 (with 0.1% (*w/v*) surfactant)	0/50	0/50	0/50
Reference item: dimethoate			
10.0 (with 0.1% (*w/v*) surfactant)	0/9	0/5	0/0

Table 13. Summary of results for endpoints for the test item

	Test item concentration [µg test item/bumblebee]	Aminopyralid concentration [µg a.s./bumblebee]
LD_{50} /48	**> 400.0**	**> 11.6**
NOED	**≥ 400.0**	**≥ 11.6**

Conclusion

The median lethal doses (LD_{50}/24 h, LD_{50}/48 h) are higher than the dose used in the test, i.e. > 400.0 µg of test item/bumblebee (i.e. > 11.6 µg of a.s./bumblebee).
NOED value after 24 and 48 hours is higher than or equal to the dose used in the test, i.e. ≥ 400.0 µg of test item/bumblebee (i.e. ≥ 11.6 µg of a.s./bumblebee).
Obtained results are summarized in Table 13.

Results obtained at the end of the definitive test (after 48 hours):

Dose		Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}	
Test item [µg test item/bumblebee]			[no.]	[%]	[µg test item/ bumblebee]	[µg a.s./bumblebee]
Control (0.0) (0.1% (*w/v*) surfactant)		50	0	0.0	> 400.0	> 11.6
400.0 (with 0.1% (*w/v*) surfactant)		50	0	0.0		
NOED					≥ 400.0	≥ 11.6
Reference item: dimethoate						
Dose [µg/bumblebee]	10.0 (with 0.1% (*w/v*) surfactant)	30	30	100.0	–	

Validity of the study

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%).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 9

Comments of zRMS:	The study was performed fully in line with OECD 245 with minor deviations. These deviations has no impact on the quality, integrity and final results of the study. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD50 for nominal dose > 90 µg of test item/bee/day (>2.6 µg a.s/bee/day), NOEDD for nominal dose ≥ 90 µg if test item/bee/day (≥ 2.6 µg a.s/bee/day), The following validity criteria were met during the test: - At the end of the experiment average mortality of the control groups was 2.0% (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 (0.017 µg/bee/day), after Abbott correction, was 51.7% (must be ≥ 50% on day 10 of exposure).

Reference:	KCP 10.3.1/03
Report	AMINO 30 SL Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, 2024; Dybek Marcin; Study Code: B-94-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL
content: 29.67 g/L of aminopyralid (CAS No: 150114-71-92)
batch no.: 1/24
production date: 07.2024
expiry date: not specified by the Sponsor

Biological test system:
species: the honeybee, Apis mellifera L.; strain: carnica, source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: freshly emerged worker honeybees (max. 2 days old) from the same queen-right colony

Experimental design:
−the test item:
number of concentrations: 1 and the control
number of replicates: 5
number of insects: 10 bees/replicate
−the reference item:
number of concentrations: 1
number of replicates: 3
number of insects: 10 bees/replicate
exposure duration: 10 days

Nominal concentration of the test item:
3000.0 mg/kg

Nominal dose of the test item:
90.0 µg/bee/day

Test item dietary dose:
114.9 µg/bee/day

Nominal concentration of the reference item (dimethoate):
0.8 mg/kg

Nominal dose of the reference item (dimethoate):
0.024 µg bee/day

Reference item dietary dose:
0.017 µg/bee/day

Test conditions:
temperature: 32.1 – 34.2°C;
relative humidity: 51.2 – 70.0%;

Statistical method:
due to the lack of mortality in group treated with the test item, statistical analysis was not performed

Endpoints:
honeybee mortality after 10 days of exposure.

Results and discussions

The definitive test design was chosen based on the preliminary range-finding, non-GLP test results.

Preliminary range-finding non-GLP test
Food intake during 10 days and the consumed (ingested) doses are presented in Table 6. Average consumption of a 50% sucrose solution in the control group was 40.5 mg/bee/day. Average consumption in the groups treated with the test item at the concentrations of 120.0, 600.0 and 3000.0 mg/kg (i.e. 3.6, 18.0 and 90.0 µg of test item/30 mg of diet, respectively) was 35.2, 34.2 and 39.7 mg/bee/day, respectively. On the basis of average consumption of treated 50% sucrose solution in the study groups, it may be concluded that each bee treated with the test item at the nominal concentrations of 120.0, 600.0 and 3000.0 mg/kg of diet ingested 4.2, 20.5 and 119.0 µg of the test item/day.

Table 6. Consumption of 50% sucrose solution after 10 days of exposure – preliminary non-GLP range - finding test

Nominal test item concentration/ dose		Ingested test item dose [µg of test item /bee/day]	Average consumption of a 50% sucrose solution[b] [mg of diet/bee/day]
[mg of test item/kg of diet]	[µg of test item /30 mg] [µg of test item /bee/day]		
Control			40.5
120.0	3.6	4.2	35.2
600.0	18.0	20.5	34.2
3000.0	90.0	119.0	39.7

[b]: food consumption on each day of exposure (µg/bee/day) was determined by weighing the feeders with a sucrose solution and dividing the amount of food by the number of surviving bees

After 10 days, no dead bees in the control group (50% sucrose solution) were observed. Mortality of the bees exposed to the test item at the concentrations of 120.0, 600.0 and 3000.0 mg/kg (i.e. 3.6, 18.0 and 90.0 µg of test item/30 mg of diet, respectively) was 0.0% for all doses.
During the preliminary non-GLP test, no behavioural abnormalities were observed in the groups treated with the test item.

Definitive test
Average consumption of a 50% sucrose solution in the control group was 40.8 mg of diet/bee/day. Aver-

age consumption in the group treated with the test item at the concentration of 3000.0 mg/kg (i.e. 90.0 µg of test item/30 mg of diet) was 38.3 mg of treated diet/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg (i.e. 0.024 µg of reference item/30 mg of diet) was 20.7 mg of treated diet /bee/day (Table 9).

Table 9. Consumption of 50% sucrose solution after 10 days of exposure – definitive test

Nominal test item concentration/ dose		Ingested test item dose [µg/bee/ day]	Consumption of a 50% sucrose solution[c] [mg/bee/day]					
[mg/kg of diet]	[µg/bee/ day] [µg/30 mg/ day]		replicates					Mean
			I	II	III	IV	V	
AMINO 30 SL								
Control			43.0	40.7	38.2	42.4	39.8	**40.8**
3000.0	**90.0**	**114.9**	36.3	38.3	36.2	42.5	38.2	**38.3**
Dimethoate (reference item)								
0.8	**0.024**	**0.017**	21.7	20.0	20.5	-	-	**20.7**

[c]: food consumption on each day of exposure (mg/bee/day) was determined by weighing the feeders with a sucrose solution and dividing the amount of food by the number of surviving bees subtracting evaporation

On the basis of average consumption of a 50% sucrose solution in the study groups, it may be concluded that each bee treated with the test item at the nominal concentration of 3000.0 mg/kg (i.e. 90.0 µg of test item/30 mg of diet) ingested 114.9 µg of test item/day. In the group treated with the reference item, at the concentration of 0.8 mg/kg (i.e. 0.024 µg/30 mg) each bee ingested 0.017 µg of reference item/day (Table 8). All doses consumed by the bees were calculated taking into account the mean evaporation value (Table 10.

Table 8. Honeybee mortality and the LC_{50} and LDD_{50}/10 d – definitive test

Nominal test item concentration/ dose		Ingested[a] test item dose [µg/bee/ day]	Number of tested bees [no]	Total mortality			LC_{50} [mg/kg] LDD_{50} [µg/bee/day]	
[mg/kg of diet]	[µg/bee/ day] [µg of test item/30 mg/ day]			No.	[%]	[%][b]		
AMINO 30 SL								
Control			50	1	2.0	-	**> 3000.0**	**> 114.9**
3000.0	**90.0**	**114.9**	50	0	0.0	**(-2.0)**		
Dimethoate (reference item)								
0.8	**0.024**	**0.017**	30	16	53.3	**51.7**	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 according to the Abbott formula [7]

The exposure was performed between 23.08 – 02.09.2024

Table 10. Evaporation of the 50% sucrose solution during 10 days of exposure – definitive test

Day of exposure	Evaporation [g/day]			Mean evaporation [g/day]
	replicates			
	I	II	III	
1	0.032	0.039	0.032	**0.034**
2	0.030	0.033	0.028	**0.030**
3	0.035	0.034	0.032	**0.034**
4	0.033	0.032	0.029	**0.031**
5	0.035	0.036	0.035	**0.035**
6	0.031	0.032	0.026	**0.030**
7	0.035	0.036	0.025	**0.032**
8	0.029	0.026	0.030	**0.028**
9	0.028	0.032	0.030	**0.030**
10	0.030	0.035	0.035	**0.033**

Mortality in the control was 2.0% after 10 days of exposure. The percentage of mortality of the honeybees exposed to the test item, at the concentration of 3000.0 mg/kg (dietary dose 114.9 µg of test item/bee/day) at exposure termination (after 10 days), corrected according to the formula of Abbott, was (-2.0)% (Table 8). The negative value indicates that mortality in the group treated with the test item was lower than in the control group. Due to the lack of mortality in the group treated with the test item, statistical analysis was not performed [SOP/B/64]. The obtained results allow to define that the LC_{50} is higher than 3000.0 mg/kg, and the LDD_{50} value is higher than 114.9 µg of test item/bee/day.

The mortality of the bees treated with the reference item at the concentration of 0.8 mg/kg (dietary dose of 0.017 µg of reference item/bee/day) at the exposure termination (on day 10), corrected according to the formula of Abbott, was 51.7% (Table 8). The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

In the definitive test no sublethal effects were observed in the group treated with the test item. In the group treated with the reference item sublethal effects were observed (Table 12).

Table 12. Behavioural effects on honeybees – definitive test

Nominal test item dose [µg/bee/day] [µg/30 mg/ day]	Time of exposure / replicate	after 1 day	after 2 days	after 3 days	after 4 days	after 5 days	after 6 days	after 7 days	after 8 days	after 9 days	after 10 days
		Number of bees with toxic symptoms*/number of living bees									
0.0 (Control)	I	0/10	0/10	0/10	0/10	0/10	0/10	0/9	0/9	0/9	0/9
	II	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	III	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	IV	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	V	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
AMINO 30 SL											
90.0	I	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	II	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	III	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	IV	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	V	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Dimethoate (reference item)											
0.024	I	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/9	0/9	0/5
	II	0/10	0/10	0/10	0/10	0/10	0/9	0/9	0/8	0/6	0/3
	III	0/10	0/10	0/10	0/10	0/10	0/9	0/9	0/7	1ap/6	6ap/6

*Bees with sublethal toxicity effects are classified according to the following criteria:
a – affected
ap – apathy
c – cramps
m – moribund
v - vomiting

Conclusion

The validity criterion concerning mortality was met, because mortality in the control was 2.0% after 10 days of exposure.
The percentage of mortality of the honeybees exposed to the test item, at the concentration of 3000.0 mg/kg (nominal dose 90.0 µg of test item/bee/day, dietary dose 114.9 µg of test item/bee/day) at exposure termination (after 10 days), corrected according to the formula of Abbott, was (–2.0)%. The negative value indicates that mortality in the group treated with the test item was lower than in the control group.
On the basis of the obtained mortality results the LC_{50} is higher than 3000.0 mg/kg, the LDD_{50} value for nominal dose is higher than 90.0 µg of test item/bee/day (i.e. higher than 2.6 µg of a.s./bee/day) and the LDD_{50} value for dietary dose is higher than 114.9 µg of test item/bee/day (i.e. higher than 3.3 µg of a.s./bee/day). Due to the lack of mortality in group treated with solution of the test item, statistical analysis was not performed. The NOEC value is higher than or equal to 3000.0 mg/kg and the calculated NOEDD value for nominal dose is higher than or equal to 90.0 µg/bee/day. The NOEDD value for dietary dose is higher than or equal to 114.9 µg/bee/day.
The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality, after Abbott correction, was equal to 51.7% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

The effects of AMINO 30 SL on mortality of honeybees are summarized below:

Nominal test item concentration/dose		Ingested[a] dose	Number of tested bees	Total mortality			LC_{50}	LDD_{50}	LDD_{50}
[mg of test item/kg of diet]	[µg of test item/30 mg of diet/day] [µg of test item/bee/day]	[µg of test item/bee/day]	[no]	No.	[%]	Corr[b] [%]	[mg of test item /kg of diet]	[µg/bee/day] for nominal dose	[µg of test item/bee/day] for ingested dose
AMINO 30 SL									
0.0 (Control)			50	1	**2.0**	-	**> 3000.0**	**> 90.0** (i.e. > 2.6 a.s/bee/day)	**> 114.9** (i.e. > 3.3 a.s/bee/day)
3000.0	**90.0**	**114.9**	50	0	**0.0**	**(-2.0)**			
NOEC [mg/kg]			**≥ 3000.0**						
NOEDD [µg/bee/day] for nominal dose			**≥ 90.0** (i.e. ≥ 2.6 a.s/bee/day)						
NOEDD [µg/bee/day] for ingested dose			**≥ 114.9** (i.e. ≥ 3.3 a.s/bee/day)						
Dimethoate (reference item)									
0.8	**0.024**	**0.017**	30	16	**53.3**	**51.7**	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 according to the Abbott formula [7]

Test validity criteria

The following validity criteria were met during the test:
-At the end of the experiment average mortality of the control groups was 2.0% (criterion: it must not exceed 15%) [1].
-After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.017 µg/bee/day), after Abbott correction [7], was 51.7% (criterion: it must be ≥ 50% on day 10 of exposure).

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

A 2.3.1.3.1 Study 10

Comments of zRMS:	The study was performed fully in line with OECD 239 with minor deviations. These deviations has no impact on the quality, integrity and final results of the study. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: NOED = 33.33 µg if test item/bee/day

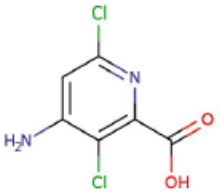
	The study met the validity criteria : - larval mortality in control I on days 3-8 was 8.33% in replicate 1, 2 and 3, and in control II it was 8.33% in replicate 1, 2 and 3 (required: ≤15%), - the adults emergence rate in control I on day 22 was 83.33% in replicate 1, 2 and in 3, and in control II it was 83.33% in replicate 1, 2 and in 3 (required: ≥70%), - for fenoxycarb as reference item, the emergence rate was 0.00% in replicate 1, 2 and in 3 (required: ≤20%).

Reference:	KCP 10.3.1/04
Report	Honey bee larval toxicity test following repeated exposure of the test item AMINO 30 SL according to OECD GD 239 ENV/JM/MONO(2016)34, 2024; Niśkiewicz, M.; Study Code: 0038/0215/E; SORBOLAB Research Laboratory LLC; Zaniemyska Street 11 61-029 Poznań, Poland GLP, Unpublished
Guideline(s):	according to the OECD GD 239 ENV/JM/MONO(2016)34
Deviations:	Yes Deviations from the Study plan and OECD GD 239 ENV/JM/MONO(2016)34 were found: 1. During definitive test the decrease in temperature during larval stage (to min. 33.1°C), pre-pupal stage (to min. 33.1°C) and pupae/imago stage (to 33.8°C) were observed. Additionally, during larval stage decrease in humidity (to 39.0% RH) and during pre-pupae stage (to 69.3% RH) were observed. Increase in humidity during pre-pupae stage (to 99.9% RH) and during pupae/imago stage (to 83.8% RH) were also observed. Requirements: temperature: 34.5±0.5°C, humidity: larvae 95±5% RH, pre-pupae 80±5% RH, pupae/imago 50-80% RH. 2. During range-finding test decrease in temperature during larval stage (to min. 33.8°C). Additionally, during larval stage decrease in humidity (to min. 58.0%RH) was observed. During pre-pupae stage decrease in humidity (to min. 69.1% RH) was observed. Requirements: temperature: 34.5±0.5°C, humidity: larvae 95±5% RH, pre-pupae 80±5% RH, pupae/imago 50-80% RH. The above deviations did not affect the test result. The study met the validity criteria.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

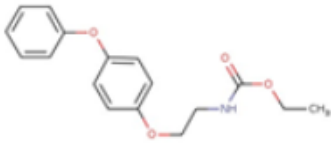
Test item
Name- AMINO 30 SL
Type of packaging material- HDPE
Appearance- liquid
Date of receipt- 30.07.2024
Batch No.- 1/24
Production date- 07.2024
Expiry date- not available

Name of the active substance- Aminopyralid
IUPAC name of the active substance- 2-Pyridinecarboxylic acid, 4-amino-3,6-dichloro-
Content of the active substance- 30 g/kg
CAS of the active substance- 150114-71-9
Molecular weight of the active substance- 207.01 g/mol



Molecular structure of the active substance
Molecular formula of the active substance- $C_6H_4Cl_2N_2O_2$
Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion - soluble in water
The storage conditions - temperature: 10-30°C
Reference number - LBS/58/24
Certificate of analysis - Appendix 1

Reference item
Name - Fenoxycarb
CAS - 72490-01-8
IUPAC nomenclature - [2-(4-phenoxyphenoxy)ethyl]-carbamic acid ethyl ester
Molecular weight - 301.34 g/mol



Structural formula
Molecular formula - $C_{17}H_{19}NO_4$
Producer - Sigma-Aldrich
Batch no.- BCCG3127
Purity - 99.2% (w/w)
Expiration date - 30.06.2026
Certificate of analysis - Appendix 2

Test species
The test organisms were the honey bees Apis mellifera carnica from the breeding of Mr Wiesław Londzin, registered in the District Veterinary Office in Bielsko-Biała under the identification number 240252110. The insects were not treated with chemicals during the month before the start of the test. In the study 1 - day first instar larvae of honey bees originated from 3 different, healthy, well-maintained breedings (36 larvae i.e. 12 larvae from each colony) were used. The physiological condition of the bees was checked on the basis of study with reference item.

Preparation of the organisms
Three days before the start of the experiment, queens in each colony were isolated using a one-frame insulator. After 30 hours, the queens were released from the isolator (after checking for the presence of freshly laid eggs). The frames containing the eggs remained in the isolation cage adjacent to the frame with freshly hatched larvae until the larvae hatch. On day 1, the frames with freshly hatched larvae were transferred from the hive to the laboratory in an insulated container in order to avoid temperature variation, and maintained at temperature 34.5°C. The frames were placed under clean conditions for grafting which was performed on a warming plate at temperature 34.5°C. Larvae that have not yet acquired a C-shape or that laying on a royal jelly layer were selected for the test. They were gently placed in the same position on the bottom of a well of a culture plate filled with diet A. To ensure the minimumnumber of larvae (36 larvae, i.e. 12 larvae from each of 3 colonies) needed to commence exposure to the test item on

day 3, larvae were collected in excess (48 larvae, i.e.16 larvae from 3 colonies).

Course of the study
The test was carried out based on the OECD GD 239 ENV/JM/MONO(2016)34 and in accordance with the SPB-E/53 procedure. The larvae were carefully placed in the same position at the bottom of queen-cell cup filled with diet A placed in breeding plate's well (Wuxi NEST Biotechnology Co., Ltd.). Plates were placed in desiccators (Laboplay, series E13PP), in which, during 1-8 days, humidity was maintained on level of 95±5% using saturated solution of K_2SO_4 placed in the dish at the bottom of desiccator. On day 8, larvae plates were placed in the desiccator, in which humidity was maintained at the level of 80±5% with saturated solution of NaCl placed ate the bottom of desiccator. On day 15, plates were transferred to plastic cages (18.0 cm × 12.0 cm × 7.5 cm) with ad libitum access to 50% (w/v) sucrose solution and pine pollen. Cages were placed in test room of humidity 50-80% maintained with humidifier.

Preparation of test item and reference item for the test
The appropriate weight of the test item was dissolved in deionized water. A series of dilutions of the test solutions were prepared from the solution obtained. The reference item was selected based on the mode of action of the test item during range-finding test. The appropriate weight of reference item – fenoxycarb was dissolved in 2.5% (v/v) of acetone in deionized water and next dissolved in deionized water to obtain solution of fenoxycarb in 0.5% (v/v) of acetone. The final concentrations of the test item or reference item were prepared by addition of the appropriate volume of the stock solution to subsequent batches of diet (larval diets).

Solubility test
Solubility test will not be performed. According to the information provided by the Sponsor the test item is water soluble.

Stability test
The stability of the test item under storage conditions was determined (temperature 6±2°C, darkness). Due to the highly complex composition of the diet for the larvae (royal jelly), the stability of the test item in the diet was not tested under the experimental conditions. Based on the results, test item was found to be stable for 72 h in storage conditions.

Preparation of diet
Larval diet was disposed depending on the developmental stage (all solutions were prepared in weight percentage). The diet was freshly prepared each day and warmed in room in temperature 34.5°C for 30 min. before administration. Larvae were fed on warming plate at 34.5°C. The diet was dispensed using an automatic pipette with care to avoid touching the larvae and drowning them into the diet.

Range-finding test
The range-finding test was performed to determine the number and range of doses of the test item to be used in the definitive test.

Definitive test
In definitive test, test item was administrated in larvae diet in proper concentration during 4-days exposition. Mortality and behavior changes were observed during the test. As reference substance the fenoxycarb was used. The mode of action of the test item during the range-finding test suggested an effect on pupae, therefore fenoxycarb was used as the reference item during the definitive test.

Test design
The definitive test was performed using a range of the concentrations of the test item and controls. The test design is presented in Table 3.

Table 3. Concentrations of the test item and reference item - definitive test

Test item		
Dose [µg of the test item/ larva]	**Concentration [mg of the test item/kg of diet]**	**Test item stock solution*) [g of the test item/L of deionized water solution]**
0.00	0.00	0.00 – Control I
1.23	8.02	0.62
3.70	24.07	1.85
11.11	72.22	5.56
33.33	216.67	16.67
100.00	650.00	50.00
Reference item		
Dose [µg of the reference item/larva]	**Concentration [mg of reference item/kg of diet]**	**Reference item stock solution**) [g of the reference item/L of 0.5% (v/v) acetone solution]**
0.00000	0.00	0.0000 – Control II
0.04928	0.32	0.0352

*) The volume of the test item stock solution mixed into the diet was 1.43% (v/v) of the final volume of diet.
**) The volume of the reference item stock solution mixed into the diet was 1.00% (v/v) of the final volume of diet.

Test conditions

The definitive test was conducted in the following conditions:
- Time of exposure: 4 days.
- Number of bee larvae in one plate: 36 (12 larvae from 3 colonies).
- Test vessels: up to day 15 – 48-well culture plates with queen cells, placed in a desiccator and incubator; after the 15th day – transparent plastic boxes (18.0 cm × 12.0 cm × 7.5 cm), placed in the test room.
- Temperature and humidity summary is presented in Table 12.

Table 12. Temperature and humidity summary – definitive test

Honey bee development stage	Temperature[1] [°C]			Humidity[1] [%RH]		
	Average	Minimum	Maximum	Average	Minimum	Maximum
Larvae (day 1-8)	34.3	33.1	34.6	98.8	39.0	99.9
Pre-pupae (day 8-15)	34.2	33.1	34.4	85.7	69.3	99.9
Pupae/imago (day 15-22)	34.2	33.8	34.4	76.9	64.7	83.8

- Lightning: darkness (excluding observations).
- Feeding: diet according to Table 2.

Table 2. Diet application scheme

Place	In hive		In laboratory							
Stage	Eggs		Larval stage						Pre-pupal stage	Pupal/imago stage
Time [day]	-3 to -2	-2 to 0	1	2	3	4	5	6	8-15	15-22
Description/diet	egg laying	incubation	diet A*	none	diet B** + solution of test item	diet C*** + solution of test item			none	50.00% (w/v) aqueous solution of sucrose and pine pollen
Volume of diet [µL/larva]	none		20.00	none	20.00	30.00	40.00	50.00	none	*ad libitum*

* Diet A: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose, 12% weight of fructose.
** Diet B: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose, 15% weight of fructose.
*** Diet C: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose, 18% weight of fructose.

Results and discussions

During definitive test, no statistically significant effect on larval mortality was observed on day 8 at any tested concentrations.
The test item showed statistically significant effect on pupal mortality on days 15 and 22 at concentration 650.00 mg test item/kg of diet.
For emerged adults, a statistically significant effect was observed at concentration 650.00 mg test item /kg of diet.
The final results of the study are presented in Table 1.

Table 1. Final results of the study

Larval mortality results				
Concentration [mg of test item/kg of diet]	Time [day]			
	8			
	Mortality [%]	Statistical significance*)	LOEC	NOEC
			[mg of test item/kg of diet]	
Control I	8.33	not applicable	>650.00	≥650.00
8.02	11.11	-		
24.07	13.89	-		
72.22	13.89	-		
216.67	16.67	-		
650.00	19.44	-		

- statistically insignificant
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowest test item concentration causing statistically significant differences in relations to the control
*) values calculated using ToxRat Professional using multiple sequentially-rejective Fisher test after Bonferroni-Holm with significance level p=0.05

Pupal mortality results						
Concentration [mg of test item/kg of diet]	Time [day]					
	15		22			
	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	LOEC	NOEC
					[mg of test item/kg of diet]	
Control I	9.09	not applicable	9.09	not applicable	650.00	216.67
8.02	15.63	-	18.75	-		
24.07	19.35	-	22.58	-		
72.22	25.81	-	25.81	-		
216.67	26.67	-	26.67	-		
650.00	37.93	+	37.93	+		

- statistically insignificant
+ statistically significant
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowest test item concentration causing statistically significant differences in relations to the control
*) values calculated using ToxRat Professional using Chi2 2x2 table test with Bonferroni correction with significance level p=0.05

Emergence results							
Concentration [mg of test item/kg of diet]	Dose [µg of test item/larva]	Time [day]					
		22					
		Mortality [%]	Statistical significance*)	LOED	NOED	LOEC	NOEC
				[µg of test item/larva]		[mg of test item/kg of diet]	
Control I	Control I	16.67	not applicable	>100.00**)	33.33	>50.00**)	216.67
8.02	1.23	27.78	-				
24.07	3.70	33.33	-				
72.22	11.11	36.11	-				
216.67	33.33	38.89	-				
650.00	100.00	50.00	+				

- statistically insignificant
+ statistically significant
*) values calculated using ToxRat Professional using Chi2 2x2 table test with Bonferroni correction with significance level p=0.05
**) values determined based on the analysis of the results
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowest test item concentration causing statistically significant differences in relations to the control
NOED the highest test item dose not causing statistically significant differences in relations to the control
LOED the lowest test item dose causing statistically significant differences in relations to the control

Emergence results			
Parameter	Concentration [mg of test item/kg of diet]	Parameter	Dose [µg of test item/larva]
EC_{10}	2.90	ED_{10}	0.45
EC_{20}	34.59	ED_{20}	5.32
EC_{50}	n.d.	ED_{50}	n.d.

n.d. not determined
EC_{10} test item concentration causing effect on 10% population
EC_{20} test item concentration causing effect on 20% population
EC_{50} test item concentration causing effect on 50% population
ED_{10} test item dose causing effect on 10% population
ED_{20} test item dose causing effect on 20% population
ED_{50} test item dose causing effect on 50% population

Emergence results of reference item treatment		
Concentration [mg of the reference item/kg of diet]	Time [day]	
	22	
	Mortality [%]	Statistical significance
Control II	16.67	-*)
0.32	100.00	+**)

- statistically insignificant
+ statistically significant
*) values calculated using ToxRat Professional using Fisher's exact binomial test at significance level p=0.05 with comparison to Control I
**) values calculated using ToxRat Professional using Fisher's exact binomial test at significance level p=0.05 with comparison to Control II

Conclusion

During definitive test, no statistically significant effect on larval mortality was observed on day 8 at any tested concentrations.
There was statistically significant effect on the mortality of pupae observed on day 15 and 22 at concentrations 650.00 mg test item/kg of diet.
For emerged adults, statistically significant effect was observed at concentration 650.00 mg test item/kg of diet.
On the 8th day of the experiment, the observations of presence of diet not consumed or consumed by the larvae were performed. The diet was ingested entirely by the larvae at concentration 8.02 mg test item/kg of diet and at control I. The diet was not ingested entirely by 3.33% larvae at concentration 24.07 mg test item/kg of diet, 10.71% larvae at concentration 72.22 mg test item/kg of diet, 7.14% larvae at concentration 216.67 mg test item/kg of diet and by 25.00% larvae at concentration 650.00 mg test item/kg of diet.
As a result of exposition to reference item, the emergence rate observed on day 22 was 0.00% (required: ≤20%). The obtained results are in accordance with the requirements of the OECD GD 239 ENV/JM/MONO(2016)34 i.e. mortality ≤20% and confirm the correct reaction of the test system.

Test validity criteria

The study met the validity criteria (acc. to OECD GD 239 ENV/JM/MONO(2016)34):
- larval mortality in control I on days 3-8 was 8.33% in replicate 1, 2 and 3, and in control II it was 8.33% in replicate 1, 2 and 3 (required: ≤15%),
- the adults emergence rate in control I on day 22 was 83.33% in replicate 1, 2 and in 3,
and in control II it was 83.33% in replicate 1, 2 and in 3 (required: ≥70%),
- for fenoxycarb as reference item, the emergence rate was 0.00% in replicate 1, 2 and in 3 (required: ≤20%).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional studies were performed.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional studies were performed.

A 2.3.1.6 KCP 10.3.2 Non target arthropods studies

A 2.3.1.6.1 Study 11

Comments of zRMS:	The study was conducted according to the method Vogt H. et al., (2000). In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. Deviations have no impact on the quality, integrity and final results of the study. Validity criteria were met: - pre-imaginal mortality of the control group was 20.0% (criterion: a maximum of 20.0%), - mortality of the reference item group, after Abbott's correction, was 100.0% (criterion: a minimum of 50%), - the mean number of eggs per female per day in the control group (fecundity) was 15.9 (criterion: ≥ 15.0), - the mean hatching rate in the control group (fertility) was 71.4 (criterion: ≥ 70%). The study is acceptable and suitable for the use in the risk assessment. LR_{50}> 0.300 L product/ha NOERmortality ≥ 300 L/ha

Reference:	KCP 10.3.2/01
Report	An extended laboratory test for evaluating the effects of AMINO 30 SL on the green lacewing, *Chrysoperla carnea*, 2024; Dybek Marcin; Study Code: B-93-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Vogt H. et al., 2000)
Deviations:	Yes In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/62. This deviation has no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
Content:
29.67 g/L of aminopyralid
(CAS No: 150114-71-92)
Batch number:
1/24
Production date:
07.2024
Expiry date:
not determined by the Sponsor

Biological test system:
the green lacewing, Chrysoperla carnea (Steph.), Neuroptera: Chrysopidae
– age:
first instars' larvae (2 days old)
– source:
a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from a commercial breeder (Katz Biotech AG, Germany), [SOP/B/78]

Experimental design:
5 study groups:
−a control group (0.0 L/ha)
−AMINO 30 SL at the rates of:
−0.075 L/ha
−0.150 L/ha
−0.300 L/ha
−dimethoate at the rate of 15.0 g/ha
number of replicates: 30 replicates/group
number of larvae: 1 larva of Chrysoperla carnea /replicate

Test conditions:
– temperature: 23.0 − 27.0°C
– relative air humidity: 60.0 − 78.4%
– photoperiod: 16 hours light : 8 hours dark
– light intensity 1704 lux

Statistical analysis:
Probit analysis using linear max. likelihood regression,
Chi2 2x2 Table Test with Bonferroni Correction

Endpoints:
–cumulative mortality of larvae, pupae, and adults after emergence
–LR_{50} value
–reproduction of the lacewings: −fecundity (mean number of eggs/female/day), −fertility (mean hatching rate)

Results and discussions

Mortality of *Chrysoperla carnea*
Mortality of the lacewings recorded in the definitive test is presented in Table 2. In the definitive test mortality of the control group was 20.0%. The percentages of mortality of the green lacewings exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha of the test item, after Abbott's correction, were

20.8, 12.5 and 25.0% respectively.
There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha in comparison to the control group (Chi2 2x2 Table Test with Bonferroni Correction, p(z) > Alpha*sign, Alpha=0.05).
On the basis of the obtained results it can be concluded that the LR_{50} value is higher than 0.300 L/ha of test item and the $NOER_{mortality}$ value is higher than or equal to 0.300 L/ha of test item (Table 2).
The percentage of mortality of *Chrysoperla carnea* exposed to dimethoate at rate of 15.0 g/ha, after Abbott's correction, was 100.0%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate (Table 2).

Table 2. Mortality of *Chrysoperla carnea* – definitive test

Study group [L/ha]	Number of tested green lacewings [no.]	Mortality					
		Number of dead individuals [no.]			Total		
		larvae	pupae	adults	[no.]	[%]	Corr. [%][a]
Test item: AMINO 30 SL							
Control	30	4	2	0	6	**20.0**	-
0.075	30	6	4	1	11	**36.7**	**20.8**
0.150	30	6	3	0	9	**30.0**	**12.5**
0.300	30	7	2	3	12	**40.0**	**25.0**
LR_{50}		**> 0.300 [L/ha]**					
$NOER_{mortality}$		**≥ 0.300 [L/ha]**					
[g/ha]	**Reference item: dimethoate**						
15.0	30	29	1	0	30	**100.0**	**100.0**

[a]: Mortality corrected according to the Abbott's correction [9]

The definitive test was performed between: 18.09 – 06.11.2024 (mortality: 18.09 – 18.10.2024 for control group and groups with the test item, 18.09 – 11.10.2024 for group with the reference item).

Reproductive performance

Reproduction of the lacewings from the control group and the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha was assessed, since the mortality were < 50%. Deformed specimens are counted as living, but they were excluded from the reproductive performance. Sex of the lacewings at the pre-oviposition period is presented in Table 4, whereas the results of the reproduction test are presented in Table 5. The mean number of eggs/female/day in the control group was equal to 15.9 (criterion: ≥ 15.0). The mean numbers of eggs/female/day in the groups treated with test item at the rates of 0.075, 0.150 and 0.300 L/ha were 15.4, 17.5 and 16.0, respectively. The mean hatching rate in the control group was 71.4% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha were 78.9, 83.2 and 76.7% (Table 5).
Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha were (-10.5), (-16.5) and (-7.5)%, respectively (Table 5). The negative value indicates that hatching rate in the group treated with the test item was higher than in the control group.
Based on the results it can be stated that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on mortality of the tested organisms. The test item at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on the mean number of layed eggs by green lacewings and no adverse effect on mean hatching rate at all tested rates, i.e. 0.075, 0.150 and 0.300 L/ha.

Table 4. Sex of the green lacewings *Chrysoperla carnea* – pre-oviposition period

Study group [L/ha]	Number of males and females [no.]*		Number of tested insects [no.]
	♀	♂	
Control	12	12	24
0.075	12	7	19
0.150	11	9	20
0.300	11	6	17

*: adults with deformities were not included in the reproduction assessment

Table 5. Reproduction of *Chrysoperla carnea*

Study group/ application rate [L/ha]	DAT	Number of [no.]							Mean hatching rate (Fertility) [%]	Fecundity reduction relative to the control (Pr) [%][r]
		Eggs (total)	Viability females ♀	Eggs/female /day	Mean	Eggs used for hatching assessment	Hatched larvae	Hatching rate [%]		
Control	**38**	162	10	16.2	**15.9**	85	60	70.6	**71.4**	–
	43	156	10	15.6		104	75	72.1		
0.075	**38**	184	12	15.3	**15.4**	82	61	74.4	**78.9**	**(-10.5)[b]**
	43	171	11	15.5		126	105	83.3		
0.150	**38**	188	11	17.1	**17.5**	113	86	76.1	**83.2**	**(-16.5)[b]**
	43	161	9	17.9		102	92	90.2		
0.300	**38**	177	11	16.1	**16.0**	79	64	81.0	**76.7**	**(-7.5)[b]**
	43	143	9	15.9		98	71	72.4		

DAT: day after the treatment
r: The percentage of reproduction reduction (Pr) was calculated according to equation no. 1 (5.2. Reproductive performance),
b: The negative value indicates that hatching rate in the group treated with the test item was higher than in the control group

Conclusion

The effects of the test item, AMINO 30 SL on mortality and reproductive capacity the green lacewings, *Chrysoperla carnea* in the laboratory test are summarized below.

Study group	Parameters (endpoints)						
	Mortality				Reproduction		
Test item [L/ha]	[no.]	[%]	Corr. [%][a]	LR_{50} [L/ha]	Mean number of eggs/female/day [no.]	Mean hatching rate [%]	Reduction [%]
Test item: AMINO 30 SL							
Control (0.0)	6	20.0	-	> 0.300	15.9	71.4	–
0.075	11	36.7	20.8		15.4	78.9	(-10.5)[b]
0.150	9	30.0	12.5		17.5	83.2	(-16.5)[b]
0.300	12	40.0	25.0		16.0	76.7	(-7.5)[b]
$NOER_{mortality}$	≥ 0.300 [L test item/ha]						
Reference item: dimethoate							
[g/ha]	[no.]	[%]	Corr. [%][a]		Reproduction		
15.0	30	100.0	100.0		not assessed		

[a]: Mortality corrected according to the Abbott's correction [9]
[b]: The negative value indicates that hatching rate in the group treated with the test item was higher than in the control group

The validity criterion concerning mortality was met, because mortality of the green lacewings, *Chrysoperla carnea* in the control group was 20.0%. The percentages of mortality of the green lacewings exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha of the test item, after Abbott's correction, were 20.8, 12.5 and 25.0% respectively.
There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha in comparison to the control group (Chi2 2x2 Table Test with Bonferroni Correction, p(z) > Alpha*sign, Alpha = 0.05).
On the basis of the obtained results it can be concluded that the LR_{50} value is higher than 0.300 L/ha. The $NOER_{mortality}$ value is higher than or equal to 0.300 L/ha.
The percentage of mortality of *Chrysoperla carnea* exposed to dimethoate at rate of 15.0 g/ha, after Abbott's correction, was 100.0%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.
The mean number of eggs/female/day in the control group was equal to 15.9 (criterion: ≥ 15.0). The mean numbers of eggs/female/day in the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha were equal to 15.4, 17.5 and 16.0, respectively. The mean hatching rate in the control group was 71.4% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of

0.075, 0.150 and 0.300 L/ha were 78.9, 83.2 and 76.7%, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates 0.075, 0.150 and 0.300 L/ha were (-10.5), (-16.5) and (-7.5)%, respectively. The negative value indicates that hatching rate in the group treated with the test item was higher than in the control group.
Based on the results it can be stated that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on mortality of the tested organisms. The test item at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on the mean number of layed eggs by green lacewings and no adverse effect on mean hatching rate at all tested rates, i.e. 0.075, 0.150 and 0.300 L/ha.

Test validity criteria
The following validity criteria were met during the study [4]:
-pre-imaginal mortality of the control group was 20.0% (criterion: a maximum of 20.0%),
-mortality of the reference item group, after Abbott's correction [9], was 100.0% (criterion: a minimum of 50%),
-the mean number of eggs per female per day in the control group (fecundity) was 15.9 (criterion: ≥ 15.0),
-the mean hatching rate in the control group (fertility) was 71.4 (criterion: ≥ 70%).

A 2.3.1.6.2 Study 12

Comments of zRMS:	The study was conducted according to the method Schmuck et al., (2000). In the experimental part of the study a deviation from the guidelines devel-oped by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This deviation has no impact on the quality, integrity and final results of the study. Validity criteria were met: - pre-imaginal mortality of the control group was 17.5% (criterion: ≤ 30.0%), - mean corrected mortality of the reference item group was 81.8% (criterion: a minimum of 40%), - fertility (the mean number of fertile eggs/female/day) in the control group was 5.4 (criterion: ≥ 2 fertile eggs/female). - The study is acceptable and suitable for the use in the risk assessment. LR_{50}> 0.300 L product/ha $NOER_{mortality}$ ≥ 300 L/ha

Reference:	KCP 10.3.2/02
Report	An extended laboratory test for evaluating the effects of AMINO 30 SL on the ladybird beetle, *Coccinella septempunctata* (L.), 2024; Dybek Marcin; Study Code: B-90-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Schmuck et al., 2000)
Deviations:	Yes In the experimental part of the study a deviation from the guidelines devel-

	oped by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/63. This deviation has no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL1
Content:
29.67 g/L of aminopyralid
(CAS No: 150114-71-92)
Batch number:
1/24
Manufacturing date:
07.2024
Expiry date:
not determined by the Sponsor

Biological test system:
the ladybird beetle, C. septempunctata L. (Arthropoda: Coccinellidae)
– age: 5-day-old larvae
– source: a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna according to the SOP/B/77.

Experimental design:
5 study groups:
−a control group (0.0 L/ha)
−AMINO 30 SL at the rates of:
-the test item at the rate of 0.075 L/ha
-the test item at the rate of 0.150 L/ha
-the test item at the rate of 0.300 L/ha
−pyrazophos at the rate of 20.0 g/ha
number of replicates: 40 replicates/group
number of larvae: 1 larva of Coccinella septempunctata /replicate

Test conditions:
– temperature: 23.0 – 27.0°C
– relative air humidity: 60.0 − 78.0%
– photoperiod: 16 hours light : 8 hours dark
– light intensity 1704 lx

Statistical analysis:
probit analysis using linear max. likelihood regression, Chi2 2x2 Table Test with Bonferroni Correction

Endpoints:
–preimaginal mortality of the ladybird beetles
–LR_{50}

–NOERmortality
–reproductive performance of the moulted beetles (the mean number of fertile eggs/female/day) reproduction reduction (Pr)

Results and discussions

Mortality of *Coccinella septempunctata*
In the definitive test mortality in the control group was 17.5%. The mortality of the ladybird beetles exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha, after Abbott correction, were 6.1, 12.1 and 24.2%, respectively (Table 2).
At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha of AMINO 30 SL and the control group (Chi2 2x2 Table Test with Bonferroni Correction p(z) > Alpha*).
The LR_{50} value is higher than 0.300 L/ha of AMINO 30 SL. The $NOER_{mortality}$ is higher than or equal to 0.300 L/ha of AMINO 30 SL (Table 2).
The mortality of the ladybird beetles exposed to the reference item at the rate of 20.0 g of pyrazophos/ha, after Abbott correction, was equal to 81.8%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to pyrazophos (Table 2).

Table 2. Mortality of *C. septempunctata* – definitive test

Study group [L/ha]	Number of tested beetles [no.]	Mortality					
		Number of dead individuals [no.]			Total		
		larvae	pupae	adults	[no.]	[%]	Corr. [%][a]
Control	40	5	2	0	7	17.5	-
0.075	40	5	4	0	9	22.5	**6.1**
0.150	40	7	4	0	11	27.5	**12.1**
0.300	40	9	6	0	15	37.5	**24.2**
LR_{50}		**> 0.300 [L/ha]**					
$NOER_{mortality}$		**≥ 0.300 [L/ha]**					
[g/ha]	**pyrazophos**						
20.0	40	25	9	0	34	85.0	**81.8**

[a]: Mortality corrected according to the Abbott formula [10]
The definitive test was performed between 18.09 – 28.10.2024 (mortality: 18.09 – 07.10.2024)

Reproduction test
In the definitive test reproduction of the ladybird beetles from the control group and the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha were assessed, since the mortality, after Abbott correction [10], was ≤ 50%.
The adults, grouped in the terrariums, were observed until the moment of first egg batch laying in the control group. After the first egg batch had been observed in the control group, sex of adult beetles was determined again and reproductive performance observations were made for the next days.
The beetles were provided pine pollen (*Pinus sp.*), honey-water solution (2:1), broad bean stems infested with the aphids, *A. pisum* and water (min. 3 times a week). Fresh aphids on broad bean stems were introduced to the reproduction units every day during the egg collection stage. Every week, the beetles were transferred to clean reproduction units containing fresh bean stems infested with the aphids, a fresh source of pollen, and a fresh honey-water solution.
Folded sheets of paper towel were offered to the beetles for egg laying. They were checked once a day.

Freshly laid eggs were cut out of the paper sheets and stored in plastic containers.
Mortality of the beetles during the egg laying period was recorded daily, and sex of all dead insects was determined. The laid eggs were counted daily over a period of a reproductive performance. Egg batches were stored under laboratory conditions until the larval hatch. To assess the larval hatch, live larvae were counted over the hatching period (up to 3 days after the first larvae occurred). If the mean number of fertile eggs per viable female per day was below 2 only in treated group, the effect on the reproductive performance was considered as treatment related.

Observations
Condition of the ladybird beetles will be recorded as follows:
Larvae:
–alive: apparently unaffected,
–affected: behavioural abnormalities (showing signs of reduced coordination to the larvae in the control group),
–moribund: lying on their backs with quivering legs,
–dead: not moving after gentle agitation,
–not visible: not observed (escaped larvae),
Pupae:
–alive: apparently unaffected,
–dead: not emerged (black pupae),
Adult beetles:
–hatched alive: adults in good condition,
–hatched deformed: adults with deformities (e.g. abdomen or wings),
–dead: died during emergence.

Conclusion

The effects of the test item, AMINO 30 SL on mortality and reproductive capacity of the ladybird beetle, *Coccinella septempunctata* L. in the laboratory test are summarized below.

Study group	Parameters (endpoints)						
	Mortality				Reproduction		
Test item [L/ha]	[no.]	[%]	Corr. [%][a]	LR_{50} [L/ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]
Test item: AMINO 30 SL							
Control (0.0)	7	17.5	-	> 0.300	7.4	5.4	–
0.075	9	22.5	6.1		7.7	5.8	(-6.8)[b]
0.150	11	27.5	12.1		9.7	8.1	(-51.0)[b]
0.300	15	37.5	24.2		5.0	4.1	24.4
$NOER_{mortality}$	≥ 0.300 [L test item/ha]						
Reference item: pyrazophos							
[g/ha]	[no.]	[%]	Corr. [%][a]		Reproduction		
20.0	34	85.0	81.8		not assessed		

[a]: Mortality corrected according to the Abbott formula [10]
[b]: The negative value indicates that mean numbers of fertile eggs/female/day in the group treated with the test item was higher than in the control group.

The validity criterion concerning mortality was met, because mortality of the ladybird beetle, Coccinella septempunctata L. in the control group was equal to 17.5% (≤ 30.0%). The mortality of the ladybird beetles exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha, after Abbott correction, were 6.1, 12.1 and 24.2%, respectively.
At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha of AMINO 30 SL and the control group (Chi2 2x2 Table Test with Bonferroni Correstion (p(z) > Alpha*).
The LR_{50} value is higher than 0.300 L/ha of AMINO 30 SL. The NOERmortality is higher than or equal to 0.300 L/ha of AMINO 30 SL.
The mortality of the ladybird beetles exposed to the reference item at the rate of 20.0 g of pyrazophos/ha, after Abbott correction [10], was equal to 81.8%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to pyrazophos.
The mean number of fertile eggs/female/day in the control group was 7.4 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha were equal to 7.7, 9.7 and 5.0, it refers to (-6.8), (-51.0) and 24.4% of reproduction reduction. The negative value indicates that mean numbers of fertile eggs/female/day in the group treated with the test item was higher than in the control group.

It can be concluded that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha had no adverse effect on mortality of the ladybird beetle.
Based on the results, it can be stated that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on the reproduction capacity of the ladybird beetle.

Test validity criteria:
The following validity criteria were met during the study [6]:
-pre-imaginal mortality of the control group was 17.5% (criterion: a maximum of 30.0%),
-mean corrected mortality of the reference item group was 81.8% (criterion: a minimum of 40%),
-fertility (the mean number of fertile eggs/female/day) in the control group was 5.4 (criterion: ≥ 2 fertile eggs/female).

A 2.3.1.6.3 Study 13

Comments of zRMS:	The study was conducted according to the method Mead-Briggs M.A. et al., 2010. There are minor deviation which has no impact on the quality, integrity and final results of the study. Validity criteria were met: - after 48 hours, mortality of the control group was 6.7% (criterion: a maximum of 10.0%), - after 48 hours, mortality of the group treated with the reference item at the rate of 20.0 g/ha, after Abbott correction, was 82.1% (criterion: a minimum of 50%), - all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity), - the mean number of mummies per female in the control group was 33.4 (criterion: a minimum of 5.0 mummies/female), - all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring). The study is acceptable and suitable for the use in the risk assessment. LR_{50} > 0.300 L product/ha ER_{50} > 0.300 L product/ha $NOER_{mortality}$ ≥ 300 L/ha $NOER_{fecundity}$< 0.075 L/ha

Reference:	KCP 10.3.2/03
Report	An extended laboratory test for evaluating the effects of AMINO 30 SL on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez), 2024; Dybek Marcin; Study Code: B-92-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2010)
Deviations:	No

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

Materials and methods

Test item:
AMINO 30 SL1
Batch number:
1/24
Production date:
01.07.2024
Expiry date:
not determined by the Sponsor
Active substance:
29.67 g/L of aminopyralid
(CAS No: 150114-71-92)

Biological test system:
the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae
– age: imago (24 – 48 hours after emerging from mummies)
– source: breeding of the parasitic wasps at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from a commercial breeder (Katz Biotech AG)

Experimental design:
5 study groups:
−a control group (0.0 L/ha)
−test item at the rate of 0.075 L/ha
−test item at the rate of 0.150 L/ha
−test item at the rate of 0.300 L/ha
−reference item: dimethoate at the rate of 20.0 g/ha
mortality assessment: 6 replicates/group, 5 females/replicate
fecundity assessment: 15 replicates/group, 1 female/replicate

Test conditions:
– temperature: 19 – 20°C
– relative air humidity: 64 – 67%
– photoperiod: 16 hours light : 8 hours dark
– light intensity: mortality and oviposition assessment: 2730 lx
fecundity phase: 5407 lx

Statistical analyses:
Mortality:
–Probit analysis using linear max. likelihood regression,
–Chi 2x2 Table Test with Bonferroni Correction
Fecundity:
–Probit analysis using linear max. likelihood regression,
–Shapiro-Wilk’s Test on Normal Distribution,
–Levene’s Test on Variance Homogeneity,
–Williams Multiple Sequential t-test Procedure
Repellency:
–Shapiro-Wilk´s Test on Normal Distribution,

–Levene's Test on Variance Homogeneity,
–Dunnett's Multiple t-test Procedure

Endpoints:
–wasp mortality after 48 hours of exposure
–LR50 and the NOERmortality
–ER50 and the NOERfecundity
–reduction in fecundity (Pr) of the surviving female wasps exposed to test item, 12 days after the oviposition period

Results and discussions

In the definitive test, after 4, 24 and 48 hours, mortality of the control wasps was 0.0, 3.3 and 6.7%, respectively.
After 4, 24 and 48 hours of the exposure to AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha, the percentages mortality of *A. rhopalosiphi*, after Abbott correction, were 0.0, (-3.5) and (-7.1)% for each group. The negative value indicate that the mortality in the groups treated with the test item was lower than in the control group.
Based on the obtained mortality results it could be assumed that the LR50 is higher than 0.300 L/ha and the $NOER_{mortality}$ is higher than or equal to 0.300 L/ha.
The mortality of the wasps exposed to dimethoate at the rate of 20.0 g/ha, after Abbott correction, was 82.1% after 48 hours. Therefore, the validity criterion specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.
At the significance level of 0.05, there were no statistically significant differences in the mean percentages of wasps settled on the plants between the test item groups at the rates of 0.075, 0.150 and 0.300 L/ha and the control group (Dunnett's Multiple t-test Procedure, $|t| \leq |t^*|$).
The fecundity assessment showed that the mean number of mummies per female in the control group was 33.4 (after 12 days after oviposition). As for the wasps treated with test item at the rates of 0.075, 0.150 and 0.300 L/ha the mean number of mummies per female were 23.7, 19.7 and 21.4, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha were 29.1, 40.9 and 35.9%, respectively.
At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the rates of 0.075, 0.150 and 0.300 L/ha and the control group (Williams Multiple Sequential t-test Procedure, $|t| \leq |t^*|$).
Based on the obtained fecundity results it could be assumed that the ER_{50} value is higher than 0.300 L/ha and the $NOER_{fecundity}$ is lower than 0.075 L/ha.

The effects of the test item on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Parametr (endpoint)							
Mortality after 48 hours				Fecundity			
Test item [L/ha]	Total [%]	Total [%][a]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	6.7	-	–	Control	33.4	–	–
0.075	0.0	(-7.1)*	> 0.300	0.075+	23.7	29.1	> 0.300
0.150	0.0	(-7.1)*		0.150+	19.7	40.9	
0.300	0.0	(-7.1)*		0.300+	21.4	35.9	
$NOER_{mortality}$	≥ 0.300 L/ha			$NOER_{fecundity}$	< 0.075 L/ha		
Reference item: dimethoate							
Rate [g/ha]	Total mortality [%]			Fecundity			
20.0	83.3	82.1		not assessed			

[a]: Mortality corrected according to the Abbott formula [11]
*: The negative value indicates that mortality in the group treated with the test item was lower than in the control group.
+: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3], [SOP/B/67]

Conclusion

On the basis of the obtained repellency results it can be concluded that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has no repellent properties.
On the basis of the obtained mortality results it can be concluded that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has no adverse effect on the mortality of the wasps, *A. rhopalosiphi*.
On the basis of the obtained fecundity results it can be concluded that AMINO 30 SL at the rates of 0.075, 0.150 and 0.300 L/ha has adverse effect on the fecundity of the wasps, *A. rhopalosiphi*.

Test validity criteria

The following validity criteria were met during the study:
–after 48 hours, mortality of the control group was 6.7% (criterion: a maximum of 10.0%),
–after 48 hours, mortality of the group treated with the reference item at the rate of 20.0 g/ha, after Abbott correction [11], was 82.1% (criterion: a minimum of 50%),
–all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
–the mean number of mummies per female in the control group was 33.4 (criterion: a minimum of 5.0 mummies/female),
–all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

A 2.3.1.6.4 Study 14

Comments of zRMS:	The study was conducted according to the method Blümel S. et al., (2000). There are minor deviation which has no impact on the quality, integrity and final results of the study. Validity criteria were met: - mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%), - mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 95.0% on day 7 of exposure (criterion: from 50 to 100%), - the cumulative mean number of eggs per female in the control group was 6.7 (required: ≥ 4 eggs per female).The study is acceptable and suitable for the use in the risk assessment. LR_{50} > 0.300 L product/ha ER_{50} > 0.569 L product/ha $NOER_{mortality}$ ≥ 300 L/ha $NOER_{fecundity}$< 0.075 L/ha

Reference:	KCP 10.3.2/04
Report	An extended laboratory test for evaluating the effects of AMINO 30 SL on the predatory mite, *Typhlodromus pyri* (Sch.), 2024; Dybek Marcin; Study Code: B-91-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	Yes According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen should be used. However, in the experiment additional food in the form of the two-spotted spider mite (T. *urticae*) eggs, was used. Another food source prevents the mites from escaping from discs This deviation has no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
AMINO 30 SL
Active substances:
29.67 g/L of aminopyralid (CAS No: 150114-71-92)
Batch number:

1/24
Production date:
07.2024
Expiry date:
not specified by Sponsor 3

Biological test system:
the predatory mite, Typhlodromus pyri (Sch.) (Acari: Phytoseiidae)
– age:24-hour-old protonymphs
– source:a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented from a commercial breeder

5 study groups:
−a control group (0.0 L/ha)
−AMINO 30 SL at the rate of 0.075 L/ha
−AMINO 30 SL at the rate of 0.150 L/ha
−AMINO 30 SL at the rate of 0.300 L/ha
−reference item: dimethoate at the rate of 4.0 g/ha

number of replicates: 3/group
number of mites in each replicate: 20

Test conditions:
– temperature: 24 – 25 °C
– relative air humidity: 65 – 69 %
– photoperiod:16 h light : 8 h dark
– light intensity:624 lx

Statistical analysis:
Probit analysis using linear max. likelihood regression
Chi2 2x2 Table Test with Bonferroni Correction
Shapiro Wilk's Test on Normal Distribution
Levene's Test on Variance Homogenity (with Residuals) Williams Multiple Sequential t-test Procedure

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

Results and discussions

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to AMINO 30 SL at rates of 0.075, 0.150 and 0.300 L/ha, the percentage of mortality, were 0.0, 1.7 and 1.7%, respectively.
There were no statistically significant differences in mortality in the group treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha in comparison to the control group.
The LR_{50} value is higher than 0.300 L/ha. $NOER_{mortality}$ is higher than or equal to 0.300 L/ha.
After 7 days of exposure to dimethoate at the rate of 4.0 g/ha, mortality was 95.0%. Therefore, the validity criterion specified in the method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.
Reproduction of the surviving mites from the control group and groups treated with test item at the rates of 0.075, 0.150 and 0.300 L/ha were assessed since mortality of these groups was ≤ 50.0%.
The mean reproduction rate (Rr) in the control group was 6.7 eggs/female. The mean Rr after 14 days of

exposure to test item at the rates of 0.075, 0.150 and 0.300 L/ha were 5.6, 4.8 and 4.2 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.075, 0.150 and 0.300 L/ha were 16.6, 28.4 and 37.8%, respectively.
There were statistically significant differences in reproduction between the groups treated with the test item at the rates of 0.075, 0.150 and 0.300 L/ha and the control group (Williams Multiple Sequential t-test Procedure, |t|>|t*|).
The calculated ER_{50} value is 0.569 L/ha (95% confidence limit: 0.317 – 17.085). $NOER_{reproduction}$ is lower than 0.075 L/ha.

The effects of test item on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized in the table.

Parameter (endpoint)				
Mortality (dead + escaped mites)		**Reproduction**		
Test item rate [L/ha]	**Total [%]**	**Test item rate [kg/ha]**	**Mean number of eggs/ female (Rr) [no.]**	**Reproduction reduction Pr [%]**
Control (0.0)	0.0	**control (0.0)**	6.7	–
0.075	0.0	**0.075⁺**	5.6	16.6
0.150	1.7	**0.150⁺**	4.8	28.4
0.300	1.7	**0.300⁺**	4.2	37.8
LR_{50}	**> 0.300 L/ha**	**ER_{50}**	**0.569 L/ha** *(0.317 – 17.085)**	
$NOER_{mortality}$	**≥ 0.300 L/ha**	**$NOER_{reproduction}$**	**< 0.075 L/ha**	
Reference item: dimethoate				
Rate [g/ha]	**Total [%]**	**Reproduction**		
4.0	95.0	not assessed		

⁺: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [11], [SOP/B/67]
*: 95%-confidence limits

Conclusion

Based on the results it can be stated that AMINO 30 SL at the rates 0.075, 0.150 and 0.300 L/ha has no adverse effect on mortality and at the rates 0.075, 0.150 and 0.300 L/ha has an adverse effect on reproduction of the mites.

Test validity criteria
The following validity criteria were met during the study:
-mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
-mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 95.0% on day 7 of exposure (criterion: from 50 to 100%),

-the cumulative mean number of eggs per female in the control group was 6.7 (required: ≥ 4 eggs per female).

A 2.3.1.7 KCP 10.3.2 Field tests with honeybees

No additional studies were performed.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1.1 Study 15

Comments of zRMS:	The study was performed according to OECD TG 222 and principles of GLP. The validity criteria are met: For the control group: - Adult mortality: ≤ 10 % (being 3.8 %) - Number of juveniles per replicate: ≥ 30 (being 87 to 115) - Coefficient of variation of reproduction: ≤ 30 % (being 9.2 %). The following deviation to the study plan was recorded: According to the OECD Guideline No. 222 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations were used. This deviation did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4/01
Report	AMINO 30 SL Earthworm (*Eisenia andrei*) reproduction test, 2024; Gierbuszewska Aneta; Study Code: G-54-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 222 (2016)
Deviations:	Yes Deviation from the OECD Guideline No. 222 (2016): According to the OECD Guideline No. 222 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study ten concentrations were used (Chapter 3.6.1).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the impact of AMINO 30 SL on reproduction of the earthworm, Eisenia andrei and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous solution was mixed with a suitable amount of the artificial soil. Ten concentrations of the test item were used: 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Materials and methods

Test item:
AMINO 30 SL
batch no.: 1/24

Active substance:
aminopyralid – 29.67 g/L

Artificial soil:
10% sphagnum peat, 20% kaolin clay, 69.70% air-dried quartz sand, 0.30% calcium carbonate;

Test organism:
the earthworm, Eisenia andrei obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology

Test design: test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate

Concentrations of the test item:
control, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg/kg dry weight of the artificial soil

Test conditions: temperature: 19.6 – 22.0°C;
pH at the beginning of the experiment: 5.58 – 5.76;
pH at the end of the experiment: 5.54 – 5.64;
soil moisture content at the beginning of the experiment: 23.6 – 26.7% (53.0 – 60.0% of the maximum water holding capacity);
soil moisture content at the end of the experiment: 22.2 – 25.3% (49.9 – 56.8% of the maximum water holding capacity);
light-dark cycle: 16h : 8h;
light intensity at the beginning of the experiment:627.5 – 633.1 lux
light intensity at the end of the experiment: 623.8 – 631.2 lux

Statistical analysis:
EC_{10}, EC_{20}, EC_{50} – logit analysis using linear max. likelihood regression
LC_{10}, LC_{20}, LC_{50} – probit analysis using linear max. likelihood regression
NOEC (reproduction):
-Shapiro-Wilk's Test on Normal Distribution,
-Levene's Test on Variance Homogeneity (with Residuals),
-Dunnett's Multiple 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

Endpoint:
EC_{10}, EC_{20}, EC_{50}, NOEC, LOEC (reproduction)
LC_{50}, NOEC, LOEC (survival)

Results and discussions

Mortality of the adult earthworms
After 4 weeks of the experiment, at the control group mortality of adult earthworms was equal to 3.8%. At concentrations ranging from 5.0 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0% and 5.0%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is higher than 1000.0 mg/kg dry weight of the artificial (Table 12).

Table 12. Endpoint values determined during the earthworm reproduction test (*Eisenia andrei*).

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of aminopyralid/kg dry weight of artificial soil]
EC_{10}	262.0 (144.1 – 398.2)	7.61 (4.18 – 11.56)
EC_{20}	> 1000.0	> 29.03
EC_{50}	> 1000.0	> 29.03
NOEC (reproduction)	171.5	4.98
LOEC (reproduction)	308.6	8.96
LC_{10}	> 1000.0	> 29.03
LC_{20}	> 1000.0	> 29.03
LC_{50}	> 1000.0	> 29.03
NOEC (survival)	≥ 1000.0	≥ 29.03
LOEC (survival)	> 1000.0	> 29.03

Observations of the adult earthworm
After 4 weeks of the experiment, at the concentrations between 5.0 and 1000.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the adult earthworms were not observed.

Body weights of the living adult earthworms
After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of artificial soil, the body weight change was between 6.8 and 25.2%. As for the control group, the body weight increase was equal to 7.9%.

Impact of the test item on reproduction of the earthworms
After the application of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 82.0 and 105.8 per replicate. The mean number of juveniles in the control group was equal to 97.9 per replicate.
After 8 weeks of the experiment, it was concluded that AMINO 30 SL had statistically significant impact on reproduction of the earthworms at the concentrations ranging from 308.6 to 1000.0 mg/kg dry weight of artificial soil.
The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 262.0 mg/kg dry weight of the artificial soil.
The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is above 1000.0 mg/kg dry weight of the artificial soil.
The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is above 1000.0 mg/kg dry weight of the artificial soil.
The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal to 171.5 mg/kg dry weight of the artificial soil.
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is equal to 308.6 mg/kg dry weight of the artificial soil.

Observations of the juveniles of earthworms
After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Results of the reference test
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is equal to 5.00 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 222, the LOEC should be between 1 – 5 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Conclusion

At concentrations ranging from 5.0 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 5.0%. In the control group mortality of the adult earthworms was equal to 3.8%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is higher than 1000.0 mg/kg dry weight of the artificial soil.
No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed.
After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of artificial soil, the body weight change was between 6.8 and 25.2%. As for the control group, the body weight increase was equal to 7.9%.
After the application of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 82.0 and 105.8 per replicate. The mean number of juveniles in the control group was equal to 97.9 per replicate.
No changes in the appearance (morphology) and behaviour of the juveniles earthworms were noticed.
After 8 weeks of the experiment, it was concluded that AMINO 30 SL had statistically significant impact on reproduction of the earthworms at the concentrations ranging from 308.6 to 1000.0 mg/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.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of aminopyralid/kg dry weight of artificial soil]
EC_{10}	262.0 (144.1 – 398.2)	7.61 (4.18 – 11.56)
EC_{20}	> 1000.0	> 29.03
EC_{50}	> 1000.0	> 29.03
NOEC (reproduction)	171.5	4.98
LOEC (reproduction)	308.6	8.96
LC_{10}	> 1000.0	> 29.03
LC_{20}	> 1000.0	> 29.03
LC_{50}	> 1000.0	> 29.03
NOEC (survival)	≥ 1000.0	≥ 29.03
LOEC (survival)	> 1000.0	> 29.03

Test validity criteria

The results are considered valid because the following criteria were satisfied in the control:
- each replicate produced from 87 to 115 juveniles (97.9 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 9.2% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 3.8% (criterion: ≤ 10%).

A 2.4.1.2 KCP 10.4.1.1 Earthworms - sub-lethal effects

No additional studies were performed.

A 2.4.1.3 KCP 10.4.1.2 Earthworms - field studies

No additional studies were performed.

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

A 2.4.2.1.1 Study 16

Comments of zRMS:	The study was performed according to OECD TG 232 and principles of GLP. The validity criteria are met: For the control group: Mean adult mortality: ≤ 20 % (observed: 6.3%) Mean number of juveniles per test vessel: ≥ 100 (observed:456.9) Coefficient of variation for the mean number of juveniles: < 30 % (observed: 23.8%). The following deviations from the study plan were noted: At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels. According to the OECD Guideline No. 232 and SOP/G/87 eight test concentrations of the test item should be used in order to calculate ERx and NOER. In this study ten concentrations was prepared. These deviations did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4/02
Report	AMINO 30 SL Collembolan (*Folsomia candida*) Reproduction Test, 2024; Czarnynoga Magdalena; Study Code: G-55-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 232 (2016)
Deviations:	Yes Deviation from the OECD Guideline No. 232 (2016): At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (3.6.6.). Deviation from the OECD Guideline No. 232 and SOP/G/87 According to the OECD Guideline No. 232 and SOP/G/87 eight test concentrations of the test item should be used in order to calculate ERx and NOER. In this study ten concentrations was prepared. All above mentioned deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the impact of AMINO 30 SL on reproduction of the collembolans, Folsomia candida and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These were 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

Materials and methods

Test item:
AMINO 30 SL
batch no.: 1/24

Active substance:
aminopyralid: 29.67 g/L

Artificial soil:
5% sphagnum peat, 20% kaolin clay, 74.88% air-dried industrial sand and 0.12% calcium carbonate,

Test organism:
the collembolan, Folsomia candida obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The collembolans used in the study were between 9 and 12 days old.

Test design:
exposure period: 28 days
number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate
Concentrations of the test item:
a control, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg of the test item/kg of dry weight of the artificial soil

Test conditions:
temperature: 19.8 – 22.0°C; pH at the beginning of the test: 5.55 – 5.57; pH at the end of the test: 5.31 – 5.54; soil moisture content at the beginning of the test: 17.1 – 18.7% (44.5 – 48.6% of the maximum water holding capacity); soil moisture content at the end of the test: 16.2 – 17.7% (42.1 – 46.0% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 450.3 – 536.8 lux; light intensity at the end of the experiment: 507.2 – 529.4 lux;

Statistical analysis:
EC_{10}, EC_{20}, EC_{50} – logit analysis using linear max. likelihood regression
LC_{10}, LC_{20}, LC_{50} – probit analysis using linear max. likelihood regression
NOEC (number of juveniles):
-Shapiro-Wilk's Test on Normal Distribution,
-Levene's Test on Variance Homogeneity (with Residuals),
-Non-parametric Trend analysis by Contrasts (Monotonicity of Concentration/Response),
-Step-down Jonckheere-Terpstra Test Procedure
NOEC (survival):
-Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)
-Chi2 2x2 Table Test with Bonferroni Correction

Endpoints:
EC_{10}, EC_{20}, EC_{50}, NOEC

LC_{10}, LC_{20}, LC_{50}, NOEC

Results and discussions

Mortality
After the application of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 2.5 to 10.0%. As for the control group, it was equal to 6.3%.
The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000.0 mg/kg dry weight of the artificial soil (i.e. above 29.03 mg of aminopyralid/kg dry weight of the artificial soil).

Impact on reproduction
After the exposure of collembolans to the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 357.0 and 507.3 per replicate. As for the control group, the number of juveniles was equal 456.9 per replicate.
The obtained results led to the following conclusions:
- The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 664.94 mg/kg dry weight of the artificial soil (i.e. 19.30 mg of aminopyralid/kg dry weight of the artificial soil);
- The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 970.53 mg/kg dry weight of the artificial soil (i.e. 28.18 mg of aminopyralid/kg dry weight of the artificial soil);
- The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is above 1000.00 mg/kg dry weight of the artificial soil (i.e. above 29.03 mg of aminopyralid/kg dry weight of the artificial soil);
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 555.60 mg/kg dry weight of the artificial soil (i.e. equal to 16.13 mg of aminopyralid/kg dry weight of the artificial soil).

Observation of te collembolans
After 4 weeks of the experiment, at the concentrations between 5.0 and 1000.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the collembolans were not observed.

Results of the reference test
The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 97.41 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 232, the EC50 should be about 100 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper. The test was conducted 29.12.2023 – 29.01.2024.

Conclusion

After the application of the test item at the concentrations ranging from 5.0 to 1000 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 2.5 to 10.0%. As for the control group, it was equal to 6.3%.
The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of aminopyralid/kg dry weight of the artificial soil]
LC_{10}	> 1000.00	> 29.03
LC_{20}	> 1000.00	> 29.03
LC_{50}	> 1000.00	> 29.03
NOEC	≥ 1000.00	≥ 29.03

After the exposure of collembolans to the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 357.0 and 507.3 per replicate. As for the control group, the number of juveniles was equal 456.9 per replicate.
The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of aminopyralid/kg dry weight of the artificial soil]
EC_{10}	664.94 (387.67 – 788.86)	19.30 (11.25 – 22.90)
EC_{20}	970.53 (828.84 – >1000.00)	28.18 (24.06 – >29.03)
EC_{50}	> 1000.00	> 29.03
NOEC	555.60	16.13

Validity criteria

The results are considered valid because the following criteria were satisfied in the control:
- mean adult mortality: 6.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 456.9 (criterion: ≥100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 23.8% (criterion: < 30%).

A 2.4.2.1.2 Study 17

Comments of zRMS:	The study was performed according to OECD TG 226 and principles of GLP. The validity criteria are met: For the control group: - Mean mortality of adult females: ≤ 20 % (observed: 5%) - Mean number of juveniles per replicate: ≥ 50 (observed: 97.4)

	- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (observed: 12.1 %). Following deviations to the study plan were recorded: 1. According to the OECD Guideline No. 226 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study, ten concentrations were used (Chapter 3.5.2). 2. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.5.7). 3. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution (Chapter 3.5.8). 4. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites (Chapter 3.5.8). These deviations did not affect the results of the study. The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4/03
Report	AMINO 30 SL Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil, 2024; Czarnynoga Magdalena; Study Code: G-56-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 226 (2016)
Deviations:	Yes Deviations from the OECD Guideline No. 226 (2016): There are four deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: 1. According to the OECD Guideline No. 226 (2016) in order to calculate ECx and NOEC, eight test concentrations of the test item should be used. In the study, ten concentrations were used (Chapter 3.5.2). 2. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.5.7). 3. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution (Chapter 3.5.8). 4. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites (Chapter 3.5.8).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the impact of AMINO 30 SL on reproduction of the predatory mite, Hypoaspis (Geolaelaps) aculeifer and to determine the EC10, EC20, EC50, and NOEC.

Ten concentrations of the test item were used (a deviation from OECD Guideline No. 226). These included: 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, 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 solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water. The exposure period lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.

Materials and methods

Test item:
AMINO 30 SL
batch number: 1/24

Active substances:
aminopyralid – 29.67 g/L

Artificial soil:
5% sphagnum peat, 20% kaolin clay, and 74.88% air-dried industrial sand, 0.12% calcium carbonate.

Test system:
the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.

Test design: exposure time: 14 days; number of replicates: 4 replicates / concentration + 8 replicates / control;
number of mites: 10 mites / replicate
Concentrations of the test item: a control (0.0), 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, and 1000.0 mg test item/kg dry weight of the artificial soil.
Test conditions:
temperature: 20.4 – 22.0°C
pH at the beginning of the test: 5.50 – 5.59
pH at the end of the test: 5.42 – 5.61
soil moisture content at the beginning of the test: 17.8 – 18.5% (46.3 – 48.1% of the maximum water holding capacity)
soil moisture content in the middle of the test: 17.3 – 18.4% (45.0 – 47.9% of the maximum water holding capacity)
soil moisture content at the end of the test: 17.1 – 18.5% (44.5 – 48.1% of the maximum water holding capacity)
light-dark cycle: 16 h light and 8 h dark
light intensity at the beginning of the test: 601.8 – 623.3 lux
light intensity at end of the test: 609.8 – 633.5 lux

Statistical analysis:
EC_{10}, EC_{20}, EC_{50} – probit analysis using linear max. likelihood regression.
LC_{10}, LC_{20}, LC_{50} – probit analysis using linear max. likelihood regression.
NOEC:
- offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Concentration/Response), Dunnett's Multiple t-test Procedure.

- survival – Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response), Chi2 2x2 Table Test with Bonferroni Correction
Endpoints:
EC_{10}, EC_{20}, EC_{50}, NOEC
LC_{10}, LC_{20}, LC_{50}, NOEC

Results and discussions

Mortality of adult females
Mortality of the adult predatory mites exposed to the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil was between 0.0% and 7.5%.
Mortality of the control group was equal 5.0%.
The concentration of the test item causing a 50% mortality of adults within the exposure period (LC_{50}) is higher than 1000.0 mg/kg dry weight of the artificial soil.

Impact on reproduction
After the application of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 93.8 and 108.3 per replicate. The mean number of juveniles in the control group was equal to 97.4 per replicate.
The obtained results led to the following conclusions:
- The concentration of the test item causing a 10% reduction in the number of mites produced within the exposure period (EC10) is above 1000 mg/kg dry weight of the artificial soil.
- The concentration of the test item causing a 20% reduction in the number of mites produced within the exposure period (EC20) is above 1000 mg/kg dry weight of the artificial soil.
- The concentration of the test item causing a 50% reduction in the number of mites produced within the exposure period (EC50) is above 1000 mg/kg dry weight of the artificial soil.
- The highest concentration at which the test item is observed to have no statistically significant effects on mite reproduction (NOEC) is above or equal to 1000.0 mg/kg dry weight of the artificial soil.

Results of the reference test
The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) is 226.891 mg/kg dry weight of the artificial soil. According to the OECD Guideline No. 226, the EC_{50} should be between 100 and 500 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Conclusions

Mortality of the adult predatory mites exposed to the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil was between 0.0% and 7.5%. Mortality of the control group was equal to 5.0%.
After the application of the test item at the concentrations ranging from 5.0 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 93.8 and 108.3 per replicate. The mean number of juveniles in the control group was equal to 97.4 per replicate.
The results are summarized in the table given below.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days of exposure		
		No.	%	
control	80	4	5.0	97.4
5.0	40	2	5.0	106.0
9.1	40	3	7.5	104.8
16.3	40	3	7.5	95.8
29.4	40	2	5.0	93.8
52.9	40	0	0.0	108.3
95.3	40	1	2.5	104.0
171.5	40	3	7.5	94.0
308.6	40	1	2.5	94.5
555.6	40	3	7.5	97.3
1000.0	40	1	2.5	98.5

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of aminopyralid/kg dry weight of the artificial soil]
EC_{10}	> 1000.0	> 29.03
EC_{20}	> 1000.0	> 29.03
EC_{50}	> 1000.0	> 29.03
NOEC (reproduction)	≥ 1000.0	≥ 29.03
LC_{10}	> 1000.0	> 29.03
LC_{20}	> 1000.0	> 29.03
LC_{50}	> 1000.0	> 29.03
NOEC (survival)	≥ 1000.0	≥ 29.03

Validity criteria

The results are considered valid because the following criteria were satisfied in the control:
•mean adult mortality: 5.0% (criterion: ≤ 20%),
•the mean number of juveniles per vessel at the end of the test: 97.4 (criterion: ≥ 50 juveniles at the end of the test),
•the coefficient of variation for the number of juveniles: 12.1% (criterion: ≤ 30%).

A 2.4.2.2 KCP 10.4.2.1 Species level testing

No additional studies were performed.

A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

No additional studies were performed.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1.1.1 Study 18

Comments of zRMS:	The study was conducted according to OECD guideline 216 and principles of GLP. The validity criterion is met: the variation between the replicate control

	samples did not exceed the validity criterion of 15% throughout the study. There following deviation to the study plan was recorded: According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. This deviation did not affect the results of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5/01
Report	AMINO 30 SL Soil Microorganisms: Nitrogen Transformation Test, 2024; Wróbel Anna, Study Code: G-57-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 216 (2000)/EU Method C.21
Deviations:	Yes Deviation from the OECD Guideline No. 216 (2000), the EU Method C.21: According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer (point 3.4.4.4.). These deviation did not affect the results of the study. Deviation from the principles of Good Laboratory Practice: Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test item, such analyses were not carried out. The waiver of these analyses constitutes a derogation from the principles of Good Laboratory Practice.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the study was to detect long-term adverse effects of AMINO 30 SL on the processes of nitrgen transformation in aerobic surface soils.
The freshly collected agricultural soil was used in the experiment. It was manually cleared of large ojects and sieved to a particle size of 2 mm.
Two concentrations of the test item were used, i.e.:

PEC: 0.41 mg test item/kg dry weight of soil,

5 x PEC: 2.05 mg test item/kg dry weight of soil.

The treated and the control soils were divided into three replicates.
On days 0, 7, 14 and 28 of incubation, soil samples were collected to determine the quantities of nitrate.
The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using 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.

Materials and methods

Test material:
AMINO 30 SL
batch no.: 1/24
Active substance:
aminopyralid – 29.67 g/L
Soil:
Agricultural soil collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.

Test design:
Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. After adding the deionized water, every portion was divided into three replicates (3 x 530 g). Exposure period: 28 days.
Concentrations of the test item:
control;
-PEC: 0.41 mg test item/kg dry weight of soil,
-5 x PEC: 2.05 mg test item/kg dry weight of soil

Test conditions:
temperature: 20.4 – 22.0°C,
soil moisture: 44.3 – 48.8% of the maximum water holding capacity, incubation in darkness

Endpoints:
The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation.
The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28 days.

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

Table 9. 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	23.341	24.970	25.641	24.650 ± 1.18	24.581	27.167	27.731	26.493* ± 1.68	29.308	30.665	29.958	29.977* ± 0.68
0 – 14	15.382	16.100	16.932	16.138 ± 0.78	16.492	16.949	16.770	16.737* ± 0.23	16.308	17.212	16.719	16.747 ± 0.45
0 – 28	9.507	10.139	10.355	10.000 ± 0.44	10.470	10.113	10.713	10.432* ± 0.30	10.248	10.630	10.789	10.556* ± 0.28

* - 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 in comparison to the control group (Williams Multiple Sequential t-test Procedure, alpha = 0.05, two-sided)

Table 10. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	-7.48	-21.61
0 – 14	-3.71	-3.77
0 – 28	-4.32	-5.56

"-"- obtained values are higher than the ones for the control group

Results and discussions

The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 0.41 mg test item/kg dry weight of soil and 5 x PEC: 2.05 mg test item/kg dry weight of soil did not exceed 25% on 28 day of analysis.

Conclusion

On the basis of the results, it was concluded that AMINO 30 SL at the concentrations corresponding to the PEC: 0.41 mg test item/kg dry weight of soil and 5 x PEC: 2.05 mg test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.\

Validity criterion

The coefficients of variation (CV) in the control group were 2.7, 2.9, 3.2 and 3.1%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

No additional studies were performed.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1.1 Study 19

Comments of zRMS:	The study was conducted according to OECD guideline 227 and principles of GLP. All the validity criterion are met: - seedling emergence: ≥ 70 % (actual 82.5 - 100 %).

	- for control group: mean plant survival for the duration of the study: ≥ 90 % (actual 100 %), - the control plants did not exhibit any visible phytotoxic symptoms- environmental conditions for all plants belonging to the same species were identical The following deviation from the study plan was noted: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50 µE/m^2/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 213.6 and 243.7 µE/m^2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. It can be concluded that this deviation did not affect results of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/01
Report	AMINO 30 SL Terrestial Plant Test: Vegetative Vigour Test, 2024; Czarnynoga Magdalena, Study Code: G-58-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 227 (2006)
Deviations:	Yes Deviation from OECD Guideline No. 227: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50 µE/m^2/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 213.6 and 243.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. Deviation from SOP/G/70: Procedure SOP/G/70 specifies the use of spray chamber SOP/G/75. However, in the conducted study, the new spray chamber SOP/G/140 was used. This deviations did not affect the results of the experiment.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The study aimed at evaluating the effect of AMINO 30 SL on vegetative vigour of 6 terrestrial plants was conducted on 3 dicotyledonous and 3 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (4 seeds/pot for tomato; 6 seeds/pot for pea; 10 seeds/pot for carrot, onion, wheat and oats). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed.
The pot is defined as a replicate. The test item was sprayed onto the plants. Untreated control group was conducted simultaneously. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The exposure period finished 21 days after the spraying. At the end of the exposure the number of surviving plants was counted. Next, the plants were cut down and the lengths of their shoots were determined. Finally, they were dried at 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 ER_{10}, ER_{25}, ER_{50} and NOER.
Additionally, ER_{10}, ER_{25}, ER_{50} and NOER were determined for visual phytotoxicity effects basis on the results after 21 days of the experiment.

Materials and methods

Test item:
AMINO 30 SL
batch number: 1/24
active substances: aminopyralid – 29.67 g/L
Test species:
tomato (*Solanum lycopersicon*), pea (*Pisum sativum*), carrot (*Daucus carota*), onion (*Allium cepa*), winter wheat (*Triticum aestivum*), oats (*Avena sativa*),
Soil:
Sandy loam

Study design:

Plant species	Number of replicates per rate	Number of plants per replicate	Total number of plants per rate
tomato	10	2	20
pea	7	3	21
carrot	4	5	20
onion	4	5	20
winter wheat	4	5	20
oats	4	5	20

Exposure termination: 21 days after spraying

Application rates:

Test species	Application rates [mL/ha]
tomato, pea, carrot, onion, winter wheat, oats	0.5, 1.2, 3.1, 7.7, 19.2, 48.0, 120.0, 300.0

In case of each species there was one untreated control group,
volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha.

Test conditions:

temperature: 19.5 – 23.8°C;
humidity: 45.9 – 76.6%;
lighting: 16 h light : 8h dark;
light intensity: 213.6 – 243.7 µE/m2/s;
carbon dioxide concentration: 328 – 363 ppm

Statistical analysis:
In order to determine ER10, ER25, ER50 the following tests were used:
Plant number: Weibull analysis using linear max. likelihood regression
Because no change in mortality of plants was observed, no computations in plant number have been performed for pea, carrot, onion, winter wheat and oats.

Shoot length: Probit analysis using linear max. likelihood regression, logit analysis using linear max. likelihood regression, Weibull analysis using linear max. likelihood regression.
Shoot dry weight: Probit analysis using linear max. likelihood regression, logit analysis using linear max. likelihood regression.
Plant damages: Probit analysis using linear max. likelihood regression, logit analysis using linear max. likelihood regression.
In order to determine the NOER values, the following tests were used:
plant number: Qualitative Trend Analysis by Contrast (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Rao-Scott-Armitage Test Procedure.
Because no change in mortality of plants was observed, no computations in plant number have been performed for pea, carrot, onion, winter wheat and oats.
shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Dunnett`s Multiple t-test Procedure.
plant shoot dry weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Dunnett`s Multiple t-test Procedure, Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure.

Results and discussions

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements and ER50 values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Tomato *Solanum lycopersicon*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Winter wheat** *Triticum aestivum*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	130.58 (109.51– 179.35)	> 300.00	> 300.00	> 300.00	> 300.00	> 300.00
NOER	48.00	≥ 300.00	≥ 300.00	≥ 300.00	≥ 300.00	≥ 300.00
Shoot length						
ER_{50}	121.84 (121.40 – 122.63)	98.11 (68.12 – 153.47)	> 300.00	> 300.00	> 300.00	> 300.00
NOER	48.00	7.70	120.00	120.00	≥ 300.00	≥ 300.00
Plant dry weight						
ER_{50}	23.80 (16.30 – 35.30)	29.87 (20.14 – 44.39)	172.50 (156.16 – 191.63)	> 300.00	> 300.00	> 300.00
NOER	3.10	7.70	48.00	48.00	≥ 300.00	≥ 300.00
Plant Damage						
ER_{50}	6.92 (4.14 – 11.53)	24.66 (18.05 – 33.73)	185.73 (168.49 – 206.05)	> 300.00	> 300.00	> 300.00

Conclusion

The test item, i.e. AMINO 30 SL, had a varied impact on vegetative vigour of tomato, pea, carrot and onion. The test item had no impact on vegetative vigour of winter wheat and oats.
The test item caused mortality in cultivation of tomato. Mortality of pea, carrot, onion, winter wheat and oats was not observed.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of tomato and pea. Slight effect was also noticed in cultivation of carrot and onion. The test item did not inhibit the process of growth of winter wheat and oats.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of tomato, pea, carrot and onion. The test item did not inhibit the process of growth of winter wheat and oats.
During the experiment, the phytotoxic symptoms of the test item were noticed in cultivation of tomato, pea, carrot and onion. In the case of winter wheat and oats, no symptoms of phytotoxicity were observed.

Validity Criteria

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of AMINO 30 SL on vegetative vigour of terrestrial plants were met:
- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
82.5 – 95.0% – tomato,
95.2 – 100.0% – pea,
95.0 – 100.0% – carrot,
87.5 – 100.0% – onion,
92.5 – 100.0% – winter wheat,
95.0 – 100.0% – oats.
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%).
- the control plants did not exhibit any visible phytotoxic symptoms.
- environmental conditions for all plants belonging to the same species were identical.

A 2.6.2.1.2 Study 20

Comments of zRMS:	The study was conducted according to OECD guideline 208 and principles of GLP. All the validity criterion are met: - Seedling emergence in the control: ≥ 70 % (actual 75 - 100%), - mean survival of emerged control seedlings: ≥90 % (actual 100 %), - the control seedlings did not exhibit any visible phytotoxic effects, - environmental conditions for all plants of the same species were iden-tical. The following deviation was recorded: 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 209.8 and 233.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. It can be concluded that this deviation did not affect results of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/02
Report	AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling

	Growth Test, 2024; Wróbel Anna, Study Code: G-59-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	Yes Deviation from OECD Guideline No. 208: According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 209.8 and 233.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. Deviation from SOP/G/42: Procedure SOP/G/42 specifies the use of spray chamber SOP/G/75. However, in the conducted study, the new spray chamber SOP/G/140 was used. Those deviations did not affect the course and results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The study, aimed at evaluating the effect of AMINO 30 SL on seedling emergence and seedling growth of 6 terrestrial plants. The study was conducted on 3 dicotyledonous and 3 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 2 (tomato), 3 (pea), 5 (carrot, onion, oats, winter wheat) seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day to the emergence of 50% of the control seedlings and after then every 1 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The exposure period finished 14 days after the emergence of 50% of the control seedlings. At the end of the exposure, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed.

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

Additionally, the ER50 was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period.

Materials and methods

Test item:
AMINO 30 SL
batch number: 1/24
active substance: aminopyralid: 29.67 g/L

Test species:
pea (*Pisum sativum*), tomato (*Solanum lycopersicon*), carrot (*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*), winter wheat (*Triticum aestivum*).

Soil:
sandy loam

Study design:

Plant species	Number of replicates per rate	Number of seeds per replicate	Total number of seeds per rate
pea	7	3	21
tomato	10	2	20
carrot	4	5	20
onion	4	5	20
oats	4	5	20
winter wheat	4	5	20

Application rates:
0.5, 1.2, 3.1, 7.7, 19.2, 48.0, 120.0 and 300 mL/ha.

Exposure termination:
14 days after the emergence of 50% of the control seedlings;

Test conditions:
temperature: 19.3 – 23.8°C, humidity: 46.5 – 81.7%,
lighting: 16 h light : 8 h dark; light intensity: 209.8 – 233.2 µE/m2/s; carbon dioxide concentration: 358 – 376 ppm;

Statistical analysis:
The ER_{10}, ER_{25} and ER_{50} values for the emergence of plants (on the basis of maximum emergence of plants) were determined with: probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.
The ER_{10}, ER_{25} and ER_{50} values for the survival of tomato and onion plants were determined with logit analysis using linear max. likelihood regression.
Because plant mortality was not observed in cultivation of pea, carrot, oats and winter wheat no computations were performed.
The ER_{10}, ER_{25} and ER_{50} values for shoot length were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.
The ER_{10}, ER_{25} and ER_{50} values for shoot dry weight were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.
The ER_{50} for visual phytotoxicity effects (on the basis of the results obtained at the end exposure period) were determined with: probit analysis using linear max. likelihood regression.
NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on seedling emergence and seedling growth.
In order to determine the NOER values, the following tests were used:
The emergence of plants (on the basis of maximum emergence of plants):
Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone´s Test Procedure, Step-down Cochran-Armitage Test Procedure or Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm.
The survival of plants:
Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone´s Test Procedure, Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm.

Because no plants mortality was observed in cultivation of pea, carrot, oats and winter wheat no computations were performed.
The shoot length:
Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Dunnett's Multiple t-test Procedure or Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm.
The shoot dry weight:
Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Dunnett's Multiple t-test Procedure or Multiple Sequentially-rejective Median (2x2-Table) Test After Bonferroni-Holm, or Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm.

Results and discussions

The ER_{50} and NOER values determined on the basis of emergence of plants, survival of plants, shoot length and shoot dry weight measurements and ER50 values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Pea *Pisum sativum*	**Tomato** *Solanum lycopersicon*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*	**Winter wheat** *Triticum aestivum*
Emergence of plants						
ER_{50}	> 300.0	> 300.0	> 300.0	> 300.0	> 300.0	> 300.0
NOER	≥ 300.0	≥ 300.0	≥ 300.0	120.0	≥ 300.0	≥ 300.0
Survival of plants						
ER_{50}	> 300.0	> 300.0	> 300.0	> 300.0	> 300.0	> 300.0
NOER	> 300.0*	≥ 300.0	> 300.0*	≥ 300.0	> 300.0*	> 300.0*
Shoot length						
ER_{50}	> 300.0	220.6 (194.4 – 250.2)	> 300.0	> 300.0	> 300.0	> 300.0
NOER	120.0	48.0	120.0	120.0	≥ 300.0	≥ 300.0
Shoot dry weight						
ER_{50}	141.1 (124.9 – 159.5)	40.7 (33.4 – 49.6)	258.1 (249.5 – 266.9)	199.3 (177.9 – 223.4)	> 300.0	> 300.0
NOER	19.2	19.2	120.0	120.0	≥ 300.0	≥ 300.0
Plant damages						
ER_{50}	58.0 (34.8 – 96.9)	36.0 (26.1 – 49.7)	> 300.0	225.6 (157.4 – >300.0)	> 300.0	> 300.0

Conclusion

On the basis of the obtained results it was proved that the test item i.e. AMINO 30 SL had varied impact on the process of growth of selected plant species.
Mortality of plants was observed in cultivation of tomato and onion. It was one plant dead at the rate of 300 mL/ha. In case of pea, carrot, oats and winter wheat plants mortality was not observed.
Delayed emergence of plants in comparison to the control group was not noticed.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant emergence during exposure period it was proved that the test item had impact on seedling emergence of tomato and onion.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of pea, tomato, carrot and onion.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot dry weight it was proved that the test item inhibited the process of growth of pea, tomato, carrot and onion. Growth of oats and winter wheat was slightly inhibited.
During the exposure period, the phytotoxic symptoms such as stunted growth, deformations, chlorosis were observed. The heaviest plant damages were observed in cultivation of pea and tomato. In case of oats and winter wheat only stunted growth (about 10%) in some replicates were observed.

Validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of AMINO 30 SL 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% – pea,
100.0% – tomato,
80.0% – carrot,
90.0% – onion,
80.0% – oats,
75.0% – winter wheat,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

A 2.6.2.1.3 Study 21

Comments of zRMS:	The study was conducted according to OECD guideline 208 and principles of GLP. All the validity criterion are met: - Seedling emergence in the control: ≥ 70 % (actual 100%), - mean survival of emerged control seedlings: ≥90 % (actual 100 %), - the control seedlings did not exhibit any visible phytotoxic effects, - environmental conditions for all plants of the same species were identical. The following deviation was recorded: 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 211.3 and 231.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. It can be concluded that this deviation did not affect results of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/03
Report	AMINO 30 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, 2024; Wróbel Anna, Study Code: G-93-24; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group, GLP, Unpublished
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	Yes <u>Deviation from OECD Guideline No. 208:</u> 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 211.3 and

	231.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. Deviation from SOP/G/42: Procedure SOP/G/42 specifies the use of spray chamber SOP/G/75. However, in the conducted study, the new spray chamber SOP/G/140 was used. Those deviations did not affect the course and results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The study, aimed at evaluating the effect of AMINO 30 SL on seedling emergence and seedling growth of 2 terrestrial plants. The study was conducted on 1 dicotyledonous and 1 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 2 (sugar beet, corn), seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day to the emergence of 50% of the control seedlings and after then every 1 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The exposure period finished 14 days after the emergence of 50% of the control seedlings. At the end of the exposure, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed.
The results concerning the emergence, survival, the shoot length, and the shoot dry weight were statistically analyzed in order to determine the ER_{10}, ER_{25}, ER_{50}, and NOER.
Additionally, the ER_{50} was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period.

Materials and methods

Test item:
AMINO 30 SL
batch number: 1/24
active substance: aminopyralid 29.67 g/L

Test species:
sugar beet (*Beta vulgaris*), corn (*Zea mays*)

Soil:
sandy loam

Study design:

Plant species	Number of replicates per rate	Number of seeds per replicate	Total number of seeds per rate
sugar beet	10	2	20
corn	10	2	20

Application rates:
0.5, 1.2, 3.1, 7.7, 19.2, 48.0, 120.0, 300.0 mL/ha

Exposure termination:
14 days after the emergence of 50% of the control seedlings;

Test conditions:
temperature: 19.3 – 23.8°C, humidity: 46.5 – 81.7%,
lighting: 16 h light : 8 h dark; light intensity: 211.3 – 231.7 µE/m2/s; carbon dioxide concentration: 352 – 372 ppm;

Statistical analysis:
The ER_{10}, ER_{25} and ER_{50} values for the emergence of plants (on the basis of maximum emergence of plants) were determined with logit analysis using linear max. likelihood regression (corn). In case of sugar beet seedling emergence was 100 % at the control group and all application rates, therefore no computations were performed.
The ER_{10}, ER_{25} and ER_{50} values for the survival of sugar beet plants were determined with logit analysis using linear max. likelihood regression.
Because plant mortality was not observed in cultivation of corn, no computations were performed.
The ER_{10}, ER_{25} and ER_{50} values for shoot length were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.
The ER_{10}, ER_{25} and ER_{50} values for shoot dry weight were determined with probit analysis using linear max. likelihood regression or logit analysis using linear max. likelihood regression.
The ER_{50} for visual phytotoxicity effects (on the basis of the results obtained at the end exposure period) were determined with: logit analysis using linear max. likelihood regression.
NOER (no observed effect rate) – the highest rate at which the test item is observed to have no effects on seedling emergence and seedling growth.
In order to determine the NOER values, the following tests were used:
The emergence of plants (on the basis of maximum emergence of plants):
Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone´s Test Procedure, Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm
The survival of plants:
Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone´s Test Procedure, Step-down Rao-Scott-Cochran-Armitage Test Procedure (sugar beet).
Because no plants mortality was observed in cultivation of corn no computations were performed.
The shoot length:
Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure.
The shoot dry weight:
Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure or Multiple Sequentially- rejective Mediana (2x2- Table) Test After Bonferroni-Holm.

Results and discussions

The ER_{50} and NOER values determined on the basis of emergence of plants, survival of plants, shoot length and shoot dry weight measurements and ER50 values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Sugar beet *Beta vulgaris*	Corn *Zea mays*
Emergence of plants		
ER_{50}	> 300.0*	> 300.0
NOER	> 300.0*	≥ 300.0
Survival of plants		
ER_{50}	> 300.0	> 300.0*
NOER	≥ 300.0	> 300.0*
Shoot length		
ER_{50}	> 300.0	> 300.0
NOER	19.2	≥ 300.0
Shoot dry weight		
ER_{50}	104.9 (88.5 -124.4)	> 300.0
NOER	19.2	≥ 300.0
Plant damages		
ER_{50}	111.4 (98.2 -126.4)	> 300.0

* value was not determined due to mathematical reasons. It is probably above the highest application rate used in the experiment, i.e. 300.0 mL/ha.

Conclusion

On the basis of the obtained results it was proved that the test item i.e. AMINO 30 SL had varied impact on the process of growth of selected plant species.
Mortality of single plants was observed in cultivation of sugar beet.
Delayed emergence of plants in comparison to the control group was not noticed in cultivation of sugar beet and corn.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant emergence during exposure period it was proved that the test item had no impact on seedling emergence of sugar beet and corn.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of sugar beet.
On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot dry weight it was proved that the test item inhibited the process of growth of sugar beet.
During the exposure period, the phytotoxic symptoms after application of the test item were observed in cultivation of sugar beet. In case of corn slightly chlorosis at the rate of 300.0 mL/ha were observed.

Validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of AMINO 30 SL 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% – sugar beet,
100.0% – corn,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

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

No additional studies were performed.

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

No additional studies were performed.

A 2.8 KCP 10.8 Monitoring data

No additional studies were performed.