FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: H-01-2022

Product name(s): Terbutylazyna 500 SC

Chemical active substance:

terbuthylazine, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: ProAgri International Sp. z o.o.

Submission date: April 2024

MS Finalisation date: November 2024; March 2025

Version history

When	What
April 2024	Submission dRR by Applicant
November 2024	Assessment by zRMS
March 2025	The final Registration Report

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter-val between applications (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organ-isms	Bees	Non-target ar-thropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Poland	Maize	F	dicotyledonous weeds For details, please refer to dRR Part B3	broadcast spraying	BBCH 00	a) 1 b) 1	NR	a) 1.0-~~1.5~~ L/ha b) 1.0-~~1.5~~ L/ha	a) 500-~~750~~ g as/ha b) 500-~~750~~ g as/ha	100-400 L/ha	NR	Targeted range: 1.0-~~1.5~~ L/ha every 3 years	**C**	**C**	**R**	**A**	**A**	**C**	**R**
2	Poland	Maize	F		broadcast spraying	BBCH 12-16	a) 1 b) 1	NR	a) 1.0-~~1.5~~ L/ha b) 1.0-~~1.5~~ L/ha	a) 500-~~750~~ g as/ha b) 500-~~750~~ g as/ha	100-400 L/ha	NR	Targeted range: 1.0-~~1.5~~ L/ha every 3 years	**C**	**C**	**R**	**A**	**A**	**C**	**R**

* 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 as 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

*** According to the Regulation (EU) 2021/824 amending Regulations (EU) No 540/2011 and (EU) No 820/2011 the active substance terbuthylazine should be restricted to once every third year on the same field at a maximum rate of 850 g/ha.**

9.1.1 Overall conclusions

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly as text in grey. Not agreed or not relevant information is struck through and shaded for transparency.

The COMMISSION IMPLEMENTING REGULATION (EU) 2021/824 for a.s. terbuthylazine, should be taken into account by MSs in the evaluation of the zonal registration of the product.

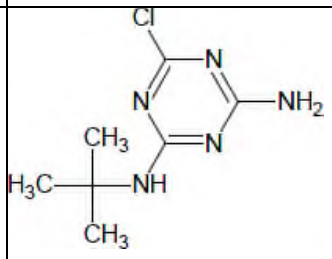
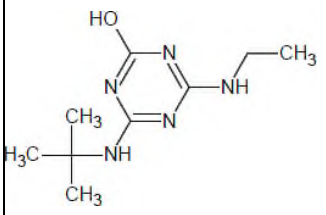
COMMISSION IMPLEMENTING REGULATION (EU) 2021/824 of 21 May 2021 amending Implementing Regulations (EU) No 540/2011 and (EU) No 820/2011 as regards the conditions of approval of the active substance terbuthylazine.

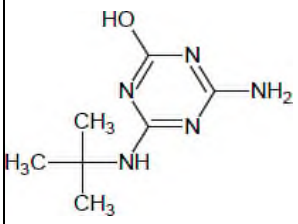
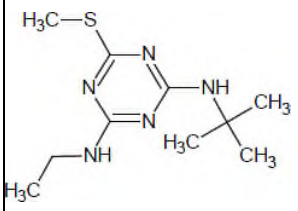
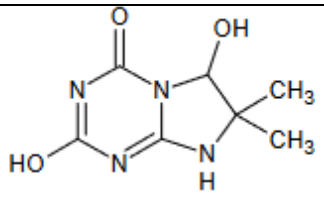
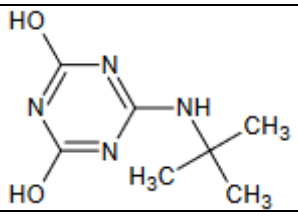
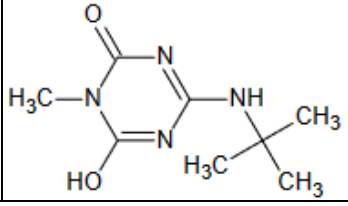
For the implementation of the uniform principles, as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the review report on terbuthylazine, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 17 June 2011 and updated by the Standing Committee on Plants, Animals, Food and Feed on 24 March 2021 shall be taken into account.

In this overall assessment Member States shall pay particular attention to:
- the consumer risk assessment from exposure to metabolites of terbuthylazine,
-the protection of groundwater, when the active substance is applied in regions with vulnerable soil and/or climatic conditions,
- the risk to mammals and earthworms.

Conditions of use shall include risk mitigation measures and the obligation to carry out monitoring programmes to verify potential groundwater contamination in vulnerable zones, where appropriate.

According to the Regulation (EU) 2021/824 amending Regulations (EU) No 540/2011 and (EU) No 820/2011 the active substance terbuthylazine should be restricted to once every third year on the same field at a maximum rate of 850 g/ha.

Metabolite	Molarmass [g/mol]	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
Desethyl- terbuthylazine (MT1)	201.7		Soil: 32.9% Water/sediment: 7.3%	PECsoil PECsw/sed PECgw
Hydroxy- terbuthylazine (MT13)	211.3		Soil: 34.5% Water/sediment: 20%	PECsoil PECsw/sed PECgw

Desethyl hydroxy- terbuthylazine (MT14)	183.2		Soil: 28% Water/sediment: n/a	PECsoil PECgw
Terbutryn(MT26)	241.4		Soil: n/a Water/sediment: 7.4%	PECsoil PECsw/sed PECgw
LM3	198.2		lysimeter metabolite	PECgw
LM5	184.2		lysimeter metabolite	PECgw
LM6	198.2		lysimeter metabolite	PECgw

9.1.1.1 * EFSA Journal 2019;17(9):5817, Updated peer review of the pesticide risk assessment for the active substance terbuthylazine in light of confirmatory data.

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

Birds

Effects on birds for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. However further data on H-01-2022 is not relevant as data for each active substance on toxicity to birds are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for H-01-2022 with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of H-01-2022 to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substances, as well as SV_{90} and SV_m. Since first-tier long-term risk assessment failed, higher-tier evaluation was performed for skylark, wood pigeon and yellow wagtail as focal species. Additionally, drinking water exposure puddle scenario and exposure for earthworm-eating birds and fish-eating birds via secondary poisoning have been performed.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to birds from the proposed use of H-01-2022. No risk management measures are required.

Terrestrial vertebrates (other than birds)

Effects on mammals for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. However further data on H-01-2022 is not relevant as data for each active substance on toxicity to mammals are considered essential. It is possible to extrapolate from data for each active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for H-01-2022 with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

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

The acute and reproductive risks of H-01-2022 to terrestrial vertebrates other than birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with terbuthylazine, as well as SV_{90} and SV_m. Since first-tier long-term risk assessment failed, higher-tier evaluation was performed for wood mouse as focal species. Additionally, drinking water exposure puddle scenario and exposure for earthworm-eating mammals and fish-eating mammals via secondary poisoning have been performed.

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

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Effects on aquatic organisms for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. The studies on effects of H-01-2022 on algae, *Daphnia magna* and aquatic plants were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022.

Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290.

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation H-01-2022. PECsw Step 3/RAC values for active substance were less than 1 for few scenarios so further evaluation with Step 4 PECsw was performed. On the basis of PEC/RAC values it was concluded that the application of H-01-2022 does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied.

For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that H-01-2022 used to protect maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition

that: 5m vegetated buffer zone is applied.

Classification of H-01-2022 was done on the basis of formulation H-01-2022 studies' results as well as active substance and co-formulants properties. The proposed classification of the product H-01-2022 is:

Aquatic Acute 1, H400
Aquatic Chronic 1, H410

9.1.1.3

9.1.1.4	**zRMS comment:**	The risk assessment for aquatic organisms for most sensitive species of each group has been accepted by zRMS. The endpoints have been approved at the European Union level or submitted by the Applicant. The highest value PECsw of scenarios D3, D4, R1 relevant to Poland was used for risk assessment. zRMS proposes refined risk assessment and mitigation measure at National level. The studies on effects of H-01-2022 on algae, *Daphnia magna* and aquatic plants were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290. PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation H-01-2022. PECsw Step 3/RAC values for active substance were less than 1 for few scenarios so further evaluation with Step 4 PECsw was performed. On the basis of PEC/RAC values it was concluded that the application of H-01-2022 does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied. For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that H-01-2022 used to protect maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition that: 5m vegetated buffer zone is applied. Based on the results with consideration FOCUS STEP 4 for scenarios relevant for Poland the following risk mitigation are required: **-5m buffer non-spray zone with 5 meter vegetated filter strip to surface water bodies** Final risk mitigation measures should be considered at MSs level.

9.1.1.5 Effects on bees (KCP 10.3.1)

Effects on bees for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. The studies on effects of H-01-2022 on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022.

Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The risks of H-01-2022 to honeybees was assessed from Hazard Quotients (HQ) and Exposure Toxicity Ratio (ETR) between toxicity endpoints, estimated from acute oral and contact studies with formulated product as well as the maximum single application rate.

All the Hazard Quotients and Exposure Toxicity Ratios were considerably less than the respective triggers, indicating H-01-2022 used to protect maize according to proposed GAP, does not pose unacceptable risk to bees. No risk management measures are required.

9.1.1.6 Effects on arthropods other than bees (KCP 10.3.2)

Effects on non-target arthropods for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. The studies on effects of H-01-2022 on arthropods were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022.

Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the guidance for risk assessment for arthropods "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and in consideration of the recommendations of the guidance document ESCORT 2.

The in-field and off-field risk of H-01-2022 to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from studies with the formulated product H-01-2022 as well as in-field and off-field predicted environmental rate. No risk was determined in-field and off-field after application of H-01-2022 according to proposed GAP. No risk management measures are required.

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

Effects on earthworms and other soil micro-organisms for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. The studies on effects of H-01-2022 on earthworms and other micro and macro-organisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022.

Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Earthworms
The risk of H-01-2022 to earthworms was assessed from toxicity exposure ratios (TERs) between the selected toxicity endpoints for metabolites and the formulated product H-01-2022 as well as the maximum soil PECs.

The chronic TER values were greater than the trigger of 5 indicating an acceptable risk to earthworms following application of H-01-2022 according to proposed GAP. No risk management measures are required.

Folsomia candida* and *Hypoaspis aculeifer
The risk of H-01-2022 to *Folsomia candida* and *Hypoaspis aculeifer* was assessed from toxicity exposure ratios (TERs) between the endpoint for the formulated product H-01-2022 as well as the maximum soil PECs.

The acute and chronic TER values were greater than the trigger of 5 indicating an acceptable risk to *Folsomia candida* and *Hypoaspis aculeifer* following application of H-01-2022 according to proposed GAP. No risk management measures are required.

Micro-organisms
The risk of H-01-2022 to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and the formulated product H-01-2022 with predicted application concentrations (PECs).

Considering to the performed risk assessment it was assessed that the application of H-01-2022 according to proposed GAP does not pose unacceptable risk to soil micro-organisms. No risk management measures are required.

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

Effects on non-target terrestrial plants for H-01-2022 were not evaluated as part of the EU review of terbuthylazine. The studies on seedling emergence and vegetative vigour for H-01-2022 were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022.

The risk of H-01-2022 to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation H-01-2022 and off-field predicted environmental rate.

The risk of H-01-2022 to non-target plants was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation H-01-2022 with application rates. According to the performed risk assessment it was assessed that the application of H-01-2022 at maximum rate of 1.5 L/ha (750 g as/ha) does not pose unacceptable risk to non-target plants provided risk mitigation measures are applied:

- 5m buffer zone or
- 50% nozzle reduction.

In case of lower application rate 1 L/ha no risk mitigation measures are required.

9.1.1.9 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).

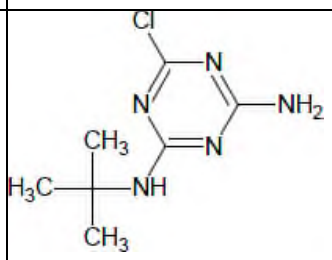
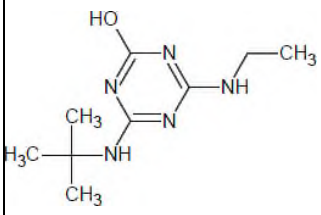
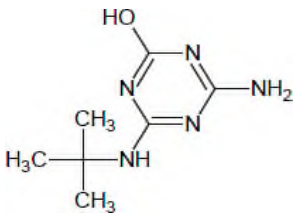
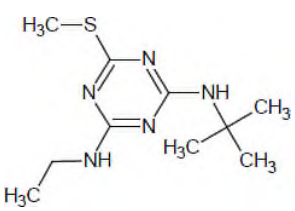
Table 9.1-2: Critical use pattern of H-01-2022 grouped according to criterion

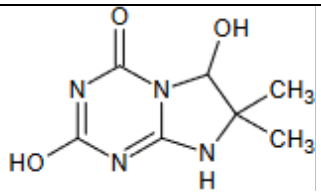
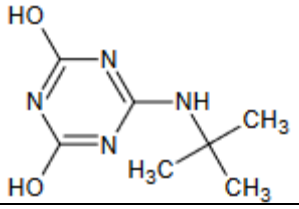
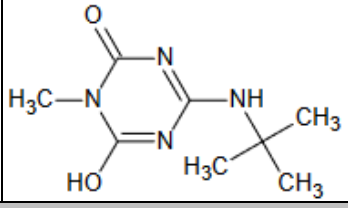
Grouping according to criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	maize	application rate: 750 g as/ha	NR
2	maize	application rate: 500 g as/ha	NR

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 H-01-2022 is indicated in the table.

Table 9.1-3 Metabolites of terbuthylazine

Metabolite	Molar mass [g/mol]	Chemical structure	Maximum observed occurrence in com-partments	Exposure assessment required due to
Desethyl- ter-buthylazine (MT1)	201.7		Soil: 32.9% Water/sediment: 7.3%	PECsoil PECsw/sed PECgw
Hydroxy- ter-buthylazine (MT13)	211.3		Soil: 34.5% Water/sediment: 20%	PECsoil PECsw/sed PECgw
Desethyl hy-droxy- ter-buthylazine (MT14)	183.2		Soil: 28% Water/sediment: n/a	PECsoil PECgw
Terbutryn (MT26)	241.4		Soil: n/a Water/sediment: 7.4%	PECsoil PECsw/sed PECgw

LM3	198.2		lysimeter metabolite	PECgw
LM5	184.2		lysimeter metabolite	PECgw
LM6	198.2		lysimeter metabolite	PECgw

* EFSA Journal 2019;17(9):5817, Updated peer review of the pesticide risk assessment for the active substance terbuthylazine in light of confirmatory data.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. However, the provision of further data on the H-01-2022 is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	terbuthylazine	Acute	**LD_{50} =1236 mg as/kg bw**	EFSA Journal 2011; 9(1):1969
Bobwhite quail	'Terbuthylazine 500 g/L SC'	Acute	LD_{50} >909 mg as/kg bw	EFSA Journal 2011; 9(1):1969
Mallard duck	terbuthylazine	Short-term	LC_{50} > 395 mg as/kg bw	EFSA Journal 2011; 9(1):1969
Japanese quail	terbuthylazine	Long-term	**NOEL = 13.85 mg as/kg bw**	EFSA Journal 2011; 9(1):1969

9.2.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: Screening and first-tier assessment of the acute and long-term risk for birds due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize				
Active substance	terbuthylazine				
Acute toxicity (mg/kg bw)	1236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
BBCH 00 750 g as/ha	small granivorous bird	24.7	1	18.53	66.70
BBCH 12-16 750 g as/ha	small omnivorous bird	158.8	1	119.1	10.38
Reprod. toxicity (mg/kg bw/d)	13.86				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
BBCH 00 750 g as/ha	small granivorous bird	11.4	1 x 0.53	4.53	**3.06**
BBCH 12-16 750 g as/ha	small omnivorous bird	64.8	1 x 0.53	25.76	**0.54**
First tier assessment					
BBCH 00 750 g as/ha	Small granivorous bird “finch” Linnet (*Carduelis cannabina*)	11.4	1 x 0.53	4.53	**3.06**
	Small omnivorous bird “lark” Woodlark (*Lullula arborea*)	8.2	1 x 0.53	3.26	**4.25**
	Small insectivorous bird “wag-tail”	5.9	1 x 0.53	2.35	5.90

	Yellow wagtail (*Motacilla flav*a)				
BBCH 12-16 750 g as/ha	Medium granivorous bird “gamebird” Partridge (*Perdix perdix*)	3.0	1 x 0.53	1.19	11.65
	Small insectivorous/ worm feeding species “thrush” Robin (*Erithacus rubecula*)	5.7	1 x 0.53	2.27	6.11
	Small omnivorous bird “lark” Woodlark (*Lullula arborea*)	10.9	1 x 0.53	4.33	**3.20**
	medium herbivorous/ granivorous bird “pigeon” Wood pigeon (*Columba palumbus*)	22.7	1 x 0.53	9.02	**1.54**
	Small insectivorous bird “wagtail” Yellow wagtail (*Motacilla flava*)	11.3	1 x 0.53	4.49	**3.09**

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

Table 9.2-3: Screening and first-tier assessment of the acute and long-term risk for birds due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize				
Active substance	terbuthylazine				
Acute toxicity (mg/kg bw)	1236				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
BBCH 00 500 g as/ha	small granivorous bird	24.7	1	12.35	100.08
BBCH 12-16 500 g as/ha	small omnivorous bird	158.8	1	79.4	15.57
Reprod. toxicity (mg/kg bw/d)	13.86				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
BBCH 00 500 g as/ha	small granivorous bird	11.4	1 x 0.53	3.02	4.59
BBCH 12-16 500 g as/ha	small omnivorous bird	64.8	1 x 0.53	17.17	**0.81**
First tier assessment					
BBCH 00 500 g as/ha	Small granivorous bird “finch” Linnet (*Carduelis cannabina*)	11.4	1 x 0.53	3.02	**4.59**
	Small omnivorous bird “lark”	8.2	1 x 0.53	2.17	6.39

	Woodlark (*Lullula arborea*)				
	Small insectivorous bird "wag-tail" Yellow wagtail (*Motacilla flav*a)	5.9	1 x 0.53	1.56	8.88
BBCH 12-16 500 g as/ha	Medium granivorous bird "gamebird" Partridge (*Perdix perdix*)	3.0	1 x 0.53	0.8	17.33
	Small insectivorous/ worm feeding species "thrush" Robin (*Erithacus rubecula*)	5.7	1 x 0.53	1.51	9.18
	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	10.9	1 x 0.53	2.89	**4.8**
	medium herbivorous/ granivorous bird "pigeon" Wood pigeon (*Columba palumbus*)	22.7	1 x 0.53	6.02	**2.3**
	Small insectivorous bird "wagtail" Yellow wagtail (*Motacilla flava*)	11.3	1 x 0.53	2.99	**4.64**

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

In accordance with DAR for refinement of the long risk assessment to birds for three species were considered relevant: skylark, wood pigeon and wagtail and considered in higher-tier risk assessment.

Skylark (*Alauda arvensis*)

In accordance with terbuthylazine Addendum to DAR skylark diet is very variable. However, it is always mixed i.e. not composed of single component so not using singe diet approach was evaluated as valid. For the purpose of risk assessment, diet according to results of generic field study of Wolf (2005) were selected as a study was done in maize in April-May. The skylark diet consists of insects 81%, seedlings 2.6%, seeds 16.4%. In accordance with terbuthylazine Addendum to DAR FIR for skylark is 21.01 g_{wet}/day and body weight 37.2 g hence FIR/bw is 0.57. The mean residues in insects (RUD of 1.93) and maize leaves (RUD of 34.5) was reported Addendum to DAR. RUD in weed seeds were used in accordance with EFSA Journal 2009; 7(12):1438, Appendix F, RUD table for different food items.

Table 9.2-4: Higher-tier assessment of long term exposure for skylark (*Alauda arvensis*)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	13.86
TER criterion	5

Crop scenario Growth stage	**PD/diet type**	**FIR/bw**	**RUDm**	**PT × DF**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Maize BBCH 00 & BBCH 12-16 750 g as/ha	Invertebrates, 81%	0.57[1]	1.93[2]	1 x 1	1 x 0.53	0.35	
	Weed seeds, 16.4%		40.2[3]	1 x 1	1 x 0.53	1.49	

	Leaves (maize & weeds), 2.6%		34.5[4]	1 x 1	1 x 0.53	0.17	
						2.01	6.79
Maize BBCH 00 & BBCH 12-16 500 g as/ha	Invertebrates, 81%	0.57[1]	1.93[2]	1 x 1	1 x 0.53	0.24	
	Weed seeds, 16.4%		40.2[3]	1 x 1	1 x 0.53	1	
	Leaves (maize & weeds), 2.6%		34.5[4]	1 x 1	1 x 0.53	0.14	
						1.38	10.04

[1] RIR/bw (Addendum to DAR)
[2] mean measured residues from a field study (Bakker, 2006) standardised to 1kg as/ha (Addendum to DAR)
[3] EFSA Journal 2009; 7(12):1438, Appendix F
[4] mean initial residues from 13 residue trials in maize standardised to 1kg as/ha (Addendum to DAR)

Wood pigeon (*Columb apalumbus*)

It was assumed wood pigeon diet consists of single component leaves (treated maize). In accordance with EFSA Journal 2009; 7(12):1438, Appendix F wood pigeon FIR/bw is 0.79. The mean residues in maize leaves (RUD of 34.5) was reported Addendum to DAR. Additionally, in accordance with Addendum to DAR twa value in maize is 0.19.

Table 9.2-5: Higher-tier assessment of long term exposure for wood pigeon (*Columb apalumbus*)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	13.86
TER criterion	5

Crop scenario Growth stage	**PD/diet type**	**FIR/bw**	**RUDm**	**PT × DF**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Maize BBCH 00 & BBCH 12-16 750 g as/ha	Leaves, 100%	0.79[1]	34.5[2]	1 x 1	1 x 0.19[3]	3.88	**3.57**
Maize BBCH 00 & BBCH 12-16 500 g as/ha	Leaves, 100%	0.79[1]	34.5[2]	1 x 1	1 x 0.19[3]	2.59	5.35

[1] EFSA Journal 2009; 7(12):1438, Appendix F
[2] mean initial residues from 13 residue trials in maize standardised to 1kg as/ha (Addendum to DAR)
[3] ftwa value for maize based on residu levels and dissipations times in maize (Addendum to DAR)

In case of higher rate 750 g as/ha further risk refinement is needed.

The woodpigeon *Columba palumbus* is a widespread and common or abundant species in agricultural and forested landscapes, and partly also in urban areas. Woodpigeons feed on a wide range of plant material, with seeds or green leaves dominating, depending on season. Seeds from newly sown cereal, pea or rape fields and all types of grain from stubble fields are apparently preferred when available. In winter, green leaves of broad-leaved crops (oil-seed rape) and different weeds are important but beech mast, acorns etc. may also be significant during autumn and win-ter. The summer diet is highly variable and may include up to 5% invertebrates (Christensen et al. 1996). Ljunggren (1968) studied adult crop contents in a rural

population of woodpigeons in SW Sweden. The results are presented as percentage of food items (by number)

According to Appendix G of EFSA/2009/1438 ratios of daily intake rate to body weight based on given diet composition was calculated.

Table 9.2-6: Calculation of daily intake and FIR/bw ratio for a woodpigeon of body mass 490 g

Food category	PD (% of diet)	FE (kJ/ dry g)	MC (%)`	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Rape seeds	28	21.7	9.9	0.76	4.16		10.03	0.02
Cereal grain	26	18.4	14.7	0.76	3.10		9.31	0.02
Peas	16	21.7	9.9	0.76	2.38		5.73	0.01
Weed seeds	15	21.7	9.9	0.76	2.23		5.37	0.01
Plant leaves	13	17.6	76.4	0.53	0.29		4.66	0.01
Total	-	-	-	-	12.15	435.25	-	-

Table 9.2-7: Higher-tier assessment of long term exposure for wood pigeon (*Columb apa-lumbus*)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	13.86
TER criterion	5

Crop scenario Growth stage	PD/diet type	FIR/bw	RUDm	PT × DF	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt
Maize BBCH 00 & BBCH 12-16 750 g as/ha	Rape seeds , 28%	0.02	28.7	1 x 1	1 x 0.53	0.228	
	Cereal grain , 26%	0.02	15.0	1 x 1	1 x 0.53	0.119	
	Peas, 16%	0.01	28.7	1 x 1	1 x 0.53	0.114	
	Weed seeds, 15%	0.01	28.7	1 x 1	1 x 0.53	0.114	
	Plant leaves, 13%	0.01	54.2	1 x 1	1 x 0.53	0.215	
						0.79	17.54

Yellow wagtail (*Motacilla flava*)

It was assumed wagtail diet consists of ground dwelling arthropods 75% and foliar dwelling arthropods 25%. In accordance with EFSA Journal 2009; 7(12):1438, Appendix F wagtail FIR/bw is 0.79.

Table 9.2-8: Higher-tier assessment of long term exposure for yellow wagtail (*Motacilla flava*)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	13.86
TER criterion	5

Crop scenario Growth stage	PD/diet type	FIR/bw	RUDm	PT × DF	MAFm × TWA	DDDm (mg/kg bw/d)	TERlt

Maize BBCH 00 & BBCH 12-16 750 g as/ha	Foliar arthropods, 25%	0.79	21	1 x 1	1 x 0.53	1.65	
	Ground-dwelling arthropods, 75%	0.79	1.93[1]	1 x 1	1 x 0.53	0.45	
						2.1	6.6
Maize BBCH 00 & BBCH 12-16 500 g as/ha	Foliar arthropods, 25%	0.79	21	1 x 1	1 x 0.53	1.1	
	Ground-dwelling arthropods, 75%	0.79	1.93[1]	1 x 1	1 x 0.53	0.3	
						1.4	9.9

[1] mean measured residues from a field study (Bakker, 2006) standardised to 1kg as/ha (Addendum to DAR)

zRMS comment:	Evaluator agrees with risk assessment for birds submitted by Applicant. Refinement risk assessment for birds has been accepted by RMS except for for maximum dose rate at 750 g s.a./ha performed by Applicant for wood pigeon based on the PD refinement value based on Ljunggren (1968). The refinement risk assessment for mammals for maximum dose rate at 750 g s.a./ha performed by Applicant for wood pigeon based on the PD refinement value based on Ljunggren (1968) study may be questioned. The Applicant should complete the informations: 1. Justification to use the study from Sweden (northern zone). 2. Please also discuss if the PD study was performed in a maize environment, in the correct season. Was the diet based on volume percentages, or mass percentage or something else? Were correct conversion factors be considered? Etc Please check appendix Q in the guidance. The refinement risk assessment for birds should be considered by MSs level.

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 H-01-2022 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). With a K(f)oc of 14-345, terbuthylazine belongs to the group of less sorptive substances.

Effective application rate (g/ha) = 750	

Acute toxicity (mg/kg bw)	= 1236		quotient	= 0.61
Reprod. toxicity (mg/kg bw/d)	= 13.86		quotient	= **54.11**

Effective application rate (g/ha)	= 500			
Acute toxicity (mg/kg bw)	= 1236		quotient	= 0.40
Reprod. toxicity (mg/kg bw/d)	= 13.86		quotient	= 36.08

Table 9.2-9: Assessment of the risk for birds due to exposure to terbuthylazine via contaminated drinking water in puddles

Intended use	maize
Active substance	terbuthylazine
Application rate (g/ha)	750 & 500
Acute toxicity (mg/kg bw)	1236
TER criterion	10
Reprod. toxicity (mg/kg bw/d)	13.86
TER criterion	5

Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**Daily dose (mg/kg bw/d)**	**TER_a** / **TER_{lt}**
750	151	0.30	0.46	0.14	8828.57
					99
500	151	0.20	0.46	0.09	13733.33
					154

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

zRMS comment:	Evaluator agrees with risk assessment for birds submitted by Applicant. Refinement risk assessment for birds has been accepted by RMS. The all refinement TER_{LT} values were above trigger of 5 indicating acceptable risk for birds. Toxicity endpoints was approved by EU level.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} values of terbuthylazine is 3.4 and it exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

Table 9.2-10: Assessment of the risk for earthworm-eating birds due to exposure to terbuthylazine bioaccumulation in earthworms (secondary poisoning)

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.859	0.573	21d TWA PECs, dRR Part B8
log P_{ow} / P_{ow}	3.4	3.4	-
Koc	151	151	Confirmatory Data Terbuthylazine November 2015
foc	0.02	0.02	default
BCF_{worm}	10.26	10.26	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	8.81	5.88	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	9.25	6.17	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	13.86	13.86	-
TER_{lt}	**1.5**	**2.25**	-

TER values shown in bold fall below the relevant trigger.

The risk assessment was refined using earthworm bioaccumulation study presented in respective EU DAR where earthworms (*Eisenia fetida*) were exposed to soils spiked with terbuthylazine for 28 days followed by an elimination phase (7 days). The test item was an SE formulation containing 17.5% w/w terbuthylazine and 29.1% w/w S-metolachlor. The test item was mixed homogeneously into artificial soil (550 g dwt soil) at nominal concentrations of 0.16 and 1.6 mg terbuthylazine/kg dry soil. The uptake of terbuthylazine was low, with concentrations in worms always being less than that of the soil (BAF<1). The mean BAF during uptake was 0.86 (low) and 0.58 (high). RMS considered this study as acceptable for use in the refined risk assessment for earthworm-eating birds. A new refined risk assessment has been performed using the measured BAF (0.86) in place of the BCF calculated above (equal 6.71 which is highly overestimated value).

Table 9.2-11: Assessment of the risk for earthworm-eating birds due to exposure via bioaccumulation in earthworms (secondary poisoning) – higher tier risk assessment

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.859	0.573	21d TWA PECs, dRR Part B8
log P_{ow} / P_{ow}	3.4	3.4	-
Koc	151	151	Confirmatory Data Terbuthylazine November 2015
foc	0.02	0.02	default
BAF_{worm}	0.86	0.86	measured BAF, DAR Terbuthylazine Volume 3 B9 2010
PEC_{worm}	0.74	0.49	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.78	0.51	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	13.86	13.86	-
TER_{lt}	17.77	27.18	-

zRMS comment:	Agreed. The refinement risk assessment for birds should be considered by MSs level.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of terbuthylazine in water.

Table 9.2-12: Assessment of the risk for fish-eating birds due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning)

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.04104	0.02735	21d TWA PECsw Step 2 (pre-emergance application, worst case), dRR Part B8
BCF_{fish}	34	34	DAR Section 9.2.4.9
BMF	NR	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.40	0.93	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.22	0.15	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	13.86	13.86	-
TER_{lt}	63	92.4	-

zRMS comment:	Agreed.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. H-01-2022 used to protect maize according to proposed GAP, does not pose unacceptable risk to birds.

zRMS comment:	Evaluator agrees with risk assessment for birds submitted by Applicant. Refinement risk assessment for birds has been accepted by RMS. The all refinement TER_{LT} values were above trigger of 5 indicating acceptable risk for birds. Toxicity endpoints was approved by EU level. The refinement risk assessment for birds should be considered by MSs level.

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

Effects on mammals of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. However, the provision of further data on the H-01-2022 is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	terbuthylazine	Oral, Acute	**LD_{50} = 1000** -1590 **mg as/kg bw**	EFSA Journal 2011; 9(1):1969 EFSA Journal 2011; 9(1):1969
Rat	MT14	Oral, Acute	LD_{50} > 2000 mg/kg bw	EFSA Journal 2011; 9(1):1969
Rat	MT13	Oral, Acute	LD_{50} > 2000 mg/kg bw	EFSA Journal 2011; 9(1):1969
Rat	MT1	Oral, Acute	LD_{50} =236 mg/kg bw	EFSA Journal 2011; 9(1):1969
Rat	terbuthylazine	Oral, Long-term	**NOAEL = 3.3 mg/kg bw/d**	EFSA Journal 2011; 9(1):1969

9.3.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: Screening and first-tier assessment of the acute and long-term risk for mammals due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize				
Active substance/product	terbuthylazine				
Acute toxicity (mg/kg bw)	1000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
Bare soil BBCH 00 750 g as/ha	small granivorous mammal	14.4	1	10.80	92.59
Maize BBCH 12-16 750 g as/ha	small herbivorous mammal	136.4	1	102.30	**9.78**
First tier assessment					
Maize BBCH 10-19 750 g as/ha	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	7.6	1	5.70	175.44
Maize BBCH 10 -29 750 g as/ha	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	136.4	1	102.30	**9.78**
Maize BBCH 10-29 750 g as/ha	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	17.2	1	12.90	77.52
Reprod. toxicity (mg/kg bw/d)	3.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
Bare soil BBCH 00 750 g as/ha	small granivorous mammal	6.6	1 x 0.53	2.62	**1.26**
Maize BBCH 12-16 750 g as/ha	small herbivorous mammal	72.3	1 x 0.53	28.74	**0.11**
First tier assessment					
Bare soil BBCH 00 750 g as/ha	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	5.7	1 x 0.53	2.27	**1.45**
Maize BBCH 10-19 750 g as/ha	Small insectivorous mammal "shrew" Common shrew (*Sorex araneus*)	4.2	1 x 0.53	1.67	**1.98**
Maize BBCH 10 -29 750 g as/ha	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	72.3	1 x 0.53	28.74	**0.11**
Maize BBCH 10-29 750 g as/ha	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	3.9	1 x 0.53	1.55	**2.13**

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

Table 9.3-3: Screening and first-tier assessment of the acute and long-term risk for mammals due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize				
Active substance/product	terbuthylazine				
Acute toxicity (mg/kg bw)	1000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
Bare soil BBCH 00 500 g as/ha	small granivorous mammal	14.4	1	7.2	138.89
Maize BBCH 12-16 500 g as/ha	small herbivorous mammal	136.4	1	68.2	14.66
Reprod. toxicity (mg/kg bw/d)	3.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening assessment					
Bare soil BBCH 00 500 g as/ha	small granivorous mammal	6.6	1 x 0.53	1.75	**1.89**
Maize BBCH 12-16 500 g as/ha	small herbivorous mammal	72.3	1 x 0.53	19.16	**0.17**
First tier assessment					
Bare soil BBCH 00 500 g as/ha	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)	5.7	1 x 0.53	1.51	**2.19**
Maize BBCH 10-19 500 g as/ha	Small insectivorous mammal “shrew” Common shrew (*Sorex araneus*)	4.2	1 x 0.53	1.11	**2.97**
Maize BBCH 10 -29 500 g as/ha	Small herbivorous mammal “vole” Common vole (*Microtus arvalis*)	72.3	1 x 0.53	19.16	**0.17**
Maize BBCH 10-29 500 g as/ha	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)	3.9	1 x 0.53	1.03	**3.2**

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

Table 9.3-4: Screening and first-tier assessment of the acute and long-term risk for mammals due to the use of H-01-2022 in maize – exposure to metabolites (max. application rate 500 g as/ha)

Intended use	maize
Metabolite	MT1 / MT13 / MT14 / MT20
MT1 acute toxicity (mg/kg bw)	236

TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
Bare soil BBCH 00 0.144 kg MT1/ha	small granivorous mammal	14.4	1	2.07	114.01
Maize BBCH 12-16 0.144 kg MT1/ha	small herbivorous mammal	136.4	1	19.64	12.02
MT13 acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
Bare soil BBCH 00 0.159 kg MT13/ha	small granivorous mammal	14.4	1	2.29	873.36
Maize BBCH 12-16 0.159 kg MT13/ha	small herbivorous mammal	136.4	1	21.69	92.21
MT14 acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening assessment					
Bare soil BBCH 00 0.112 kg MT14/ha	small granivorous mammal	14.4	1	1.61	1242.24
Maize BBCH 12-16 0.112 kg MT14/ha	small herbivorous mammal	136.4	1	15.28	130.89

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

zRMS comment:	Evaluator agrees with risk assessment for mammals at Tier 1 submitted by Applicant. Toxicity endpoints was approved by EU level.

Higher-tier risk assessment

In accordance with Additional Report to the DAR a generic field data, monitoring/trapping of mammals and radio-tracking of wood mice in maize growing farmland area, are available. The study was conducted in spring in the "Tullnerfeld' region to the west of Vienna in Austria. Based on results it was concluded that vole and shrew were not species occurring regularly in maize during pre-emergence and early post emergence and that wood mouse is the relevant focal species for these exposure scenarios.

Wood mouse (*Apodemus sylvaticus*)

Residue Unit Dose (RUD)

A field residue trials submitted in DAR for terbuthylazine have been used in refinement of risk assessment. The application rate was equivalent 844 g terbuthylazine/ha and the maize had been sown four days

before application. The residues of terbuthylazine and its metabolite desethyl-terbuthylazine in arable crop dwelling invertebrates and weeds were determined following a single application of the formulation containing nominally 500 g terbuthylazine/L. On the basis of results, the mean residues for all invertebrates were calculated to be 1.5 mg terbuthylazine/kg and 0.18 mg desethyl-terbuthylazine/kg. Total residues 1.63 mg/kg equivalent to a **vertebrates RUD of 1.93 mg/kg**. The DT50 for terbuthylazine in invertebrates was calculated as 9.98 days (ftwa of 0.53). The study has been evaluated and accepted by the RMS.

The RUD in earthworms has been calculated as PECworm=PECsoil·BAF using BCF of 0.86 from the earthworm bioaccumulation field study and 21 day TWA PECsoil of 0.859 and 0.573 mg as/kg soil following a single application of 0.75 and 0.5 kg as/ha, respectively. The **RUD in earthworms equals 0.74 and 0.49** for 0.75 and 0.5 kg as/ha, respectively.

Additionally, residue field studies in maize were submitted and evaluated in the DAR for terbuthylazine. A field study was also submitted to evaluate these residues and dissipation for weeds. Based on results it was concluded that **RUD in maize equals 34.5 mg/kg**. DT_{50} for terbuthylazine in maize equals 2.8 days resulting in reduced **TWA 0.19** that has been applied to vegetative plant tissue i.e. maize and other plants**.** Residues in other food type used in risk refinment was assumed in accordance with DAR for terbuthylazine as **RUD 24.5** for vegetative plant tissue (mean measured residues from field studies evaluated in DAR), **RUD 15.0** for cereal grain and **RUD 40.2** dicotyledon seeds (RUD values included in Appendix F, EFSA Journal 2009; 7(12):1438).

Proportion of diet obtained in habitant treated with pesticide (PT)

In accordance with Additional Report to EU DAR, additional data concerning the time budgets (PT) of the mammals trapped in each crop are present. In germinated maize, two individuals were tracked, with one of them being tracked twice. The PT values were 0, 3.1 and 3.8%, resulting in average PT of 2.3%. Wood mice spent an average PT of 16.6% on plain fields (5.3-43.8%). For the risk refinement **PT of 43.8%** was used.

Deposition factor (DF)
In higher tier risk assessment interception factors of 0 and 25% were applied for pre-emergence and post-emergence, respectively.

Diet and ratios of daily intake rate to body weight (FIR/bw)

Diet of wood mouse was based on information obtained from EU DAR (Peiz 1989). The ratios of daily intake rate to body weight (FIR/bw) were calculated based Appendix G to EFSA Journal 2009; 7(12):1438. The mouse body mass of 21.7 g was assumed (Appendix A, EFSA Journal 2009; 7(12):1438).

Table 9.3-5: Calculation of FIR/bw ratios for a wood mouse (body mass 21.7g)

Food category	PD (% of diet)	FE (kJ/ dry g)	MC (%)	AE (%)	FE (kJ/g fresh)	DEE (kJ)	Daily intake rate (g fresh weight/d)	FIR/bw
Pre-emergence application								
Insects	10	19.4	84.3	87	0.26	-	0.95	0.04
Earthworms	40	19.4	84.3	87	1.06	-	3.80	0.18
Vegetative plant tissue	16	17.8	88.1	76	0.26	-	1.52	0.07
Cereal grain	30	18.4	14.7	84	3.96	-	2.83	0.13

Dicotyledon seeds	4	21.7	9.9	84	0.66	-	0.38	0.02
Total	-	-			6.25	58.83	-	-
Post-emergence application								
Insects	10	19.4	84.3	87	0.26	-	0.94	0.04
Earthworms	40	19.4	84.3	87	1.06	-	3.77	0.17
Vegetative plant tissue	16	17.6	76.4	47	0.31	-	1.51	0.07
Cereal grain	30	18.4	14.7	84	3.96	-	2.82	0.13
Dicotyledon seeds	4	21.7	9.9	84	0.66	-	0.38	0.02
Total	-	-			6.25	58.83	-	-

Table 9.3-6: Higher-tier assessment of long-term exposure for wood mouse (*Apodemus sylvaticus*) (max. application rate 750 g as/ha)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	3.3
TER criterion	5

Crop scenario Growth stage	**PD/diet type**	**FIR/bw**	**RUDm**	**DF**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Maize BBCH 00 750 g as/ha	Insect larvae, 10%	0.04	1.93	1	0.53	0.031	
	Earthworms, 40%	0.18	0.74	1	0.53	0.053	
	Vegetative plant tissue, 16%	0.07	24.5	1	0.19	0.244	
	Cereal grain , 30%	0.13	15.0	1	0.53	0.775	
	Dicotyledon seeds (herb), 4%	0.02	40.2	1	0.53	0.320	
						1.423	**2.32**
						Additional PT=43.8%	5.29
Maize BBCH 12-16 750 g as/ha	Insect larvae, 10%	0.04	1.93	~~0.75~~ 1	0.53	~~0.023~~ 0.03	
	Earthworms, 40%	0.17	0.74	~~0.75~~ 1	0.53	~~0.038~~ 0.05	
	Vegetative plant tissue, 16%	0.07	34.5	~~0.25~~ 1	0.19	~~0.086~~ 0.344	
	Cereal grain, 30%	0.13	15.0	~~0.75~~ 1	0.53	~~0.581~~ 0.77	
	Dicotyledon seeds, 4%	0.02	40.2	~~0.75~~ 1	0.53	~~0.240~~ 0.32	

	~~0.968~~ 1.514	~~**3.41**~~ **2.18**
Additional PT=43.8%		~~7.78~~ **4.97**

Table 9.3-7: Higher-tier assessment of long-term exposure for wood mouse (*Apodemus sylvaticus*) (max. application rate 500 g as/ha)

Intended use	maize
Active substance/product	terbuthylazine
Reprod. toxicity (mg/kg bw/d)	3.3
TER criterion	5

Crop scenario Growth stage	**PD/diet type**	**FIR/bw**	**RUDm**	**DF**	**MAFm × TWA**	**DDDm (mg/kg bw/d)**	**TERlt**
Maize BBCH 00 500 g as/ha	Insect larvae, 10%	0.04	1.93	1	0.53	0.020	
	Earthworms, 40%	0.18	0.49	1	0.53	0.023	
	Vegetative plant tissue, 16%	0.07	24.5	1	0.19	0.454	
	Cereal grain , 30%	0.13	15.0	1	0.53	0.517	
	Dicotyledon seeds (herb), 4%	0.02	40.2	1	0.53	0.213	
						1.227	**2.69**
						Additional PT=43.8%	6.14
Maize BBCH 12-16 500 g as/ha	Insect larvae, 10%	0.04	1.93	~~0.75~~ 1	0.53	~~0.015~~ 0.02	
	Earthworms, 40%	0.17	0.49	~~0.75~~ 1	0.53	~~0.017~~ 0.0226	
	Vegetative plant tissue, 16%	0.07	34.5	~~0.25~~ 1	0.19	~~0.057~~ 0.228	
	Cereal grain, 30%	0.13	15.0	~~0.75~~ 1	0.53	~~0.388~~ 0.517	
	Dicotyledon seeds, 4%	0.02	40.2	~~0.75~~ 1	0.53	~~0.160~~ 0.21	
						~~0.637~~ 0.9976	~~**5.18**~~ **3.3**
						Additional PT=43.8%	~~11.83~~ 7.5

zRMS comment:	The refinement risk assessment for mammals was corrected by zRMS. According to both EFSA 2009 and EFSA 2023 as well as the zonal agreements, interception cannot be considered in early stages. Also for the use of pre-emergence application and post- emergence exposure, interception should not be used. The TER for maximum dose rate 750 g s.s./ha is slightly below trigger value 5 (4.97). In opinion this TER should be accepted. The further risk assessment is not needed. The refinement risk assessment for mammals should be considered by MSs level.

9.3.2.2 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 14-345, terbuthylazine belongs to the group of less sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the risk assessment for the maximum application rate of 750 g as/ha covers the risk for mammals from the lower rate of 500 g as/ha.

Effective application rate (g/ha)	= 750			
Acute toxicity (mg/kg bw)	= 1000		quotient	= 0.75
Reprod. toxicity (mg/kg bw/d)	= 3.3		quotient	= **227.27**

Effective application rate (g/ha)	= 500			
Acute toxicity (mg/kg bw)	= 1000		quotient	= 0.50
Reprod. toxicity (mg/kg bw/d)	= 3.3		quotient	= **151.52**

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) exceeds the critical value of 50, a quantitative risk assessment (calculation of TER values) is necessary.

zRMS comment:	Agreed.

Table 9.3-8: Assessment of the risk for mammals due to exposure to terbuthylazine via contaminated drinking water in puddles

Intended use	maize
Active substance	terbuthylazine
Application rate (g/ha)	750 & 500
Acute toxicity (mg/kg bw)	1000

TER criterion		10			
Reprod. toxicity (mg/kg bw/d)		3.3			
TER criterion		5			
Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**Daily dose (mg/kg bw/d)**	**TER_a**
					TER_{lt}
750	151	0.30	0.05	0.02	26000
					165
500	151	0.20	0.05	0.01	52000
					330
zRMS comment:	Agreed.				

9.3.2.3 Effects of secondary poisoning

The log P_{ow} values of terbuthylazine is 3.4 and it exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to terbuthylazine via bioaccumulation in earthworms (secondary poisoning)

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.859	0.573	21d TWA PECs, dRR Part B8
log P_{ow} / P_{ow}	3.4	3.4	-
Koc	151	151	Confirmatory Data Terbuthylazine November 2015
foc	0.02	0.02	default
BCF_{worm}	10.26	10.26	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	8.81	5.88	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	11.28	7.53	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	3.3	3.3	-
TER_{lt}	**0.29**	**0.44**	-

TER values shown in bold fall below the relevant trigger.

The risk assessment was refined using earthworm bioaccumulation study presented in respective DAR for terbuthylazine where earthworms (*Eisenia fetida*) were exposed to soils spiked with terbuthylazine for 28 days followed by an elimination phase (7 days). The test item was an SE formulation containing 17.5% w/w terbuthylazine and 29.1% w/w S-metolachlor. The test item was mixed homogeneously into artificial soil (550 g dwt soil) at nominal concentrations of 0.16 and 1.6 mg terbuthylazine/kg dry soil. The uptake

of terbuthylazine was low, with concentrations in worms always being less than that of the soil (BAF<1). The mean BAF during uptake was 0.86 (low) and 0.58 (high). RMS considered this study as acceptable for use in the refined risk assessment for earthworm-eating birds. A new refined risk assessment has been performed using the measured BAF (0.86) in place of the BCF calculated above (equal 6.71 which is highly overestimated value).

Table 9.3-10: Assessment of the risk for earthworm-eating mammals due to exposure via bioaccumulation in earthworms (secondary poisoning) – higher tier risk assessment

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.859	0.573	21d TWA PECs, dRR Part B8
log P_{ow} / P_{ow}	3.4	3.4	-
Koc	151	151	Confirmatory Data Terbuthylazine November 2015
foc	0.02	0.02	default
BAF_{worm}	0.86	0.86	measured BAF, DAR Terbuthylazine Volume 3 B9 2010
PEC_{worm}	0.74	0.49	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.95	0.63	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	3.3	3.3	-
TER_{lt}	**3.47**	5.24	-

The TER_{lt} for the maximum rate of 750 g as/ha is above the trigger value of 5 indicating unacceptable risk. However, in accordance with DAR for terbuthylazine no mammal species with 100% earthworm diet occurred in germinated maize field during the proposed application period (Wolf, 2005). Therefore, the risk assessment can be addressed using wood mouse as a focal species, for which the acceptable risk has been identified above.

zRMS comment:	The risk for earthworm-eating mammals due to exposure via bioaccumulation in earthworms (secondary poisoning) – higher tier risk assessment is accepted by zRMS. However, TER_{lt} for the maximum rate of 750 g as/ha is still above the trigger value of 5 indicating unacceptable risk. The justification provided by Applicant: "*in accordance with DAR for terbuthylazine no mammal species with 100% earthworm diet occurred in germinated maize field during the proposed application period (Wolf, 2005). Therefore, the risk assessment can be addressed using wood mouse as a focal species, for which the acceptable risk has been identified above*" is not sufficient. The risk for earthworm-eating mammals due to exposure via bioaccumulation in earthworms (secondary poisoning) for the maximum rate of 750 g as/ha should be provided or the maximum dose should be reduced to 500 g/ha. The refinement risk assessment for mammals should be considered by MSs level.

Risk assessment for fish-eating mammals via secondary poisoning

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

Table 9.3-11: Assessment of the risk for fish-eating mammals due to exposure to terbuthylazine via bioaccumulation in fish (secondary poisoning)

Parameter	Terbuthylazine 750 g/ha	Terbuthylazine 500 g/ha	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.04104	0.02735	21d TWA PECsw Step 2 (pre-emergance application, worst case), dRR Part B8
BCF_{fish}	34	34	-
BMF	NR	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.40	0.93	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.20	0.13	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	3.3	3.3	-
TER_{lt}	16.5	25.38	-

zRMS comment:	Agreed.

9.3.2.4 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

All the TER values exceed the trigger values of 10 for acute and 5 for reproductive/long-term risk. H-01-2022 used to protect maize according to proposed GAP, does not pose unacceptable risk to mammals.

zRMS comment:	Evaluator agrees with risk assessment for mammals submitted by Applicant. Refinement risk assessment for mammals has been accepted by RMS. **The risk for earthworm-eating mammals due to exposure via bioaccumulation in earthworms (secondary poisoning) for the maximum rate of 750 g as/ha should be provided or the maximum dose should be reduced to 500 g/ha.** The refinement risk assessment for mammals should be considered by MSs level.

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

Not relevant.

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 terbuthylazine, its metabolites and representative formulation. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on effects of H-01-2022 on *Daphnia*, algae *Pseudokirchneriella subcapitata* and aquatic plants *Lemna gibba* and *Myriophyllum spicatum* were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Terbuthylazine				
Oncorhynchus mykiss	terbuthylazine	96 h, s	**LC_{50nom} =2.2 mg as/L $_{nom}$**	EFSA Journal 2011; 9(1):1969
Oncorhynchus mykiss	terbuthylazine	90d, f	**$NOEC_{mm}$ = 0.09 mg as/L $_{nom}$**	
Daphnia magna	terbuthylazine	48 h	No definitive endpoint available	
Daphnia magna	terbuthylazine	21 d, ss	**$NOEC_{nom}$ = 0.019 mg as/L $_{nom}$**	
Chironomus riparius	terbuthylazine	27d, s	**$NOEC_{nom}$(water phase) = 0.5 mg as/L**	
Microcystis aeruginosa	terbuthylazine	72 h, s	E_bC_{50mm} = 0.016 mg as/L E_rC_{50mm} = 0.102 mg as/L	
Pseudokirchneriella subcapitata	terbuthylazine	72 h, s	E_bC_{50mm} = 0.012 mg as/L **E_rC_{50mm} = 0.028 mg as/L**	
Lemna gibba	terbuthylazine	14 d, s	$E_{fn}C_{50nom}$ = 0.0128 mg as/L E_rC_{50nom} = 0.412 mg as/L E_bC_{50nom} = 0.0133 mg as/L	
Metabolites				
Oncorhynchus mykiss	MT1	96 h, s	**LC_{50mm} =18 mg/L $_{nom}$**	EFSA Journal 2011; 9(1):1969
Daphnia magna	MT1	48 h, s	**EC_{50nom} = 42 mg/L**	
Selenastrum capricornutum	MT1	72 h, s	E_bC_{50mm} = 0.14 mg/L **E_rC_{50mm} = 0.38 mg/L**	
Oncorhynchus mykiss	MT13	96 h, s	**LC_{50mm} >2.5 mg/L $_{nom}$**	
Daphnia magna	MT13	48 h, s	**EC_{50nom} >2.8 mg/L**	

Chironomus riparius	MT13	28d, s	**$NOEC_{nom}$(sediment phase) = 400 as/kg**	
Desmodesmus subspicatus	MT13	72 h, s	E_bC_{50nom} >3.96 mg/L **E_rC_{50mm} >3.8 mg/L**	
Selenastrum capricornutum	MT13	72 h, s	E_bC5_{0nom} >3.96 mg/L E_rC_{50mm} >3.8 mg/L	
Oncorhynchus mykiss	MT26	96 h, s	**LC_{50mm} =1.1 mg/L $_{nom}$**	
Chironomus riparius	MT26	28d, s	**$NOEC_{nom}$(sediment phase) = 16 as/kg**	
Pseudokirchneriella subcapitata	MT26	72 h, s	E_bC_{50mm} = 0.0017 mg/L **E_rC_{50mm} = 0.0036 mg/L**	
Lemna gibba	MT26	14 d, s	**$E_{fn}C_{50mm}$ = 0.025 mg/L**	
Myriophyllum aquaticum	MT26	14 d, s	$E_{rfw}C_{50nom}$ = 2.0 mg/kg (sediment)	
Pseudokirchneriella subcapitata	LM3	72 h, s	E_rC_{50mm} = 80 mg/L E_yC_{50mm} = 39 mg/L **E_bC_{50mm} = 39 mg/L**	EFSA Journal 2019;17(9):5817
Pseudokirchneriella subcapitata	LM5	72 h, s	E_rC_{50mm} >100 mg/L E_yC_{50mm} >100 mg/L **E_bC_{50mm} >100 mg/L**	
Pseudokirchneriella subcapitata	LM6	72 h, s	E_rC_{50mm} >100 mg/L E_yC_{50mm} >100 mg/L **E_bC_{50mm} >100 mg/L**	
Higher-tier studies (micro- or mesocosm studies)				
Not available.				

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 – H-01-2022

Species	Substance	Exposure System	Results	Reference
Daphnia magna	Terbuthylazine 500 SC	48 h, s	EC_{50} =19.35 mg/L (**8 mg as/L**)	KCP 10.2.1.2/01 Rachana AR / 2023 / AG-G1146
Pseudokirchneriella subcapitata	Terbuthylazine 500 SC	72 h, s	E_rC_{50} = 2.427 mg/L (1.003 mg as/L) E_yC_{50} = 0.7564 mg/L (0.3126 mg as/L)	KCP 10.2.1.3/01 Rachana AR / 2023 / AG-G1147
Lemna gibba	Terbuthylazine 500 SC	7 d, ss	Frond number E_rC_{50} = 0.601 mg/L (**0.2484 mg as/L**) E_yC_{50} = 0.4 mg/L (0.1653 mg as/L) Dry weight E_rC_{50} = 1.07 mg/L (0.4401 mg as/L) E_yC_{50} = 0.82 mg/L (0.3372 mg as/L)	KCP 10.2.1.4/01 / Likith NG / 2023/ AG-G1148
Myriophyllum	Terbuthylazine	14 d	Shoot length	KCP 10.2.1.4/02 /

Species	Substance	Exposure System	Results	Reference
spicatum	500 SC		E_rC_{50} = 3.298 mg/L (1.363 mg as/L) E_yC_{50} = 2.830 mg/L (1.170 mg as/L) Dry weight E_rC_{50} = 3.757 mg/L (1.553 mg as/L) E_yC_{50} = 2.785 mg/L (1.151 mg as/L) Fresh weight E_rC_{50} = 3.610 mg/L (1.492 mg as/L) E_yC_{50} = 2.706 mg/L (1.118 mg as/L)	Likith NG / 2023/ AG-G1158
Higher-tier studies (micro- or mesocosm studies)				
Not available.				

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

9.5.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.5-2. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. Risk assessment was performed with active substance endpoints and formulation endpoints.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for terbuthylazine for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of H-01-2022 in maize (pre-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna minor***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		2200	90	8000*	19	28	500	248.4*
AF		100	10	100	10	10	10	10
RAC (µg/L)		22	9	80	1.9	2.8	50	24.84
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
maize BBCH 00 (pre-emergence application rate 750 g as/ha)								
Step 1	214.9996	**9.77**	**23.89**	**2.69**	**113.16**	**76.79**	**4.30**	**8.66**
Step 2 NEU	42.2782	**1.92**	**4.70**	0.53	**22.25**	**15.10**	0.85	**1.70**
Step 3								
D3/ditch	3.935	0.18	0.44	0.05	**2.07**	**1.41**	0.08	0.16
D4/pond	0.2505	0.01	0.03	0.00	0.13	0.09	0.01	0.01
D4/stream	3.377	0.15	0.38	0.04	**1.78**	**1.21**	0.07	0.14
D5/pond	0.2413	0.01	0.03	0.00	0.13	0.09	0.00	0.01
D5/stream	3.386	0.15	0.38	0.04	**1.78**	**1.21**	0.07	0.14
D6/ditch	3.949	0.18	0.44	0.05	**2.08**	**1.41**	0.08	0.16
R1/pond	0.3481	0.02	0.04	0.00	0.18	0.12	0.01	0.01
R1/stream	10.85	0.49	**1.21**	0.14	**5.71**	**3.88**	0.22	0.44

R2/stream	8.229	0.37	0.91	0.10	**4.33**	**2.94**	0.16	0.33
R3/stream	3.846	0.17	0.43	0.05	**2.02**	**1.37**	0.08	0.15
R4/stream	26.38	**1.20**	**2.93**	0.33	**13.88**	**9.42**	0.53	**1.06**
maize BBCH 00 (pre-emergence application rate 500 g as/ha)								
Step 1	143.3331	6.52	15.93	1.79	75.44	51.19	2.87	5.77
Step 2 NEU	28.1854	1.28	3.13	0.35	14.83	10.07	0.56	1.13
Step 3								
D3/ditch	2.623	0.12	0.29	0.03	**1.38**	0.94	0.05	0.11
D4/pond	0.1661	0.01	0.02	0.00	0.09	0.06	0.00	0.01
D4/stream	2.250	0.10	0.25	0.03	**1.18**	0.80	0.05	0.09
D5/pond	0.1588	0.01	0.02	0.00	0.08	0.06	0.00	0.01
D5/stream	2.256	0.10	0.25	0.03	**1.19**	0.81	0.05	0.09
D6/ditch	2.631	0.12	0.29	0.03	**1.38**	0.94	0.05	0.11
R1/pond	0.2347	0.01	0.03	0.00	0.12	0.08	0.00	0.01
R1/stream	7.160	0.33	0.80	0.09	**3.77**	**2.56**	0.14	0.29
R2/stream	5.391	0.25	0.60	0.07	**2.84**	**1.93**	0.11	0.22
R3/stream	2.564	0.12	0.28	0.03	**1.35**	0.92	0.05	0.10
R4/stream	17.45	0.79	**1.94**	0.22	**9.18**	**6.23**	0.35	0.70

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for terbuthylazine for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of H-01-2022 in maize (post-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna minor***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		2200	90	8000*	19	28	500	248.4*
AF		100	10	100	10	10	10	10
RAC (µg/L)		22	9	80	1.9	2.8	50	24.84
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)								
Step 1	214.9996	**9.77**	**23.89**	**2.69**	**113.16**	**76.79**	**4.30**	**8.66**
Step 2 NEU	33.2200	**1.51**	**3.69**	0.42	**17.48**	**11.86**	0.66	**1.34**
Step 3								
D3/ditch	3.936	0.18	0.44	0.05	**2.07**	**1.41**	0.08	0.16
D4/pond	0.2602	0.01	0.03	0.00	0.14	0.09	0.01	0.01
D4/stream	3.379	0.15	0.38	0.04	**1.78**	**1.21**	0.07	0.14
D5/pond	0.2773	0.01	0.03	0.00	0.15	0.10	0.01	0.01
D5/stream	3.551	0.16	0.39	0.04	**1.87**	**1.27**	0.07	0.14
D6/ditch	3.948	0.18	0.44	0.05	**2.08**	**1.41**	0.08	0.16
R1/pond	1.236	0.06	0.14	0.02	0.65	0.44	0.02	0.05
R1/stream	17.97	0.82	**2.00**	0.22	**9.46**	**6.42**	0.36	0.72

R2/stream	9.993	0.45	**1.11**	0.12	**5.26**	**3.57**	0.20	0.40
R3/stream	24.39	**1.11**	**2.71**	0.30	**12.84**	**8.71**	0.49	0.98
R4/stream	25.81	**1.17**	**2.87**	0.32	**13.58**	**9.22**	0.52	**1.04**
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)								
Step 1	143.3331	6.52	15.93	1.79	75.44	51.19	2.87	5.77
Step 2 NEU	22.1467	1.01	2.46	0.28	11.66	7.91	0.44	0.89
Step 3								
D3/ditch	2.624	0.12	0.29	0.03	**1.38**	0.94	0.05	0.11
D4/pond	0.1724	0.01	0.02	0.00	0.09	0.06	0.00	0.01
D4/stream	2.252	0.10	0.25	0.03	**1.19**	0.80	0.05	0.09
D5/pond	0.1816	0.01	0.02	0.00	0.10	0.06	0.00	0.01
D5/stream	2.366	0.11	0.26	0.03	**1.25**	0.85	0.05	0.10
D6/ditch	2.631	0.12	0.29	0.03	**1.38**	0.94	0.05	0.11
R1/pond	0.8149	0.04	0.09	0.01	0.43	0.29	0.02	0.03
R1/stream	11.84	0.54	**1.32**	0.15	**6.23**	**4.23**	0.24	0.48
R2/stream	6.518	0.30	0.72	0.08	**3.43**	**2.33**	0.13	0.26
R3/stream	16.07	0.73	**1.79**	0.20	**8.46**	**5.74**	0.32	0.65
R4/stream	17.07	0.78	**1.90**	0.21	**8.98**	**6.10**	0.34	0.69

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

For the intended uses, calculated PEC/RAC ratios for terbuthylazine did not indicate an acceptable risk for aquatic organisms in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PECsw considering reduced exposure of surface water bodies and the most sensitive group of aquatic organisms (NOEC for *Daphnia magna* of 0.019 mg as/L in connection with an assessment factor of 10).

Table 9.5-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for terbuthylazine based on FOCUS Step 4 calculations and toxicity data for *Daphnia magna* (chronic exposure) with mitigation of spray drift and run-off for the use of H-01-2022 in maize (pre-emergence application)

Intended use		maize					
Active substance		terbuthylazine					
Application rate (g/ha)		750					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	D3/ditch	3.935	1.290	0.6839	-	-	-
None	D4/stream	3.377	1.425	0.7589	-	-	-
None	D5/stream	3.386	1.442	0.7779	-	-	-
None	D6/ditch	3.949	1.303	0.6969	-	-	-
None	R1/stream	10.85	10.85	10.85	1.145	0.6071	-
None	R2/stream	8.229	8.229	8.229	1.522	0.8071	-
None	R3/stream	3.846	1.736	1.736	1.619	0.8589	-
None	R4/stream	26.38	26.38	26.38	1.144	0.6070	-
RAC = 1.9 µg/L		**PEC/RAC ratio**					
None	D3/ditch	**2.07**	0.68	0.36	-	-	-
None	D4/stream	**1.78**	0.75	0.40	-	-	-
None	D5/stream	**1.78**	0.76	0.41	-	-	-
None	D6/ditch	**2.08**	0.69	0.37	-	-	-
None	R1/stream	**5.71**	**5.71**	**5.71**	0.60	0.32	-
None	R2/stream	**4.33**	**4.33**	**4.33**	0.80	0.42	-
None	R3/stream	**2.02**	0.91	0.91	0.85	0.45	-
None	R4/stream	**13.88**	**13.88**	**13.88**	0.60	0.32	-
Intended use		maize					
Active substance		terbuthylazine					
Application rate (g/ha)		500					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	D3/ditch	2.623	0.8597	0.4558	-	-	-
None	D4/stream	2.250	0.9499	0.5055	-	-	-
None	D5/stream	2.256	0.9605	0.5176	-	-	-
None	D6/ditch	2.631	0.8674	0.4635	-	-	-
None	R1/stream	7.160	7.160	7.160	0.7631	0.4046	-

None	R2/stream	5.391	5.391	5.391	1.015	0.5378	-
None	R3/stream	2.564	1.217	1.217	1.080	0.5724	-
None	R4/stream	17.45	17.45	17.45	0.7630	0.4045	-
RAC = 1.9 µg/L		**PEC/RAC ratio**					
None	D3/ditch	**1.38**	0.45	0.24	-	-	-
None	D4/stream	**1.18**	0.50	0.27	-	-	-
None	D5/stream	**1.19**	0.51	0.27	-	-	-
None	D6/ditch	**1.38**	0.46	0.24	-	-	-
None	R1/stream	**3.77**	**3.77**	**3.77**	0.40	0.21	-
None	R2/stream	**2.84**	**2.84**	**2.84**	0.53	0.28	-
None	R3/stream	**1.35**	0.64	0.64	0.57	0.30	-
None	R4/stream	**9.18**	**9.18**	**9.18**	0.40	0.21	-

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

Table 9.5-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for terbuthylazine based on FOCUS Step 4 calculations and toxicity data for *Daphnia magna* (chronic exposure) with mitigation of spray drift and run-off for the use of H-01-2022 in maize (post-emergence application)

Intended use		maize					
Active substance		terbuthylazine					
Application rate (g/ha)		750					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	D3/ditch	3.936	1.290	0.6841	-	-	-
None	D4/stream	3.379	1.428	0.7622	-	-	-
None	D5/stream	3.551	1.515	0.8198	-	-	-
None	D6/ditch	3.948	1.308	0.7039	-	-	-
None	R1/stream	17.97	17.97	17.97	1.268	0.6088	-
None	R2/stream	9.993	9.993	9.993	1.535	0.8144	-
None	R3/stream	24.39	24.39	24.39	1.611	0.8547	-
None	R4/stream	25.81	25.81	25.81	1.144	0.6070	-
RAC = 1.9 µg/L		**PEC/RAC ratio**					
None	D3/ditch	**2.07**	0.68	0.36	-	-	-
None	D4/stream	**1.78**	0.75	0.40	-	-	-
None	D5/stream	**1.87**	0.80	0.43	-	-	-
None	D6/ditch	**2.08**	0.69	0.37	-	-	-
None	R1/stream	**9.46**	**9.46**	**9.46**	0.67	0.32	-
None	R2/stream	**5.26**	**5.26**	**5.26**	0.81	0.43	-
None	R3/stream	**12.84**	**12.84**	**12.84**	0.85	0.45	-
None	R4/stream	**13.58**	**13.58**	**13.58**	0.60	0.32	-
Intended use		maize					
Active substance		terbuthylazine					
Application rate (g/ha)		500					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	D3/ditch	2.624	0.8598	0.4559	-	-	-
None	D4/stream	2.252	0.9520	0.5076	-	-	-
None	D5/stream	2.366	1.009	0.5452	-	-	-
None	D6/ditch	2.631	0.8713	0.4683	-	-	-
None	R1/stream	11.84	11.84	11.84	0.8354	0.4057	-

None	R2/stream	6.518	6.518	6.518	1.024	0.5427	-
None	R3/stream	16.07	16.07	16.07	1.074	0.5696	-
None	R4/stream	17.07	17.07	17.07	0.7630	0.4045	-
RAC = 1.9 µg/L		**PEC/RAC ratio**					
None	D3/ditch	**1.38**	0.45	0.24	-	-	-
None	D4/stream	**1.19**	0.50	0.27	-	-	-
None	D5/stream	**1.25**	0.53	0.29	-	-	-
None	D6/ditch	**1.38**	0.46	0.25	-	-	-
None	R1/stream	**6.23**	**6.23**	**6.23**	0.44	0.21	-
None	R2/stream	**3.43**	**3.43**	**3.43**	0.54	0.29	-
None	R3/stream	**8.46**	**8.46**	**8.46**	0.57	0.30	-
None	R4/stream	**8.98**	**8.98**	**8.98**	0.40	0.21	-

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 for terbuthylazine indicate an acceptable risk in all scenarios provided the following risk mitigations are applied.

BBCH 00 pre-emergence application 750 g as/ha & 500 g as/ha
5m buffer zone for scenarios D3 ditch, D4 stream, D5 stream, D6 ditch and R3 stream
5m vegetated buffer zone for scenarios R1 stream, R2 stream and R4 stream

BBCH 12--16 post-emergence application 750 g as/ha & 500 g as/ha
5m buffer zone for scenarios D3 ditch, D4 stream, D5 stream and D6 ditch,
5m vegetated buffer zone for scenarios R1 stream, R2 stream, R3 stream and R4 stream

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (pre-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**-**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	**-**
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		18000	-	42000	-	380	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		180	-	420	-	38	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 00 (pre-emergence application rate 750 g as/ha)								
Step 1	106.7594	0.59	-	0.25	-	**2.81**	-	-
Step 2 NEU	20.7462	0.12	-	0.05	-	0.55	-	-
maize BBCH 00 (pre-emergence application rate 500 g as/ha)								
Step 1	71.1730	0.40	-	0.17	-	**1.87**	-	-
Step 2 NEU	13.8308	0.08	-	0.03	-	0.36	-	-

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (post-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**-**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	**-**
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		18000	-	42000	-	380	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		180	-	420	-	38	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)								
Step 1	106.7594	0.59	-	0.25	-	**2.81**	-	-
Step 2 NEU	16.1953	0.09	-	0.04	-	0.43	-	-
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)								
Step 1	71.1730	0.40	-	0.17	-	**1.87**	-	-
Step 2 NEU	10.7969	0.06	-	0.03	-	0.28	-	-

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 for MT1 indicate an acceptable risk for aquatic organisms. Therefore, further PEC/RAC ratios are not required.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT13 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (pre-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	-
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	-
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		2500	-	2800	-	3800	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		25	-	28	-	380	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 00 (pre-emergence application rate 750 g as/ha)								
Step 1	101.5911	**4.06**	-	**3.63**	-	0.27	-	-
Step 2 NEU	20.1189	0.80	-	0.72	-	0.05	-	-
maize BBCH 00 (pre-emergence application rate 500 g as/ha)								
Step 1	67.7274	**2.71**	-	**2.42**	-	0.18	-	-
Step 2 NEU	13.4126	0.54	-	0.48	-	0.04	-	-

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

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT13 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (post-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	-
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	-
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		2500	-	2800	-	3800	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		25	-	28	-	380	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)								
Step 1	101.5911	**4.06**	-	**3.63**	-	0.27	-	-
Step 2 NEU	15.3604	0.61	-	0.55	-	0.04	-	-
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)								
Step 1	67.7274	**2.71**	-	**2.42**	-	0.18	-	-
Step 2 NEU	10.2403	0.41	-	0.37	-	0.03	-	-

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 for MT13 indicate an acceptable risk for aquatic organisms. Therefore, further PEC/RAC ratios are not required.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT14 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (pre-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	-
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	-
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		15000*	-	15000*	-	15000*	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		150	-	150	-	1500	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 00 (pre-emergence application rate 750 g as/ha)								
Step 1	48.6336	0.32	-	0.32	-	0.03	-	-
Step 2 NEU	9.4779	0.06	-	0.06	-	0.01	-	-
maize BBCH 00 (pre-emergence application rate 500 g as/ha)								
Step 1	32.4224	0.22	-	0.22	-	0.02	-	-
Step 2 NEU	6.3186	0.04	-	0.04	-	0.00	-	-

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

* The study authors proposed the acute fish and *Daphnia magna* L/EC50 for MT14 was >100 mg/L and the EC50 to algae to be 30.7 mg/L. However, these values are greater than the water solubility of MT14 (18 mg/L) and therefore the Rapporteur has reservations in accepting quantified toxicity endpoints. The water solubility of MT14 is 18 mg/L and therefore to assume the saturation level (the amount of MT14 in solution under the conditions of the study) of 15 mg as/L is not unreasonable (EFSA Journal 2019;17(9):5817).

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT14 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of H-01-2022 in maize (post-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	-
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna minor***	-
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	-
(µg/L)		15000*	-	15000*	-	15000*	-	-
AF		100	10	100	10	10	10	-
RAC (µg/L)		150	-	150	-	1500	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)								
Step 1	48.6336	0.32	-	0.32	-	0.03	-	-
Step 2 NEU	7.1855	0.05	-	0.05	-	0.00	-	-
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)								
Step 1	32.4224	0.22	-	0.22	-	0.02	-	-
Step 2 NEU	4.7903	0.03	-	0.03	-	0.00	-	-

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

* The study authors proposed the acute fish and *Daphnia magna* L/EC50 for MT14 was >100 mg/L and the EC50 to algae to be 30.7 mg/L. However, these values are greater than the water solubility of MT14 (18 mg/L) and therefore the Rapporteur has reservations in accepting quantified toxicity endpoints. The water solubility of MT14 is 18 mg/L and therefore to assume the saturation level (the amount of MT14 in solution under the conditions of the study) of 15 mg as/L is not unreasonable (EFSA Journal 2019;17(9):5817).

For the intended uses, calculated PEC/RAC ratios for MT14 indicate an acceptable risk for aquatic organisms. Therefore, further PEC/RAC ratios are not required.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT26 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of H-01-2022 in maize (pre-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna minor***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC
(µg/L)		1100	-	-	-	3.6	25	16000
AF		100	10	100	10	10	10	10
RAC (µg/L)		11	-	-	-	0.36	2.5	1600
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 00 (pre-emergence application rate 750 g as/ha)								
Step 1	12.0378	1.09	-	-	-	**33.44**	**4.82**	0.01
Step 2 NEU	2.3677	0.22	-	-	-	**6.58**	0.95	0.00
Step 3								
D3/ditch	0.306	0.03	-	-	-	0.85	0.12	0.00
D4/pond	0.019	0.00	-	-	-	0.05	0.01	0.00
D4/stream	0.263	0.02	-	-	-	0.73	0.11	0.00
D5/pond	0.019	0.00	-	-	-	0.05	0.01	0.00
D5/stream	0.263	0.02	-	-	-	0.73	0.11	0.00
D6/ditch	0.307	0.03	-	-	-	0.85	0.12	0.00
R1/pond	0.027	0.00	-	-	-	0.08	0.01	0.00
R1/stream	0.844	0.08	-	-	-	**2.34**	0.34	0.00

R2/stream	0.640	0.06	-	-	-	**1.78**	0.26	0.00
R3/stream	0.299	0.03	-	-	-	0.83	0.12	0.00
R4/stream	2.052	0.19	-	-	-	**5.70**	0.82	0.00
maize BBCH 00 (pre-emergence application rate 500 g as/ha)								
Step 1	8.0252	0.73	-	-	-	**22.29**	**3.21**	0.01
Step 2 NEU	1.5784	0.14	-	-	-	**4.38**	0.63	0.00
Step 3								
D3/ditch	0.204	0.02	-	-	-	0.57	0.08	0.00
D4/pond	0.013	0.00	-	-	-	0.04	0.01	0.00
D4/stream	0.175	0.02	-	-	-	0.49	0.07	0.00
D5/pond	0.012	0.00	-	-	-	0.03	0.00	0.00
D5/stream	0.175	0.02	-	-	-	0.49	0.07	0.00
D6/ditch	0.205	0.02	-	-	-	0.57	0.08	0.00
R1/pond	0.018	0.00	-	-	-	0.05	0.01	0.00
R1/stream	0.557	0.05	-	-	-	**1.55**	0.22	0.00
R2/stream	0.419	0.04	-	-	-	**1.16**	0.17	0.00
R3/stream	0.199	0.02	-	-	-	0.55	0.08	0.00
R4/stream	1.357	0.12	-	-	-	**3.77**	0.54	0.00

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

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MT26 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of H-01-2022 in maize (post-emergence application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna minor***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC
(µg/L)		1100	-	-	-	3.6	25	16000
AF		100	10	100	10	10	10	10
RAC (µg/L)		11	-	-	-	0.36	2.5	1600
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)								
Step 1	12.0378	1.09	-	-	-	**33.44**	**4.82**	0.01
Step 2 NEU	1.8671	0.17	-	-	-	**5.19**	0.75	0.00
Step 3								
D3/ditch	0.306	0.03	-	-	-	0.85	0.12	0.00
D4/pond	0.020	0.00	-	-	-	0.06	0.01	0.00
D4/stream	0.263	0.02	-	-	-	0.73	0.11	0.00
D5/pond	0.022	0.00	-	-	-	0.06	0.01	0.00
D5/stream	0.276	0.03	-	-	-	0.77	0.11	0.00
D6/ditch	0.307	0.03	-	-	-	0.85	0.12	0.00
R1/pond	0.096	0.01	-	-	-	0.27	0.04	0.00
R1/stream	1.398	0.13	-	-	-	**3.88**	0.56	0.00

R2/stream	0.777	0.07	-	-	-	**2.16**	0.31	0.00
R3/stream	1.897	0.17	-	-	-	**5.27**	0.76	0.00
R4/stream	2.007	0.18	-	-	-	**5.58**	0.80	0.00
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)								
Step 1	8.0252	0.73	-	-	-	**22.29**	**3.21**	0.01
Step 2 NEU	1.2447	0.11	-	-	-	**3.46**	0.50	0.00
Step 3								
D3/ditch	0.204	0.02	-	-	-	0.57	0.08	0.00
D4/pond	0.013	0.00	-	-	-	0.04	0.01	0.00
D4/stream	0.175	0.02	-	-	-	0.49	0.07	0.00
D5/pond	0.014	0.00	-	-	-	0.04	0.01	0.00
D5/stream	0.184	0.02	-	-	-	0.51	0.07	0.00
D6/ditch	0.205	0.02	-	-	-	0.57	0.08	0.00
R1/pond	0.063	0.01	-	-	-	0.18	0.03	0.00
R1/stream	0.921	0.08	-	-	-	**2.56**	0.37	0.00
R2/stream	0.507	0.05	-	-	-	**1.41**	0.20	0.00
R3/stream	1.250	0.11	-	-	-	**3.47**	0.50	0.00
R4/stream	1.328	0.12	-	-	-	**3.69**	0.53	0.00

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 for MT26 did not indicate an acceptable risk for aquatic organisms in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PECsw considering reduced exposure of surface water bodies and the most sensitive group of aquatic organisms (E_rC_{50} for *Pseudokirchneriella subcapitata* of 0.0036 mg/L in connection with an assessment factor of 10).

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for MT26 based on FOCUS Step 4 calculations and toxicity data for *Daphnia magna* (chronic exposure) with mitigation of spray drift and run-off for the use of H-01-2022 in maize (pre-emergence application)

Intended use		maize					
Active substance		MT26					
Application rate (g as/ha)		750					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	R1/stream	0.844	0.844	0.844	0.089	0.047	-
None	R2/stream	0.64	0.64	0.64	0.118	0.063	-
None	R4/stream	2.052	2.052	2.052	0.089	0.047	-
RAC = 0.36 µg/L		**PEC/RAC ratio**					
None	R1/stream	**2.34**	**2.34**	**2.34**	0.25	0.13	-
None	R2/stream	**1.78**	**1.78**	**1.78**	0.33	0.18	-
None	R4/stream	**5.70**	**5.70**	**5.70**	0.25	0.13	-
Intended use		maize					
Active substance		MT26					
Application rate (g as/ha)		500					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	R1/stream	0.557	0.557	0.557	0.059	0.031	-
None	R2/stream	0.419	0.419	0.419	0.079	0.042	-
None	R4/stream	1.357	1.357	1.357	0.059	0.031	-
RAC = 0.36 µg/L		**PEC/RAC ratio**					
None	R1/stream	**1.55**	**1.55**	**1.55**	0.16	0.09	-
None	R2/stream	**1.16**	**1.16**	**1.16**	0.22	0.12	-
None	R4/stream	**3.77**	**3.77**	**3.77**	0.16	0.09	-

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

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for MT26 based on FOCUS Step 4 calculations and toxicity data for *Daphnia magna* (chronic exposure) with mitigation of spray drift and run-off for the use of H-01-2022 in maize (post-emergence application)

Intended use		maize					
Active substance		MT26					
Application rate (g as/ha)		750					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	R1/stream	1.398	1.398	1.398	0.099	0.047	-
None	R2/stream	0.777	0.777	0.777	0.119	0.063	-
None	R3/stream	1.897	1.897	1.897	0.125	0.066	-
None	R4/stream	2.007	2.007	2.007	0.089	0.047	-
RAC = 0.36 µg/L		**PEC/RAC ratio**					
None	R1/stream	**3.88**	**3.88**	**3.88**	0.28	0.13	-
None	R2/stream	**2.16**	**2.16**	**2.16**	0.33	0.18	-
None	R3/stream	**5.27**	**5.27**	**5.27**	0.35	0.18	-
None	R4/stream	**5.58**	**5.58**	**5.58**	0.25	0.13	-
Intended use		maize					
Active substance		MT26					
Application rate (g as/ha)		500					
Nozzle reduction	**Vegetated filter strip (m)**	None	None	None	5 VFS	10 VFS	20 VFS
	No-spray buffer (m)	1/3	5	10	5	10	20
None	R1/stream	0.921	0.921	0.921	0.065	0.032	-
None	R2/stream	0.507	0.507	0.507	0.08	0.042	-
None	R3/stream	1.25	1.25	1.25	0.084	0.044	-
None	R4/stream	1.328	1.328	1.328	0.059	0.031	-
RAC = 0.36 µg/L		**PEC/RAC ratio**					
None	R1/stream	**2.56**	**2.56**	**2.56**	0.18	0.09	-
None	R2/stream	**1.41**	**1.41**	**1.41**	0.22	0.12	-
None	R3/stream	**3.47**	**3.47**	**3.47**	0.23	0.12	-
None	R4/stream	**3.69**	**3.69**	**3.69**	0.16	0.09	-

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 for MT26 indicate an acceptable risk in all scenarios provided the following risk mitigations are applied.

BBCH 00 pre-emergence application 750 g as/ha & 500 g as/ha
5m vegetated buffer zone for scenarios R1 stream, R2 stream and R4 stream

BBCH 12--16 post-emergence application 750 g as/ha & 500 g as/ha
5m vegetated buffer zone for scenarios R1 stream, R2 stream, R3 stream and R4 stream

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for LM3, LM5 and LM6 for each organism group based on PECgw calculations for the use of H-01-2022 in maize (pre-emergence and post-emergence application)

Group		**Algae**		**Algae**		**Algae**
Test species		***Pseudokirchneriella subcapitata***		***Pseudokirchneriella subcapitata***		***Pseudokirchneriella subcapitata***
Endpoint		E_bC_{50}		E_bC_{50}		E_bC_{50}
(µg/L)		39000		100000		100000
AF		10		10		10
RAC (µg/L)		3900		10000		10000
FOCUS Scenario	**LM3 PEC $_{gw}$ (µg/L)**		**LM5 PEC $_{gw}$ (µg/L)**		**LM6 PEC $_{gw}$ (µg/L)**	
maize BBCH 00 (pre-emergence application rate 750 g as/ha)						
-	0.945830*	0.00	1.212409*	0.00	3.911373 T	0.00
maize BBCH 00 (pre-emergence application rate 500 g as/ha)						
-	0.627337*	0.00	0.77529*	0.00	2.585014	0.00
maize BBCH 12-16 (post-emergence application rate 750 g as/ha)						
-	0.711260*	0.00	0.884346*	0.00	2.929205	0.00
maize BBCH 12-16 (post-emergence application rate 500 g as/ha)						
-	0.471827*	0.00	0.564185*	0.00	1.934892	0.00

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* the max. PECgw value for Hamburg scenario application every 3 years ** the max. PECgw value for Thiva scenario application every 3 years

9.5.3 Overall conclusions

PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity end-points from studies for active substance, metabolites and formulation H-01-2022. On the basis of PEC/RAC values it was concluded that the application of H-01-2022 does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied.

zRMS comment:	The risk assessment for aquatic organisms for most sensitive species of each group has been accepted by zRMS. The endpoints have been approved at the European Union level or submitted by the Applicant. The highest value PECsw of scenarios D3, D4, R1 relevant to Poland was used for risk assessment. zRMS proposes refined risk assessment and mitigation measure at National level. The studies on effects of H-01-2022 on algae, *Daphnia magna* and aquatic plants were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. Risk assessments for H-01-2022 with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290. PEC/RAC values were calculated on the basis of PECsw calculations as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation H-01-2022. PECsw Step 3/RAC values for active substance were less than 1 for few scenarios so further evaluation with Step 4 PECsw was performed. On the basis of PEC/RAC values it was concluded that the application of H-01-2022 does not pose unacceptable risk for aquatic organisms under condition that appropriate risk mitigations are applied. For Poland D3, D4 and R1 scenarios are relevant so it can be concluded that H-01-2022 used to protect maize according to proposed GAP does not pose unacceptable risk to aquatic organisms under condition that: 5m vegetated buffer zone is applied. Based on the results with consideration FOCUS STEP 4 for scenarios relevant for Poland the following risk mitigation are required: **-5m buffer non-spray zone with 5 meter vegetated filter strip to surface water bodies** Final risk mitigation measures should be considered at MSs level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with terbuthylazine and representative formulations. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on effects of H-01-2022 on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	terbuthylazine	Acute, Oral	LD_{50} >22.6 µg as/bee	EFSA Journal 2011; 9(1):1969
Apis mellifera	terbuthylazine	Acute, Contact	LD_{50} >32 µg as/bee	
Apis mellifera	H-01-2022	Acute, Oral	**LD_{50} = 124.087 µg/bee (57.39 µg as /bee)**	KCP 10.3.1.1.1/01 Gangadhar RS /2023/ AG-G1150
Apis mellifera	H-01-2022	Acute, Contact	**LD_{50} = 110.14 µg/bee (50.94 µg as/bee)**	KCP 10.3.1.1.2/01 Gangadhar RS/2023/ AG-G1151
Apis mellifera	H-01-2022	Chronic, Oral	LC_{50} = 10.85 mg/kg (5.02 mg as/kg) **LDD_{50} = 0.362205 µg/bee/day (0.16752 µg as/bee/day)** NOEC = 1.35 mg/kg (0.625 mg as/kg) NOEDD = 51.20 mg/kg (23.6792 mg as/kg)	KCP 10.3.1.1.2/02 Gangadhar RS /2024/ AG-G1152
Apis mellifera	H-01-2022	Acute, Larval 72h	**LD_{50} = 36.99 µg/larva (17.109 µg as/larva)** NOEC = 3.243 µg/larva (1.5 µg as/larva)	KCP 10.3.1.4/01 Gangadhar RS /2024/ AG-G1149
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.6-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.6.2 Risk assessment

9.6.2.1 Hazard quotients for bees

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SAN-

CO/10329/2002 rev.2 (final), October 17, 2002).

Risk assessment acc. to SANCO/10329/2002 rev.2 (final), October 17, 2002

Table 9.6-2: First-tier assessment of the risk for bees due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize		
Product	H-01-2022		
Application rate (g/ha)	1 × 1678.5* (equivalent to 1500 ml/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	124.087	1678.5	13.53
Acute contact toxicity	110.14		15.24

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*density of H-01-2022 is 1.119 g/ml

Table 9.6-3: First-tier assessment of the risk for bees due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize		
Product	H-01-2022		
Application rate (g/ha)	1 × 1119* (equivalent to 1000 ml/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	124.087	1119	9.02
Acute contact toxicity	110.14		10.16

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*density of H-01-2022 is 1.119 g/ml

zRMS comment:	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). The required study on oral and contact toxicity of the formulated product H-01-2022 to honeybees was conducted and considered to be valid. The endpoints as proposed by the Notifier are considered acceptable and are used in the risk assessment. All hazard quotients for acute oral and acute contact exposure were below 50, the Commission Regulation (EU) No. 546/2011 criterion, indicating low risk to honey bees. The chronic studies with formulation H-01-2022 were submitted. The studies were accepted by zRMS. The risk assessment based on these studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

The acute risk of H-01-2022 to honeybees was assessed from HQ between toxicity endpoints, estimated from acute oral and contact studies with formulated product H-01-2022 as well as the maximum single application rate. The HQ values were considerably less than 50. It can be concluded that H-01-2022 used in accordance with GAP does not pose unacceptable risk to bees. No risk mitigations are required.

zRMS comment:	Agreed.

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 representative formulations containing terbuthylazine. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on effects of H-01-2022 on arthropods were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Aphidius rhopalosiphi	H-01-2022	Tier I glass plates	Mortality 48h LR_{50} > 4500 mL/ha 48h NOER ≥ 4500 mL/ha Reproduction 12d LR_{50} > 4500 mL/ha NOER ≥ 4500 mL/ha	KCP 10.3.2.1/01/ Mautino G/ 2023/ 1013.H.SAG23/r
Typhlodromus pyri	H-01-2022	Tier I glass plates	Mortality 7d LR_{50} > 6000 mL/ha 7d NOER ≤ 750 mL/ha Fecundity 14d LR_{50} > 6000 mL/ha	KCP 10.3.2.1/02/ Mautino G/ 2023/ 1014.H.SAG23/r

			14d NOER = 3000 mL/ha	
Field or semi-field tests				
-				

9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.7-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize		
Product	H-01-2022		
Application rate (ml/ha)	1 × 1500		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**$PER_{in\text{-}field}$ (ml/ha)**	**$HQ_{in\text{-}field}$ criterion HQ ≤ 2**
Aphidius rhopalosiphi	> 4500	1500	0.33
Typhlodromus pyri	> 6000		0.25

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize		
Product	H-01-2022		
Application rate (ml/ha)	1 × 1000		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**$PER_{in\text{-}field}$ (ml/ha)**	**$HQ_{in\text{-}field}$ criterion HQ ≤ 2**
Aphidius rhopalosiphi	> 4500	1000	0.22
Typhlodromus pyri	> 6000		0.17

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient;

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize				
Active substance/product	terbuthylazine				
Application rate (g/ha)	1 × 1500				
MAF	1				
VDF	5[1]				
Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**Drift rate**	**CF**	**$PER_{off\text{-}field}$ (ml/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Aphidius rhopalosiphi	> 4500	2.77%	10	83.10	0.02
Typhlodromus pyri	> 6000				0.01

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.
[1] value for Tier I in accordance with EFSA Supporting publication 2019:EN-1673 and Working Document on Risk Assessment of Plant Protection Products in the Central Zone (Version 1.0, May 2021)

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize				
Active substance/product	terbuthylazine				
Application rate (g/ha)	1 × 1000				
MAF	1				
VDF	5[1]				
Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**Drift rate**	**CF**	**$PER_{off\text{-}field}$ (ml/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Aphidius rhopalosiphi	> 4500	2.77%	10	55.10	0.01
Typhlodromus pyri	> 6000				0.01

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.
[1] value for Tier I in accordance with EFSA Supporting publication 2019:EN-1673 and Working Document on Risk Assessment of Plant Protection Products in the Central Zone (Version 1.0, May 2021)

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 risk of H-01-2022 to non-target arthropods was assessed from in-field and off-field HQ between toxicity endpoints, estimated from laboratory studies with formulated product H-01-2022 as well as applica-

tion rate. The HQ values were considerably less than 2, indicating that the product poses a low risk to non-target arthropods. It can be concluded that H-01-2022 used in accordance with GAP does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk mitigations are required.

zRMS comment:	Agreed. The calculations of the risk assessment for in – field and off-field for **H-01-2022** for two indicator species were accepted by zRMS. HQ in - field and HQ-off field are below 2 based on laboratory studies (Tier1). The PER-in and PER_{off}-field for *T.pyri* and *A.rhopalosiphi* (based on the laboratory studies) are below trigger value 2. Therefore, this assessment indicates that **H-01-2022** poses low risk to in-field and off-field non-target arthropods following application according to the proposed use patterns.

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 terbuthylazine, its metabolites and representative formulations. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on effects of H-01-2022 on earthworms were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Eisenia foetida	MT1	Chronic, 56 days	**$NOEC_{corr}$ =2.8 mg/ kg soil dw**	EFSA Journal 2011; 9(1):1969
Eisenia foetida	MT13	Chronic, 56 days	**NOEC = 7 mg/kg soil dw**	
Eisenia fetida	H-01-2022	56 d, chronic	NOEC= 5.83 mg/kg dw (2.7 mg as/kg dw) **$NOEC_{corr}$= 2.915 mg/kg dw (1.35 mg as/kg dw)**	KCP 10.4.1.1/01/ Visala N/ 2023/ AG-G1153
Hypoaspis aculeifer	H-01-2022	14 d, chronic	NOEC ≥ 2238 mg/kg dw (≥ 1000 mg as/kg dw) **$NOEC_{corr}$ ≥ 1119 mg/kg dw (≥ 500 mg as/kg dw)**	KCP 10.4.2.1/01/ Mautino G / 2023/ 1015.H.SAG23/r

Species	Substance	Exposure System	Results	Reference
Folsomia candida	H-01-2022	28 d, chronic	NOEC= 95.3 mg/kg dw (44.076 mg as/kg dw) **$NOEC_{corr}$ = 47.65 mg/kg dw (22.038 mg as/kg dw)**	KCP 10.4.2.1/02/ Gangadhar RS./ 2023/AG-G1154
Field studies				
Not applicable	Preparation - 'Gardo Gold' ('A-9476 C')	Field study – 1 yr (Denmark)	No significant ecologically adverse effects at 4.5 L form.n/ha (844 g as/ha) after 1 yr SYN	EFSA Journal 2011; 9(1):1969
Not applicable		Field study – 1 yr (Germany)	Significant adverse effects on biomass of Epilobous juveniles at 4.0 & 4.5 L form.n/ha (750 & 844 g as/ha) after 1 yr. No other significant ecological-ly adverse effects found SYN	
Not applicable	Preparation - 'Gardoprim' /'GS 13529 SC 500' ('A-5435 E')	Field study – 1 yr (Denmark)	No significant ecologically adverse effects at 1.69 L form.n/ha (844 g as/ha) after 1 yr SYN (Oxon acess)	
Not applicable		Field study – 1 yr (Germany)	No significant ecologically adverse effects at 1.69 L form.n/ha (844 g as/ha) after 1 yr SYN (Oxon acess)	
Not applicable	Preparation - 'Terbuthylazine 500 g/L SC'	Field study – 1 yr	No significant ecologically adverse effects at 1.5 L form.n/ha (750 g tba/ha) after 1 yr. OX-ON *	
Litter bag test				
No data.				

[1] corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 (substance with logPow of > 2)

9.8.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.8-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

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 H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg as/met/kg dw)**	**PEC_{soil} (mg as/met/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
H-01-2022	1.35	1.000	**1.4**
MT1	2.8	0.2889	9.7
MT13	7	0.3174	22.1
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg as/met/kg dw)**	**PEC_{soil} (mg as/met/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
H-01-2022	22.038	1.000	22.04
Chronic effects on *Hypoaspis aculeifer*			
H-01-2022	500	1.000	500

Table 9.8-3: 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 H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg as/met/kg dw)**	**PEC_{soil} (mg as/met/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
H-01-2022	1.35	0.667	**2.0**
MT1	2.8	0.193	14.5
MT13	7	0.212	33.0
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg as/met/kg dw)**	**PEC_{soil} (mg as/met/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
H-01-2022	22.038	0.667	33
Chronic effects on *Hypoaspis aculeifer*			
H-01-2022	500	0.667	749.6

In case of earthworms, risk exposure cannot be excluded since calculated TER values are below the trigger of 5. Further refinement is required.

9.8.2.2 Higher-tier risk assessment

In higher tier risk assessment for earthworms few field studies conducted for similar formulations and summarised in EU DAR were used.

Studies conducted with Gardo Gold (A-9476 C) containing two active substances terbuthylazine and S-metolachlor in Denmark, showed no significant adverse effects on earthworm during one year after application at rate equivalent to 844 g terbuthylazine/ha. In another study conducted in Germany, a statistically significant reduction in the biomass of juveniles was noted in Gardo Gold treatment groups (tested up to 844 g terbuthylazine/ha) after one year. However, this could be due to the effect of the formulation, since Gardo Gold contains terbuthylazine and S-metolachlor and the use of the two active substances together could result in an additive toxicity to earthworms. Formulation Gardo Gold contains the same amount of active substance 500 g/L as formulation H-01-2022.

The studies conducted with Gardoprim / GS 13529 in Denmark and Germany showed no significant effects on earthworm biomass or abundance and no significant ecologically adverse effects after one year at rate of 844 g as/ha which is much higher dose than the maximum proposed use of H-01-2022 (750 g terbuthylazine /ha). Formulation Gardoprim is deemed to be comparable to H-01-2022 i.e. the same formulation type SC, content of active substance 500 g/L.

In the field study with Terbuthylazine 500 SC applied at rate of 0.75 kg terbuthylazine/ha, no significant ecologically adverse effects were found on earthworms. Nonetheless, this study was conducted on grassland field and that could result in much higher foliar interception compared to the proposed use in maize. Formulation Terbuthylazine 500 SC is deemed to be comparable to H-01-2022 i.e. the same formulation type SC, content of active substance 500 g/L.

Considering results of above studies, it was concluded than formulation containing terbuthylazine used at rate equivalent to 844 g terbuthylazine/ha is unlikely to pose a long-term risk to earthworms. Therefore, it is assumed that H-01-2022 (containing 500 g terbuthylazine / L in SC formulation) used at the maximum rate of 750 g as/ha, will not pose unacceptable long-term risk to earthworms.

9.8.3 Overall conclusions

The risk of H-01-2022 to soil meso- and macrofauna was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation H-01-2022 with predicted concentrations in soil PECs. The higher tier risk assessment was performed with results of field studies performed with formulations similar to H-01-2022. According to the performed risk assessment it was concluded that the application of H-01-2022 in accordance with GAP does not pose unacceptable risk to soil meso- and macrofauna. No risk mitigations are required.

zRMS comment:	**Earthworms** The long-term risk assessment for terbuthylazine and H-01-2022 indicates unacceptable long term risk to earthworms. Therefore, further refinement was needed. zRMS considered refinement based on the results from two field studies evaluated in the DAR (2007) where technical terbuthylazine was applied at rate of 844 g a.s/ha. In the DAR (2007) Terbuthylazine Vol 3 B9 for dose of 844 g a.s./ha an acceptable long-term risk to earthworms was concluded. Therefore it is considered that the application of formulation H-01-2022 at rate 1.0 kg prod/ha which is equal to 500 g tbt/ha or at rate 1.5 kg prod/ha which is equal to 750 g tbt/ha is unlikely to pose a long term risk to earthworms.

In EU DAR there is a few field studies for earthworms conducted with different formulations, for example:

- ☑ **TERBUTHYLAZINE 500 SC** – the field study assessed the effects of Terbuthylazine 500 SC applied to established grassland at 1.5l form./ha (equivalent to 0.75 kg terbuthylazine/ha) on earthworms. According to EFSA Conclusion 2011 no significant ecologically adverse effect at 1.5 l form, TERBUTHYLAZINE 500 SC/ha (750 g tba/ha) after 1 year, which is higher than the maximum proposed rate of 1 l/ha of Terbutyloazyna 500 SC) equivalent to 0.5 kg terbuthylazine/ha). However, use on established grassland differs from the proposed pre-emergent and early post-emergent use in maize, with possible differing levels of earthworm exposure in the two situations. It is considered that application to grassland could result in much greater foliar interception (90% interception from grassland, according to the Focus, May 2014) compared to the proposed use in maize (0-25% interception). Therefore, it is very possible that the actual soil exposure to terbuthylazine in the field study was significantly less than that which would occur from the proposed uses on maize.

	Field study	GAP
Test dose	750 g tba/ha	500-750 g tba/ha
Crop	established grassland	maize
Foliar interception	90%	0-25%
Actual soil exposure to terbuthylazine	75 g tba/ha	375-562.5 g tba/ha

The effective dose getting into the soil in a field study does not cover the application proposed in GAP. Therefore, an acceptable long-term risk to earthworms from Terbutyloazyna 500 SC at this application rate on the proposed crops could not be identified from this field study.

- ☑ **'GARDOPRIM ('A5435E')** which contains only the active substance terbuthylazine (TBA). (formulation closely related to Terbutyloazyna 500 SC: the same formulation type SC, the same the amount of active substance contained in formulation 500 g terbuthylazine/L)

The long-term risk to earthworms from the proposed uses of technical terbuthylazine up to 500 g/ha is also considered to be acceptable. This conclusion is based on two field studies included in DAR for formulation Gardoprim / GS 13529 (A-5435 E) in Denmark and Germany. No significant effects on earthworm biomass or abundance were found in these treatment groups 1 yr after application. No significant ecologically adverse effects at 1.69 l form./ha (844 g a.s./ha) after 1 yr., which is higher than the maximum proposed rate of 1 l/ha of Terbutyloazyna 500 SC) equivalent to 0.5 kg terbuthylazine/ha).

Not applicable	Gardoprim / GS 13529 (A-5435 E)	Field study – 1 yr (Denmark)	No significant ecologically adverse effects at 1.69 l form./ha (844 g a.s./ha) after 1 yr	EFSA Journal 2011; 9(1):1969
		Field study – 1 yr (Germany)	No significant ecologically adverse effects at 1.69 l form./ha (844 g a.s./ha) after 1 yr	

An acceptable long-term risk to earthworms from the maximum proposed rate of H-01-2022 (equivalent to 0.5 kg terbuthylazine/ha or 0.750 kg terbuthylazine/ha) could be identified. Acceptable long-term risk for earthworms.

The Applicant should provide a comparison of these formulations in terms of their toxicity to different groups of organisms and also a comparison of their physicochemical properties (amount of active substance in the formulation, type of formulation, composition) in dRR B9 and in document C. In order to demonstrate that both formulations are comparable in terms of ecotoxicology or that the formulation used in the higher-tier risk assessment is a worse case. The risk assessment for earthworms should be considered at the level of the Member States.

Macro-organisms such as *Folsomia candida* and *Hypoaspis aculeifer*
The risk for H-01-2022 is above the trigger value of 5, indicating acceptable chronic risk to other macro-organisms such as *Folsomia candida* and *Hypoaspis aculeifer* from the proposed uses of H-01-2022.

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

Effects on soil microorganisms of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on effects of H-01-2022 on microorganisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	terbuthylazine	28 d	no significant effects of > 25 % on nitrogen transformation up to a max tested concentration of 10.9 mg/kg soil dw	EFSA Journal 2011; 9(1):1969
N-mineralisation	MT1	28 d	no significant effects of > 25 % on nitrogen transformation up to a max tested concentration of 1.84 mg/kg soil dw	
N-mineralisation	MT13	28 d	no significant effects of > 25 % on nitrogen transformation up to a max tested concentration of 3.45 mg/kg soil dw	
N-mineralisation	MT14	28 d	no significant effects of > 25 % on nitrogen transformation up to a max tested concentration of 0.52 mg/kg soil dw	
N-mineralisation	H-01-2022	28 d	no significant effects of > 25 % on nitrogen transformation at: 2.4 and 12 mg/kg soil dw (1.11 and 5.55 mg as/kg dw)	KCP 10.5/01/ Kiran YC/ 2023/ AG-G1155

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.9-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). The metabolite desethy-hydroxy-terbuthylazine (MT14) was also included in the DAR but it is considered to be a minor metabolite, present at only 1.9% AR. Therefore, PECs was not calculated.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of H-01-2022 in maize (max. application rate 750 g as/ha)

Intended use	maize		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**

terbuthylazine	10.9	1.000	yes
terbuthylazine as H-01-2022	1.11 & 5.5	1.000	yes
MT1	1.84	0.2889	yes
MT13	3.45	0.3174	yes

Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of H-01-2022 in maize (max. application rate 500 g as/ha)

Intended use	maize		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
terbuthylazine	10.9	0.667	yes
terbuthylazine as H-01-2022	1.11 & 5.5	0.667	yes
MT1	1.84	0.193	yes
MT13	3.45	0.212	yes

9.9.3 Overall conclusions

The risk of H-01-2022 to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and H-01-2022 with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of H-01-2022 in accordance with GAP does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

zRMS comment:	Agreed. H-01-2022 has no significant effect on soil micro-organisms at 5.5 mg a.s./kg dry soil. Based on it, can be concluded that H-01-2022 under field conditions, use at the proposed rates poses no unacceptable risk to non-target soil microorganisms.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of H-01-2022 were not evaluated as part of the EU assessment of terbuthylazine. The studies on seedling emergence and vegetative vigour for H-01-2022 were submitted in this dossier and deemed acceptable for evaluation and authorisation of H-01-2022. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
monocotyledonous & dicotyledonous	H-01-2022	21 d Seedling emergence	**Onion** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} = 0.3685 L/ha Shoot height ER_{50} = 0.2750 L/ha Dry shoot weight ER_{50} = 0.2769 L/ha Phytotoxicity **ER_{50} = 0.1834 L/ha** **Cabbage** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} = 0.3685 L/ha Shoot height ER_{50} = 0.2425 L /ha Dry shoot weight ER_{50} = 0.2422 L/ha Phytotoxicity **ER_{50} = 0.1834 L/ha** **Carrot** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} = 0.3685 L/ha Shoot height ER_{50} = 0.2398 L /ha Dry shoot weight ER_{50} = 0.2320 L/ha Phytotoxicity **ER_{50} = 0.1834 L/ha** **Pea** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} >0.48 L/ha Shoot height ER_{50} >0.48 L/ha Dry shoot weight ER_{50} >0.48 L/ha Phytotoxicity ER_{50} >0.48 L/ha **Perennial ryegrass** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} = 0.3685 L/ha Shoot height ER_{50} = 0.2749 L /ha Dry shoot weight ER_{50} = 0.2516 L/ha Phytotoxicity **ER_{50} = 0.1834 L/ha** **Sunflower** Seedling emergence ER_{50} >0.48 L/ha Seedling survival ER_{50} = 0.3685 L/ha Shoot height ER_{50} = 0.2609 L /ha Dry shoot weight ER_{50} = 0.2854 L/ha Phytotoxicity **ER_{50} = 0.1834 L/ha**	KCP 10.6.2/01/ Vishala N / 2023 / AG-G1156
monocotyledonous & dicotyledonous	H-01-2022	21 d Vegetative vigour	**Onion, Cabbage, Sunflower** Plant mortality ER_{50} >0.8 L /ha Shoot height ER_{50} >0.8 L /ha Dry shoot weight ER_{50} >0.8 L /ha Phytotoxicity ER_{50} = 0.3069 L /ha **Carrot** Plant mortality ER_{50} =0.6452 L /ha Shoot height ER_{50} >0.8 L /ha Dry shoot weight ER_{50} >0.8 L /ha Phytotoxicity **ER_{50} = 0.1535 L /ha** **Pea** Plant mortality ER_{50} =0.6710 L /ha Shoot height ER_{50} >0.8 L /ha Dry shoot weight ER_{50} >0.8 L /ha Phytotoxicity **ER_{50} = 0.1535 L /ha** **Perennial ryegrass**	KCP 10.6.2/02/ Vishala N / 2023 / AG-G1157

			Plant mortality ER_{50} =0.6854 L /ha Shoot height ER_{50} >0.8 L /ha Dry shoot weight ER_{50} >0.8 L /ha Phytotoxicity ER_{50} = 0.6135 L /ha	

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product H-01-2022. Details of studies and results are included in Table 9.10-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of H-01-2022 in maize (worst case scenario, max. application rate 1.5 L/ha)

Intended use	maize			
Product	H-01-2022			
Application rate (ml/ha)	1 × 1500			
MAF	1			
Test species	**ER_{50} (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER criterion: TER ≥ 5**
Seedling emergence (Tier I)				
onion, cabbage, carrot, perennial ryegrass, sunflower	183.4	2.77	41.55	**4.4**
Vegetative vigour (Tier I)				
carrot, pea	153.5	2.77	41.55	**3.7**

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

Table 9.10-3: Assessment of the risk for non-target plants due to the use of H-01-2022 in maize (worst case scenario, max. application rate 1 L/ha)

Intended use	maize
Product	H-01-2022
Application rate (ml/ha)	1 × 1000

MAF	1			
Test species	**ER_{50} (ml/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER criterion: TER ≥ 5**
Seedling emergence (Tier I)				
onion, cabbage, carrot, perennial ryegrass, sunflower	183.4	2.77	27.7	6.6
Vegetative vigour (Tier I)				
carrot, pea	153.5	2.77	27.7	5.5

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

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 for the highest application rate 1.5 L/ha, 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 9.10-4: Risk assessment for non-target terrestrial plants due to the use of H-01-2022 in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) (worst case scenario, max. application rate 1.5 L/ha)

Intended use		maize			
Product		H-01-2022			
Application rate (ml/ha)		1 × 1500			
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	2.77	41.55	20.78	10.39	4.16
5	0.57	8.55	4.28	2.14	0.86
10	0.29	4.35	2.18	1.09	0.44
Toxicity value		**TER**			
ER_{50} = 153.5 ml/ha		**criterion: TER ≥ 5**			
1		**3.7**	7.4	14.8	36.9
5		18.0	35.9	71.7	178.5
10		35.3	70.4	140.8	348.9

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

9.10.3 Overall conclusions

The risk of H-01-2022 to non-target plants was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation H-01-2022 with application rates. According to the performed risk assessment it was assessed that the application of H-01-2022 at maximum rate of 1.5 L/ha (750 g as/ha) does not pose unacceptable risk to non-target plants provided risk mitigation measures are applied:

- 5m buffer zone or
- 50% nozzle reduction.

In case of lower application rate 1 L/ha no risk mitigation measures are required.

zRMS comment:	Accepted. The following risk mitigation measures should be applied to non-crop area: ***SPe 3:*** **-*5* m buffer zone or** **-1 m and use of 50% drift reducing nozzles**

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

Not available.

9.12 Monitoring data (KCP 10.8)

Not available.

9.13 Classification and Labelling

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

Table 9.13-1: Justified proposals for classification and labelling for H-01-2022 according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS09
Signal word:	Warning
Hazard statement(s):	Very toxic to aquatic life. [H400] Very toxic to aquatic life with long lasting effects. [H410]
Precautionary statement(s):	Collect spillage [P391]
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farm-

	yards and roads). [SP 1] To protect aquatic organisms, respect an 5m vegetated unsprayed buffer zone of to surface water bodies. [SPe 3]

Table 9.13-2: Summary of evaluation of the ecotoxicological studies for H-01-2022

Type of test, species, model system (Guide-line)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	E_yC_{50} = 0.4 mg/L	**A**	Aquatic Acute 1, H400	KCP 10.2.1.4/01 / Likith NG / 2023/ AG-G1148
Chronic toxicity to aquatic organisms	no data for formu-lation, classifica-tion based on com-position	**A**	Aquatic Chronic 1, H410	Please refer to dRR Part C

zRMS comment: Accepted.

Type of test, species, model system (Guide-line)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	E_yC_{50} = 0.4 mg/L	**A**	Aquatic Acute 1, H400	KCP 10.2.1.4/01 / Likith NG / 2023/ AG-G1148
Chronic toxicity to aquatic organisms	no data for formulation, classification based on composition	**A**	Aquatic Chronic 1, H410	Please refer to dRR Part C

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2.1.2/01	Rachana AR	2023	H-01-2022: *Daphnia magna*, Acute Immobilisation Test. Study Code: AG-G1146 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.2.1.3/01	Rachana AR	2023	H-01-2022: Alga, growth inhibition test with *Raphidocelis subcapitata.* Study Code: AG-G1147 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.2.1.4/01	Likith NG	2023	H-01-2022 *Lemna*, Growth Inhibition Test. Study Code: AG-G1148 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.2.1.4/02	Likith NG	2023	H-01-2022 Water-sediment *Myriophyllum spicatum* toxicity Study Code: AG-G1158 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP	N	ProAgri International 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**
			Unpublished		
KCP 10.3.1.1.1/01	Gangadhar RS	2023	H-01-2022: Acute oral toxicity test in honey bees. Study code: AG-G1150 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.3.1.1.2/01	Gangadhar RS	2023	H-01-2022: Acute contact toxicity test in honey bees. Study Code: AG-G1151 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.3.1.1.2/02	Gangadhar RS	2023	H-01-2022: Chronic oral toxicity test in honey bee (*Apis mellifera* L.). Study Code: AG-G1152 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.3.1.4/01	Gangadhar RS	2023	H-01-2022: Honeybee (*Apis mellifera* L.) larval toxicity test, repeated exposure Study Code: AG-G1149 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.3.2.1/01	Mautino G	2023	Effects of H-01-2022 (terbuthylazine 500 g/L) on parasitoid *Aphidius rhopalosiphi* in the laboratory – Standard laboratory test Study Code: 1013.H.SAG23/r Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.3.2.1/02	Mautino G	2023	Effects of H-01-2022 (terbuthylazine 500 g/L) on predator mite *Typhlodromus pyri* in the laboratory –	N	ProAgri

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**
			Standard laboratory test Study Code: 1014.H.SAG23/r Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished		International Sp. z o.o.
KCP 10.4.1.1/01	Vishala N	2023	H-01-2022: Earthworm reproduction test (*Eisenia fetida*) Study Code: AG-G1153 Source: Eurofins Advinus Agrosciences Services India Private Limited Unpublished GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.4.2.1/01	Mautino G	2023	Predatory mite *Hypoaspis (Geolaelaps) aculeifer* reproduction test in soil with H-01-2022 (terbuthylazine 500 g/L) Study Code: 1015.H.SAG23/r Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.4.2.1/02	Gangadhar RS	2023	H-01-2022: *Folsomia candida*, collembolan reproduction test in soil Study Code: AG-G1154 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.5/01	Kiran YC	2023	Soil Microorganisms: Nitrogen Transformation Test of H-01-2022. Study Code: AG-G1155 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International Sp. z o.o.
KCP 10.6.2/01	Vishala N	2023	H-01-2022: Seedling emergence and seedling growth test with terrestrial plants Study Code: AG-G1156 Source: Eurofins Advinus Agrosciences Services India Private Limited	N	ProAgri International 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 Unpublished		
KCP 10.6.2/02	Vishala N	2023	H-01-2022: Vegetative Vigour Test Study Code: AG-G1157 Source: Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	ProAgri International 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**

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

Not relevant. No studies submitted. The formulation study is not considered essential, because active substance data on toxicity to birds are used. It is possible to extrapolate from active substance data. Additionally, duplication of vertebrate's studies should be avoided.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Not relevant. No studies submitted. The formulation study is not considered essential, because active substance data on toxicity to mammals are used. It is possible to extrapolate from active substance data. Additionally, duplication of vertebrate's studies should be avoided.

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

Not relevant. No studies submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 KCP 10.2.1.1 Acute toxicity to fish

Not relevant. No studies submitted. The formulation study is not considered essential, because active substance data on toxicity to fish are used. It is possible to extrapolate from active substance data. Additionally, duplication of vertebrate's studies should be avoided.

A 2.2.1.2 KCP 10.2.1.2 Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 202 of the test were met.

Stability of the test item in the test medium was determined under same study and found to be stable at 101.00 µg/L and 101.0 mg/L for 48 hours at room temperature The results of analysis for 3.125, 6.25, 12.5, 25.0 and 50.000 mg/L showed that the recovery for H-01-2022 was 98.616 to 100.196% (% RSD was 0.302 to 0.717) at the start of test and 102.229 to 104.197 % (% RSD was 0.902 to 1.638) at the end of test (48 h), indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of ≤ 20%).

Validity criteria:

VALIDITY OF THE TEST

This test met all the validity criteria:

- There was no immobilization of daphnia in the negative control during the test period.
- The dissolved oxygen concentration at the end of the test was > 3 mg/L in negative control and test vessels.

Deviation of the study: none

Agreed toxicity endpoints:

Time Points	End point based on nominal concentrations				
	EC value based on nominal concentrations			95% Fiducial limits	
	mg/L		mg a.i./L	mg/L	mg a.i./L
24 Hour	E_rC_{50}	38.29	15.83	33.61- 44.69	13.89- 18.47
	LOEC	12.5	5.17	NA	NA
	NOEC	6.25	2.58	NA	NA
48 Hour	E_yC_{50}	19.35	8.00	15.43- 24.16	6.38- 9.99
	LOEC	12.5	5.17	NA	NA
	NOEC	6.25	2.58	NA	NA

Reference:	KCP 10.2.1.2/01
Report	H-01-2022: *Daphnia magna*, acute immobilization test Rachana AR; 2023; Study code: AG-G1146
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024
2. Vehicle and/or positive control:	vehicle control: test medium, positive control: potassium dichromate
3. Test organism	
Species:	*Daphnia magna* Straus
Source:	Daphnids were obtained from Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany and cultured in Ecotoxicology Section, Eurofins Advinus limited, Bengaluru, India were used in this test
Age:	less than 24-hour old neonates of daphnia
Feeding:	during the test daphnia were not fed
Test units:	glass beakers of volume 250 mL
4. Environmental conditions:	
Medium:	The medium prepared according OECD Guideline No. 202 on the basis of deionized water by adding stock solutions of reagent-grade chemicals
Stability of test compound:	Stability of the test item in the test medium was determined under same study and found to be stable at 101.00 µg/L and 101.0 mg/L for 48 hours at room temperature.
pH:	control: 7.70 – 7.76
Dissolved oxygen:	control: 6.9 – 7.3 mg/L
Temperature:	20.0-20.8 °C
Lighting:	640 to 696 lux with 16:8 h light period

STUDY DESIGN AND METHOD

Immobilisation of *Daphnia magna* exposed to the test item, H-01-2022, was investigated during a 48-hour a static test. A range finding test which was performed as a non GLP test, resulted in 0, 0, 0, 0, 0, 20, and 100 % immobilization of daphnia at the tested concentrations of 0.001, 0.01, 0.1, 1, 10 and 100 mg/L at 48 h exposure. Hence, the test concentrations of 3.125, 6.25, 12.5, 25.0 and 50.0 mg/L (factor 2.0) were selected for the definitive test. In a definitive test, 1000 mg of test items initially mixed with small volume of reconstituted water and made up to 1000 mL by using reconstituted water. This was be used as stock for remaining test concentrations preparations: 3.125, 6.25, 12.5, 25.0 and 50.00 mg/L plus the control. 1 L of test medium without any test item addition was used as negative control. 150 mL of prepared

negative control and test item concentrations was dispensed separately into 4 test containers. All the test concentrations along with the negative control were analyzed for the test item concentrations at the beginning and end of test. For analysis, triplicate composite sample was drawn from prepared test concentrations.

The test was performed in glass beakers of 250 mL capacity, containing 150 mL of either the test item concentration or the control per replicate. pH and dissolved oxygen concentration of test concentrations at the start and end of the treatment were recorded. The *Daphnia magna* were observed for immobilisation after 24 and 48 h of exposure and any abnormal behaviour or appearance. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

Test design:	4 replicates per each test item concentration and the control; 5 *Daphnia magna* in each replicate
Type of the exposure:	static
Exposure time:	48 hours
Tested concentrations, definitive test:	3.125, 6.25, 12.5, 25.0 and 50.00 mg/L plus the control
Dates:	start of the study 07.04.2023 start of the experimental part: 17.04.2023 end of the experimental part: 26.04.12.2023 end of the study: 06.11.2023
Statistic:	GraphPad Prism (Version: 9.5.0.730) (which consider the equation: Sigmoidal dose-response (variable slope); Y = bottom + (Top-Bottom) / (1+10^((LogEC_{50} X) × HillSlope), a validated computer program used to calculate EC_{50}.
Validity of the test:	This test met all the validity criteria: There was no immobilization of daphnia in the negative control during the test period. The dissolved oxygen concentration at the end of the test was > 3 mg/L in negative control and test vessels.

RESULTS

Summary of daphnia immobilization presented below. There was no immobilization of daphnia in the negative control. The immobilization of daphnia was 0, 0, 10, 35 and 60% at 24 h and 0, 0, 25, 60 and 100 % at 48 h exposure respectively at nominal concentrations of 3.125, 6.25, 12.5, 25.0 and 50.00 mg/L of H-01-2022.

Table KCP 10.2.1.2-1 Definitive Test – Results on Daphnia Immobilization

Group	Treatment (mg test item/L)	No. of Daphnia immobilized (5 Daphnia per replicate)								% Immobilization	
		24 h				48 h				24 h	48 h
		R1	R2	R3	R4	R1	R2	R3	R4		
G1	0.0 Negative Control	0	0	0	0	0	0	0	0	0	0
G2	3.125	0	0	0	0	0	0	0	0	0	0

G3	6.25	0	0	0	0	0	0	0	0	0	0
G4	12.5	0	0	0	2	1	2	1	1	10	25
G5	25.0	2	1	2	2	3	2	4	3	35	60
G6	50.0	3	2	4	3	5	5	5	5	60	100

CONCLUSION

The NOEC, LOEC and EC50 for 24h and 8h on the basis of the nominal concentrations of the test item is presented below.

Table KCP 10.2.1.2-2 Endpoints based on nominal concentrations

Time points	**mg/L**	**mg as/L**
24 hour	E_rC_{50}	38.29
	LOEC	12.5
	NOEC	6.25
48 hour	$E_\gamma C_{50}$	19.35
	LOEC	12.5
	NOEC	6.25

A 2.2.1.3 KCP 10.2.1.3 Effects on aquatic algae

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 201 of the test were met. **Validity criteria:** There was an increase in cell concentration of the negative control culture by a factor of 79.33 which is more than the required factor limit of at least 16 at the end of the test. The mean coefficient of variation for section by section specific growth rates in the negative control cultures during the course of the test was 27.51% which is within the required limit of not more than 35%. The coefficient of variation of average growth rate between replicates negative control cultures was 0.27%, which is within the required limit of not more than 7%. **Agreed toxicity endpoints:**

The EC_{50} value for growth rate and the yield based on the nominal concentration of the test item is presented below:

At 72 hours	EC values and Fiducial limits (mg test item/L)	EC values and Fiducial limits (mg a.i./L)
ErC50	2.427 (NA- 3.556)	1.003 (NA-1.470)
ErC20	1.834 (0.6960- 2.964)	0.7580 (0.2876-1.2250)
ErC10	1.556 (0.3829-2.709)	0.6431 (0.1583-1.1197)
EyC50	0.7564 (0.5040- 1.141)	0.3126 (0.2083-0.4716)
EyC20	0.2701 (0.1321-0.4686)	0.1116 (0.0546-0.1937)
EyC10	0.1479 (0.05361-0.3067)	0.0611 (0.0221-0.1268)

Note: E_rC refers to growth rate; E_yC refers to yield.

Reference:	KCP 10.2.1.3/01
Report	H-01-2022: Alga, growth inhibition test with *Raphidocelis subcapitata*. Rachana AR; 2023; Study Code: AG-G1147
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024

2. Vehicle and/or positive control: vehicle control: algal medium as described in OECD test guideline 201(OECD, 2011)

3. Test organism

Species: *Raphidocelis subcapitata* ATCC® 22662 (formerly *Pseudokirchneriella subcapitata*)

Source:	American Type Culture Collection, P.O.Box 1549 Manassas, VA 20108, USA
Age:	three days prior to the start of the test
Test units:	flask of a volume 250 mL

4. Environmental conditions:

Medium:	algal medium as described in the OECD test guideline 201(OECD, 2011)
Stability of test compound:	stability test was carried out and found to be stable at low dose 102.00 µg/L and high dose 102.00 mg/L test item concentrations in the matrix- algal water
Medium temperature:	20.1 – 20.8 ºC
pH:	8.08 to 8.14
Lighting:	mean light intensity: 7160 to 7173.7 lux

DESIGN AND METHOD

The objective of the study was to determine the chronic effects of the test item, H-01-2022, on the growth of the unicellular green algal species *Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata)* for 72 hours and to determine EC_{10}, EC_{20}, and EC_{50} as well as LOEC and NOCE endpoints values. A range finding test was carried out to determine the test concentrations for the definitive test. The reduction of algal cell biomass at 72 h exposure at the tested concentration of 0.001, 0.01, 0.1, 1, 10 and 100 mg/L was 0, 0.34, 1.01, 1.68, 2.36, 100 and 100%, respectively. In definitive test the alga was exposed to the test item at the nominal concentrations of 0.128, 0.32, 0.8, 2 and 5 mg/L (factor of 2.5) along with a negative control. The cell growth was measured at 24, 48 and 72 hours after the initiation of the test. The concentration/effect relationship was determined using two factors namely growth rate and yield at the end of the test (72 hours). Following parameters were observed and recorded during both range finding test and definitive test. The pH of the controls and the test solutions was checked at the beginning and at the end of the test. Light intensity inside the algal chamber (at four random locations) was measured once daily using a Lux meter. Temperature inside the algal chamber was recorded once daily. Shaking rate for growth chamber maintained at 150 rpm. The algal biomass in each flask was determined at 24, 48 and 72 hours after the start of the test using a haemocytometer. The measured cell concentrations in the test cultures and controls were tabulated together with the concentrations of the test item and the times of measurement. Microscopic observation of algal cells at 24hr ±1, 48 ±1, and at 72h±1 after exposure.

Test design:	tested concentrations in three replicates, control in six replicates, flasks arranged randomly
Type of the exposure:	static
Exposure time:	72 hours
Inoculum:	1 x 10^4 cells/mL
Tested concentrations, definitive test:	0.128, 0.32, 0.8, 2, and 5 mg/L
Dates:	start of the study 07.04.2023 start of the experimental part: 17.04.2023 end of the experimental part: 27.04.2023 end of the study: 07.11.2023

Statistic:	GraphPad Prism (Version: 9.5.0.730)
Validity of the test:	There was an increase in cell concentration of the negative control culture by a factor of 79.33 which is more than the required factor limit of at least 16 at the end of the test. The mean coefficient of variation for section by section specific growth rates in the negative control cultures during the course of the test was 27.51% which is within the required limit of not more than 35%. The coefficient of variation of average growth rate between replicates negative control cultures was 0.27%, which is within the required limit of not more than 7%

CONCLUSION

The EC_{50} value for growth rate and the yield based on the nominal concentration of the test item is presented below.

Table KCP 10.2.1.3-1: Freshwater alga growth inhibition test – final results

At 72 hours	EC (mg/L)	EC (mg as/L)
E_rC_{50}	**2.427**	**1.003**
E_rC_{20}	1.834	0.7580
E_rC_{10}	1.556	0.6431
E_yC_{50}	**0.7564**	**0.3126**
E_yC_{20}	0.2701	0.1116
E_yC_{10}	0.1479	0.0611

A 2.2.1.4 KCP 10.2.1.4 Effects on aquatic macrophytes

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 221 of the test were met. **Validity criteria:** The *Lemna* growth inhibition test fulfills the validity criteria of the OECD guideline 221: The factor of frond number increase, measured in the control between day 0 and day 7, was 13.5 corresponding to an average specific growth rate for frond number in the control of 0.424 per day and to a doubling time of 1.6 days (validity criterion: growth rate ≥ 0.275 per day, doubling time < 2.5 days). **Agreed toxicity endpoints:**

Parameter	End point (mg test item/L)				
	EC value			95% Fiducial limits	
		mg/L	mg a.i./L	mg/L	mg a.i./L
Inhibition in specific growth rate in frond number	E_rC_{50}	0.601	0.2484	0.5528-0.6543	0.0390-0.2704
	E_rC_{20}	0.3769	0.1558	0.34646-0.4101	0.1432-0.1687
	E_rC_{10}	0.2953	0.1220	0.27138-0.32123	0.1122-0.1321
	LOEC	0.25	0.1033	NA	NA
	NOEC	0.125	0.0517	NA	NA
Inhibition in yield rate in frond number	E_yC_{50}	0.4	0.1653	0.366-0.441	0.1505-0.1813
	E_yC_{20}	0.247	0.1020	0.2251-0.2712	0.0925-0.1115
	E_yC_{10}	0.192	0.0793	0.1745-0.2103	0.0717-
	LOEC	0.25	0.1033	NA	NA
	NOEC	0.125	0.0517	NA	NA
Inhibition in dry weight-growth rate	E_rC_{50}	1.07	0.4401	1.001-1.151	0.4117-0.4734
	E_rC_{20}	0.75	0.3085	0.6996-0.8043	0.2877-0.3308
	E_rC_{10}	0.622	0.2558	0.5801-0.667	0.2386-0.2743
	LOEC	0.25	0.1033	NA	NA
	NOEC	0.125	0.0517	NA	NA
Inhibition in dry weight - yield	E_yC_{50}	0.82	0.3372	0.531-1.239	0.2184-0.5096
	E_yC_{20}	0.574	0.2360	0.3713-0.8665	0.1527-0.3564
	E_yC_{10}	0.476	0.1957	0.3079-0.7187	0.1266-0.2956
	LOEC	0.25	0.1033	NA	NA
	NOEC	0.125	0.0517	NA	NA

Reference: KCP 10.2.1.4/01

Report: H-01-2022 *Lemna*, Growth Inhibition Test; Likith NG; 2023; Study Code: AG-G1148

Guideline(s): Yes, OECD 221

Deviations: Not relevant

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): H-01-2022

Formulation: SC (terbuthylazine 500 g/L)

Description (physical state): -

Batch no.: PA110522

Production date: 05.2022

Expiration date: 05.2024

2. Vehicle and/or positive control: vehicle: Swedish Standard (SIS) medium
positive control: 3,5-dichlorophenol

3. Test organism

Species:	*Lemna minor*
Source:	from Eurofins Agroscience Services Ecotox GmbH Aquatic Toxicology, Eutinger Strasse 24 D-75223 Niefern Öschelbronn, Germany
Test units:	glass beakers of 250 mL capacity

4. Environmental conditions:

Medium:	Swedish Standard (SIS) medium
Stability of test compound:	The test item was recoverable using the analytical method at nominal concentrations of 102.0µg/L and 102.0 mg/L in the test medium.
Medium temperature:	23.1 – 24.8ºC
pH:	6.99-7.72
Lighting:	6932 to 7162 lux; constant illumination

STUDY DESIGN AND METHOD

The effect of H-01-2022 was tested on the growth of freshwater vascular aquatic plant, *Lemna minor*. Based on the results of the range finding test, the definitive test was carried out using the test concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L; along with control. Each test item group had three replicate and control had six replicates. Lemna was exposed in semi-static mode with renewal of test medium on days 3 and 5.

Following parameters were observed and recorded during definitive test. Light intensity was measured in the growth chamber at points the same distance from the light source as the Lemna fronds. Measurements were made at the start of test. The temperature of the test medium in a surrogate vessel held under the test conditions was recorded daily. The pH of test solutions was recorded at the start and end of each renewal of test medium. Frond numbers appearing normal or abnormal were determined at the beginning of the test, on days 3, 5 and 7 (at test termination). Changes in plant development, e.g. in frond size, appearance, indication of necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, and in root length and appearance were observed. Significant features of the test medium (e.g. presence of undissolved material, growth of alga in the test vessel) was also noted. Dry weight was determined at the start of the test from a sample of the inoculum culture representative of what is used to begin the test, and at the end of the test with the plant material from each test and control(s) vessel. The measured frond numbers and dry weight in the test cultures and control(s) were tabulated together with the concentrations of the test item and the times of measurement.

Test design:	tested concentrations in three replicates, control in six replicates, 9 fronds on every replicate
Type of the exposure:	semi-static
Exposure time:	7 days
Tested concentrations, definitive test:	0.125, 0.25, 0.5, 1.0, and 2.0 mg/l

Dates:	start of the study 03.04.2023 start of the experimental part: 04.04.2023 end of the experimental part: 27.04.2023 end of the study: 07.11.2023
Statistic:	Probit analysis using a validated computer program.
Validity of the test:	The factor of frond number increase, measured in the control between day 0 and day 7, was 13.5 corresponding to an average specific growth rate for frond number in the control of 0.424 per day and to a doubling time of 1.6 days (validity criterion: growth rate ≥ 0.275 per day, doubling time < 2.5 days).

RESULTS

At measured test concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L, the observed growth rate and yield for frond numbers and dry weight resulted in toxic effects on growth of Lemna when compared with the control during the test period Fronds were found to be normal in appearance in control and test item treated group at the end of the test. Test medium was clear in control and opaques blue colored in test item treated groups. Test medium was similar in appearance on days 3, 5 and 7 as compared to the start of the exposure.

Fronds were found to be normal in appearance in control, and test item treated group at the end of the test. Test medium was clear in control and dark black colored solution in treatment group. Test medium was similar in appearance on days 3, 5 and 7 as compared at the start of the exposure.

The test item was recoverable using the analytical method at nominal concentrations of 102.0µg/L and 102.0 mg/L in the test medium. The stability test results concluded that the test item was found to be stable in the test medium at the test conditions up to 4 days at nominal concentrations of 102.0µg/L and 102.0 mg/L. The results of active ingredient analysis of 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L showed that overall percent agreement was from 97.127 to 99.531% (with % RSD 0.127 to 2.050) at the start of renewals; 101.838 to 102.453% (with % RSD 0.645 to 0.2.092) at the end of renewals indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%). No measurable concentration of test item was recorded in the control samples.

CONCLUSION

The NOEC and EC_{50} for growth rate and yield (for frond number and dry weight) on the basis of the nominal concentration of the test item is presented in table below.

Table KCP 10.2.1.4-1: *Lemna minor* growth inhibition test-final results (nominal)

	End point	mg/L	mg as/L
Inhibition in specific growth rate in frond number	**E_rC_{50}**	**0.601**	**0.2484**
	E_rC_{20}	0.3769	0.1558
	E_rC_{10}	0.2953	0.1220
	LOEC	0.25	0.1033
	NOEC	0.125	0.057
Inhibition in yield rate in frond number	**E_yC_{50}**	**0.4**	**0.1653**
	E_yC_{20}	0.247	0.1020
	E_yC_{10}	0.192	0.0793
	LOEC	0.25	0.1033

	NOEC	0.125	0.0517
Inhibition in dry weight- growth rate	**E_rC_{50}**	**0.4**	**0.4401**
	E_rC_{20}	0.247	0.0385
	E_rC_{10}	0.192	0.2558
	LOEC	0.25	0.1033
	NOEC	0.125	0.0517
Inhibition in dry weight - yield	**E_yC_{50}**	**0.41**	**0.3372**
	E_yC_{20}	0.216	0.2360
	E_yC_{10}	0.154	0.1957
	LOEC	0.25	0.1033
	NOEC	0.125	0.0517

Comments of zRMS: The study is acceptable. The validity criteria according OECD 239 of the test were met.

Validity criteria:

The Water-Sediment *Myriophyllum spicatum* toxicity test fulfills the following validity criteria of the OECD Test Guideline 239:

- The mean total shoot length was 11.5 cm and mean total shoot fresh weight was 813.0 in control plants on day 14 which doubled when compared to day 0 during the exposure phase of the test. In addition, control plants were not shown any visual symptoms of chlorosis and they were visibly free from contamination such as algae and/or bacterial films on the plants.

- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e., from test initiation to test termination) was found 31.0% in the control cultures which was within the guideline specified 35% between replicates.

Deviation of the study: none

Agreed toxicity endpoints:

***Myriophyllum spicatum* toxicity test -final results**

	Endpoints	**mg/L**	**mg as/L**
% Growth rate in shoot length	E_rC_{50}	3.298	1.363
	E_rC_{20}	2.153	0.890
	E_rC_{10}	1.667	0.689
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Yield inhibition of shoot length	E_yC_{50}	2.830	1.170
	E_yC_{20}	1.781	0.737
	E_yC_{10}	1.359	0.562
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Growth rate in dry weight	E_rC_{50}	3.757	1.553
	E_rC_{20}	2.094	0.865
	E_rC_{10}	1.487	0.615
	LOEC	2.5	1.033

		NOEC	1.25	0.517
	% Yield inhibition of dry weight	E_yC_{50}	2.785	1.151
		E_yC_{20}	1.465	0.605
		E_yC_{10}	1.007	0.416
		LOEC	2.5	1.033
		NOEC	1.25	0.517
	% Growth rate in fresh weight	E_rC_{50}	3.610	0.149
		E_rC_{20}	1.738	0.718
		E_rC_{10}	1.133	0.468
		LOEC	2.5	1.033
		NOEC	1.25	0.517
	% Yield inhibition of fresh weight	E_yC_{50}	2.706	1.118
		E_yC_{20}	1.235	0.510
		E_yC_{10}	0.7808	0.323
		LOEC	2.5	1.033
		NOEC	1.25	0.517

Reference:	KCP 10.2.1.4/02
Report	H-01-2022 Water-sediment *Myriophyllum spicatum* toxicity test; Likith NG.; 2023; Study Code: AG-G1158
Guideline(s):	Yes, OECD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024
Stability of test compound:	The test item was recoverable using the analytical method at nominal concentrations of 0.0218 and 109.0 mg/L (for water samples), 0.02112 and 100.101 mg/g (for sediment), and 0.0224 and 103.000 mg/L (for sediment pore water). The stability test results concluded that the test item was found to be stable in the test medium at the test conditions up to 14 days at nominal concentrations.

2. Vehicle and/or positive control: vehicle: Smart & Barko medium

positive control: 3,5-dichlorophenol

3. Test organism

Species:	*Myriophyllum spicatum*
Source:	Department of Ecotoxicology, Eurofins Advinus Agro-sciences Services India Private Limited Bengaluru
Test units:	glass test vessels of 2L capacity were used as test containers and glass plant pots (the sediment surface area covers more than 70% of the vessel surface area)

4. Environmental conditions:

Medium:	Smart and Barko is the test medium as described in the OECD test guideline 239 (OECD 2014)
Lighting:	light intensity 8880 to 11840 Lux within ± 15%) with 16 h light and 8 h dark cycle
Temperature:	18-22°C

STUDY DESIGN AND METHOD

The effect of test item, H-01-2022 was tested on the rooted, aquatic plant species *Myriophyllum spicatum*, growing in a water-sediment system.
Exponentially growing plant cultures of the *Myriophyllum spicatum* were allowed to grow in different concentrations of the test item over a period of 14 days. Effects on growth were determined from quantitative assessments of shoot length, fresh and dry weight, as well as qualitative observations of symptoms such as chlorosis, necrosis or growth deformities. Based on the results of the range finding test, the definitive test was carried out using the test concentrations of 0.625, 1.25, 2.5, 5.0 and 10.0 mg/L along with control. Each test item treated group had four replicates and control had six replicates. *Myriophyllum spicatum* was exposed in static mode. Total shoot length, dry weight and fresh weight were recorded on day 0 (start of the test) and day14 (end of the test).

Test design:	six replicates were maintained for the controls and 4 replicates for test item treated groups
Test type:	water spiked
Type of the exposure:	static
Exposure time:	14 days exposure phase
Tested concentrations, definitive test:	0.625, 1.25, 2.5, 5.0 and 10.0 mg/L
Dates:	start of the study 25.10.2023 start of the experimental part: 25.10.2023 end of the experimental part: 24.11.2023 end of the study: 15.12.2023
Statistic:	GraphPad Prism 8.0

RESULTS

The growth rate and yield inhibition for total shoot length, fresh and dry weight in the definitive test are as follows.

Table KCP 10.2.1.4-2: *Myriophyllum spicatum* growth rate and yield inhibition

Test concentration (mg/L)	Shoot Length		Dry weight		Fresh weight	
	% I_r	% I_Y	% I_r	% I_Y	% I_r	% I_Y
0.625	0	0.0	1.1	3.5	1.5	4.1
1.25	8.7	13.5	10.1	16.5	12.3	19.6
2.5	27.5	37.8	29.2	44.2	40.0	52.7
5.0	79.7	86.8	60.7	75.0	52.3	64.3
10.0	100.0	100.0	100	100.0	100.0	100.0

The growth and appearance of plants in the control was normal till the end of the test. The treated plants at 0.625 mg/L were found to be normal in appearance and comparable to control plants till the end of the test. At 1.25 mg/L the treated plants showed stunted growth and at 2.5 mg/L the treated group exhibited stunted growth as compared to control plants and exhibited mortality of 9 plants at the end of the test. At 5 mg/L the treated group exhibited both stunted growth and discoloration in plants as compared to control plants and mortality of 9 plants at the end of the test. At 10 mg/L all treated plants found dead at the end of test. No mortality of plants was observed in control group till the end of the test. The mortality of plants was 0, 0, 75, 75 and 100 % at 0.625, 1.25, 2.5, 5.0 and 10.0 mg/L at the end of test. Test solutions in test item treated groups appeared homogenous white colour suspension (color gradient was there across the test concentrations from lower to higher). Test solution appearance was similar on days 7 and 14 as compared to what it was at the start of the exposure.
The test item was recoverable using the analytical method at nominal concentrations of 0.0218 and 109.0 mg/L (for water samples), 0.02112 and 100.101 mg/g (for sediment), and 0.0224 and 103.000 mg/L (for sediment pore water). The stability test results concluded that the test item was found to be stable in the test medium at the test conditions up to 14 days at nominal concentrations.

CONCLUSION

The NOEC, LOEC and EC50, EC20, EC10 for growth rate and yield (for total shoot length, dry weight and fresh weight) based on the nominal concentration of the test item is as follows.

Table KCP 10.2.1.3-2: *Myriophyllum spicatum* toxicity test -final results

	Endpoints	mg/L	mg as/L
% Growth rate in shoot length	E_rC_{50}	3.298	1.363
	E_rC_{20}	2.153	0.890
	E_rC_{10}	1.667	0.689
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Yield inhibition of shoot length	E_yC_{50}	2.830	1.170
	E_yC_{20}	1.781	0.737
	E_yC_{10}	1.359	0.562
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Growth rate in dry weight	E_rC_{50}	3.757	1.553
	E_rC_{20}	2.094	0.865
	E_rC_{10}	1.487	0.615
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Yield inhibition of dry weight	E_yC_{50}	2.785	1.151
	E_yC_{20}	1.465	0.605
	E_yC_{10}	1.007	0.416
	LOEC	2.5	1.033

	NOEC	1.25	0.517
% Growth rate in fresh weight	E_rC_{50}	3.610	0.149
	E_rC_{20}	1.738	0.718
	E_rC_{10}	1.133	0.468
	LOEC	2.5	1.033
	NOEC	1.25	0.517
% Yield inhibition of fresh weight	E_yC_{50}	2.706	1.118
	E_yC_{20}	1.235	0.510
	E_yC_{10}	0.7808	0.323
	LOEC	2.5	1.033
	NOEC	1.25	0.517

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

Not relevant. No studies submitted. The long-term formulation study is not considered essential, because active substance data on long-term toxicity and acute formulation toxicity to aquatic organisms are used and deemed to be sufficient for evaluation and risk assessment.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant. No studies submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 213 of the test were met. **Validity criteria:** The current study was carried out to test the effects of H-01-2022 in honeybees, *Apis mellifera* L. when administered through oral route. The study met the validity criteria stipulated in the guideline. • No mortality was observed in control and was within the specified 10 per cent limit at the end of the test. • The LD_{50} value of toxic standard, Dimethoate at 24 h was 0.13 µg a.i./bee with fiducial limits at 95 per cent ranging from 0.11 to 0.164 µg a.i./bee. **Agreed toxicity endpoints:**

The lethal dose value based on the nominal concentration of the test item are presented below.

Nominal concentration	LD50 Endpoints	fiducial limits
Active ingredient as µg a.i./bee	57.39	50.715 to 64.948
As µg PPP/bee or µg test item/bee	124.087	109.654 to 140.428

Reference:	KCP 10.3.1.1.1/01
Report	H-01-2022: Acute oral toxicity test in honeybees; Gangadhar RS; 2023; Study code: AG-G1150
Guideline(s):	Yes, OECD 213
Deviations:	Not relevant
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024

2. Vehicle and/or positive control: vehicle: 50% sucrose solution in Milli-Q water
positive control: dimethoate

Validity of the test: The current study was carried out to test the effects of H-01-2022 in honeybees, *Apis mellifera* L. when administered through oral route. The study met the validity criteria stipulated in the guideline. No mortality was observed in control and was within the specified 10 per cent limit at the end of the test. The LD50 value of toxic standard, Dimethoate at 24 h was 0.13 µg as/bee with fiducial limits at 95 per cent ranging from 0.11 to 0.164 µg as/bee.

3. Test organism

Species: honeybee *Apis mellifera*

Source:	honeybees of uniform size were collected from the adequately fed, disease free and healthy hives of queen-right colonies, with known history and physiological status, maintained at: Ecotoxicology Laboratory, Eurofins Advinus Agrosciences Services India Private Limited, Bengaluru 560 058, India
Age:	young adult foraging workers
Acclimation period:	acclimatization period lasted 20h 25 minutes (definitive test)
Diet:	sucrose solution in water with a final concentration of 500 g/L (50% w/v) was used as food, this food also served as the control, the bees were starved up to 1.5 h during range finding test.
Test units:	stainless steel test cages of 8.5 cm length x 4 cm breadth x 6 cm height dimensions covered on four sides with 2 mm steel mesh were used for confining the bees during the experimental period
4. Environmental conditions:	
Temperature:	23.9-24.6ºC
Relative humidity:	air humidity 65-69%
Photoperiod:	dark room except while handling, treatment and during observations

STUDY DESIGN AND METHOD

The objective of the study was to determine the acute oral effects of the test item, H-01-2022 on mortality and behavior of honeybees (*Apis mellifera* L.), under laboratory conditions. Active adult foraging worker honeybees were exposed to H-01-2022 at the test concentrations of 6.25, 12.5, 25, 50 and 100 µg as/bee (13.5, 27.0, 54.1, 108.1 and 216.2 µg PPP/bee) along with sucrose (50% w/v sucrose solution in Milli-Q water) and three concentrations of the toxic standard Dimethoate at 0.075, 0.15 and 0.30 µg as/bee.
Both the range finding, and the definitive tests were conducted as follows. The bees were starved up to 1 h 30 minutes during range finding test and 1 h 00 minutes during definitive test, before the commencement of treatment to increase their appetite for food. The bees were held in the dark except while handling, treatment and during observations, which were carried out under light. A sample of 200 µL of each of the test item formulations and control for the range finding test and 200 µL of each of the test item formulations, control and toxic standard formulations for the definitive test was fed to each group of 10 bees via glass feeding tubes (2 mL syringe with 200 µL each per cage) if each bee would consume a dose of 20 µL. The amount of test formulation consumed per group was monitored. After 4 h consumption, the feeding tubes were removed from the cage and replaced with feeding tubes containing 50% w/v sucrose solution in Milli-Q throughout the experimental period. The empty weight of the tubes, initial weight of tubes with the test concentration and weight of tubes after 4 h feeding was recorded. The amount of test item, toxic standard and the control consumed per group was assessed by the difference in the weight of the tubes with respective treatment level formulation and the tubes after 4 h of feeding.

Mortality and behavioral changes (viz., sluggishness; and lying on one side and still twitching) of exposed honeybees were recorded at 4 h after start of the treatment and thereafter at 24 and 48 h after the given

dose. The amount of diet consumed per group was estimated. The presence of the test item or the toxic standard in the diet did not affect the palatability of the honeybees. In both the range finding and definitive tests, no increase in mortalities was observed between 24 and 48 h. Thus, the test was terminated after 48 h.

Test design:	tested doses and control in 3 replicates, 10 bees per replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	13.5, 27.0, 54.1, 108.1 and 216.2 µg formulation/bee (6.25, 12.5, 25, 50 and 100 µg as/bee)
Dates:	start of the study 28.03.2023 start of the experimental part: 28.03.2023 end of the experimental part: 31.03.2023 end of the study: 02.11.2023
Statistic:	Probit analysis using an in-house developed and validated computer program

RESULTS

There were no mortalities and behavioral changes of bees observed in the control group during 4, 24 and 48 h post treatment. The percent mortality was 0, 0, 0, 6.67and 33.33 % at 4 h post treatment 0, 0, 3.33, 26.67 and 93.33 % at 24 h and 0, 0, 3.33, 30 and 100 % at 48 h post treatment at the test concentration of 6.25, 12.5, 25, 50 and 100 µg as/bee (13.5, 27.0, 54.1, 108.1 and 216.2 µg PPP/bee), respectively. The per cent mortalities for the toxic standard, Dimethoate at 4 h post treatment were 0, 26.67, 46.67 %, at 24 h post treatment were 16.67, 63.33 and 86.67 % and at 48 h post treatment were 16.67, 70 and 86.67 % at the tested concentrations of 0.075, 0.15 and 0.30 µg as/bee, respectively.

CONCLUSION

The **LD_{50}** value of the test item, H-01-2022 at 48 h is **57.39 µg as/bee** based on the nominal concentration (**124.087 µg PPP/bee**) with fiducial limits at 95 per cent ranging from 50.715 to 64.948 µg as/bee (109.654 to 140.428 µg PPP/bee) based on the nominal concentration.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 214 of the test were met. **Validity criteria:** The current study was carried out to test the effects of H-01-2022 in honeybees, *Apis mellifera L.* when applied once to the dorsal thorax. The study met the validity criteria stipulated in the guideline (OECD Guideline No. 214, adopted on 21st September 1998). • There were no mortalities in control and was within the specified 10 per cent limit at the end of the test. • The LD_{50} of the positive control, Dimethoate at 24 hours was 0.13 µg a.i./bee, which smet the reported range of 0.112 to 0.157 µg a.i./bee. **Agreed toxicity endpoints:**

The lethal dose value based on the nominal concentration of the test item are presented below.

Nominal concentration	LD50 Endpoints	fiducial limits
Active ingredient as µg a.i./bee	50.94	44.115 to 58.831
As µg PPP/bee or µg test item/bee	110.14	95.384 to 127.202

Reference:	KCP 10.3.1.1.2/01
Report	H-01-2022: Acute contact toxicity test in honeybees; Gangadhar RS; 2023; Study code: AG-G1151
Guideline(s):	Yes, OECD 214
Deviations:	Not relevant.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024

2. Vehicle and/or positive control: control: Milli-Q water designated as Group 1 (G1)
positive control: dimethoate

3. Test organism

Species: honeybee *Apis mellifera*

Source: Ecotoxicology Laboratory, Eurofins Advinus Agrosciences Services India Private Limited Bengaluru 560 058, India

Age: young adult foraging workers

Acclimation period: acclimatization period lasted 21h 42 minutes (definitive test)

Diet:	sucrose solution in Milli-Q water with a final concentration of 500 g/L (50% w/v) was used as food
Test units:	stainless steel test cages of 8.5 cm length x 4 cm breadth x 6 cm height dimensions covered on four sides with 2 mm steel mesh were used for confining the bees during the experimental period

4. Environmental conditions:

Temperature:	23.9-24.6 ºC
Relative humidity:	65-69%
Photoperiod:	the bees were held in the dark except while handling, treatment and during observations, which were carried out under light

STUDY DESIGN AND METHOD

The objective of the study was to determine the acute contact effects of the test item, H-01-2022 on mortality and behavior of honeybees (*Apis mellifera* L.), under laboratory conditions. Active adult foraging worker honeybees were exposed to H-01-2022 at the test concentrations of 6.25, 12.5, 25, 50 and 100 µg as/bee (13.5, 27.0, 54.1, 108.1 and 216.2 µg PPP/bee) along with Milli-Q water control and three concentrations of the toxic standard, Dimethoate at 0.075, 0.15 and 0.30 µg as/bee. Both the range finding test and the definitive tests were conducted as follows. The bees were held in the dark except while handling, treatment and during observations, which were carried out under light. The acclimatized bees were again inactivated with carbon dioxide for 10 minutes at a flow rate of 15 L/minute in the anaesthetization chamber and collected randomly in petri plates for respective treatment groups. A sample of 1 µL each of the control, test item and toxic standard were applied on to the dorsal thorax of each bee of the respective groups using Hamilton microliter syringe. The treated bees were then transferred to respective test cages and were provided with 50% w/v sucrose solution in Milli-Q water throughout the test. Mortality and behavioral changes (viz., sluggishness; and lying on one side but still twitching) of exposed honeybees were recorded at 4, 24 and 48 h postexposure.

Test design:	tested doses and control in three replicates, 10 bees per replicate
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	13.5, 27.0, 54.1, 108.1 and 216.2 µg form/bee **(**6.25. 12.5, 25, 50 and 100 µg as/bee)
Dates:	start of the study 28.03.2023 start of the experimental part: 28.03.2023 end of the experimental part: 31.03.2023 end of the study: 02.11.2023
Statistic:	Probit analysis using an in-house developed and validated computer program.

RESULTS

There were no mortalities and behavioral changes of bees observed in the control group during 4, 24 and 48 h post treatment. The per cent mortality for test item was 0, 0, 0, 10 and 40% at 4 h post treatment, 0, 0, 6.67, 33.33 and 93.33 % at 24 h and 0, 0, 10, 40 and 100 % at 48 h post treatment at the test concentration of 6.25, 12.5, 25, 50 and 100 µg as/bee (13.5, 27.0, 54.1, 108.1 and 216.2 µg PPP/bee), respectively. The percent mortalities for the toxic standard, Dimethoate at 4 h post treatment was 0, 20, 46.67 %, at 24 h post treatment was 20, 50 and 100 % and at 48 h post treatment were 23.33, 53.33 and 100 % at the tested concentrations of 0.075, 0.15 and 0.30 µg as/bee respectively.

CONCLUSION

The **LD_{50}** value of the test item, H-01-2022 **at 48 h is 50.94 µg as/bee** based on the nominal concentration (**110.14 µg PPP/bee**) with fiducial limits at 95 per cent ranging from 44.115 to 58.831 µg as/bee based on the nominal concentration (95.384 to 127.202 µg PPP/bee).

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:

The study is acceptable. The validity criteria according OECD 245 of the test were met.

Validity criteria:

VALIDITY OF THE TEST

The current study was carried out to test the effects of H-01-2022 when administered through oral route. The study met the validity criteria stipulated in the guideline.

- No mortality was observed in control group which is within the specified ≤ 15 % limit at the end of the test.
- The average mortality in the, Dimethoate technical was 66.67 % at the end of the test and which is more than >50%, hence it met the validity.

Deviation of the study:

AS IN STUDY PLAN	DEVIATION
Page No.:22/28 **4.1 Stability Test** **4.1.2 Stability** The test samples after sampling for accuracy/precision test will be retained at room temperature till the completion of the stability test.	The stability test was not performed on the day of accuracy and precision. However, the fresh test samples were prepared on other day and 0 hr stability and 4 hr stability test was performed.
Justification and impact of deviation: The stability test was not performed on the accuracy and precision day. The 4 hr stability test was performed on other day by proving the 0 hr stability test. Hence it does not have any impact on the outcome of study.	

Agreed toxicity endpoints:

The lethal dose value based on the nominal concentration of the test item are presented below.

Nominal concentration	Endpoints	fiducial limits
Active ingredient as mg a.i./kg	LC_{50} = 5.02	3.654 to 6.907
Active ingredient as ng a.i./bee/day	LDD_{50} = 167.52	124.061 to 226.201
Active ingredient as ug a.i./bee/day	LDD_{50} = 0.16752	0.124061 to 0.226201
Nominal concentration	**Endpoints**	
Active ingredient as mg a.i./kg	LOEC = 1.25	
Active ingredient as mg a.i./kg	NOEC = 0.625	
Active ingredient as mg a.i./bee/day	LOEDD = 45.8667	
Active ingredient as ug a.i./bee/day	LOEDD = 45866.7	
Active ingredient as mg a.i./bee/day	NOEDD = 23.6792	
Active ingredient as ug a.i./bee/day	NOEDD = 23679.2	

Nominal concentration	Endpoints	fiducial limits
As mg PPP/kg	LC_{50} = 10.85	7.90 to 14.934
As ng PPP/bee/day	LDD_{50} = 362.205	268.240 to 489.08
As ug PPP/bee/day	LDD_{50} = 0.362205	0.268240 to 0.48908

Nominal concentration	Endpoints
As mg PPP/kg	LOEC = 2.703
As mg PPP/kg	NOEC = 1.35
As mg PPP/bee/day	LOEDD = 99.17
As ug PPP/bee/day	LOEDD = 99170
As mg PPP/bee/day	NOEDD = 51.20
As ug PPP/bee/day	NOEDD = 51200

Reference: KCP 10.3.1.2/01

Report: H-01-2022: Chronic oral toxicity test in honeybee (*Apis mellifera* L.) Gangadhar RS; 2023; Study Code: AG-G1152

Guideline(s): Yes, OECD 245

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05/2022
Expiration date:	05/2024

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

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	Ecotoxicology Laboratory, Eurofins Advinus Agrosciences Services India Private Limited, Bengaluru 560 058, India
Age:	max. 2-day old
Acclimation period:	one day before the test starts, bees were collected from the combs and distributed into the test cages, bees were acclimatized to test conditions for about one day (after a hatching period of one day)
Diet:	sucrose solution in Milli-Q water with a final concentration of 500 g/L (50% w/v)
Test units:	stainless steel cages of 8.5 cm length × 4 cm width × 6 cm height dimensions (that provide minimum 200 cm^3 space) covered with glass or acrylic plate at front side and other sides with mesh wall for good ventilation

4. Environmental conditions:

Temperature:	32.7 - 33.7ºC
Relative humidity:	63-67 %
Photoperiod:	darkness except while handling, treatment and during observations, which were carried out under light condition

STUDY DESIGN AND METHOD

The objective of the study was to determine the chronic oral effects of the test item, H-01-2022 on mortality and behavior of honeybees (*Apis mellifera* L.), under laboratory conditions and to determine LC50

(median Lethal Concentration) and the LDD50 (median Lethal Dietary Dose) values after 10 days of exposure, NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) NOEDD (No Observed Effect Dietary Dose) when possible.

The chronic oral toxicity of H-01-2022 in honeybees was studied on the honeybee *Apis mellifera* L. In the chronic oral toxicity test the young honeybees were exposed continuous to a 50 % (w/v) aqueous sucrose solution containing the test item by continuous and ad libitum feeding over a period of 10 days with test item H-01-2022. In range finding test, there was no mortality of honeybees in the control group and at the tested concentrations of 0.01, 0.1 and 1.0 mg a.i./kg after 10-day oral exposure. (0.02162, 0.2162 and 2.162 mg PPP/kg) There was 70.0 and 100 % mortality the tested concentration of 10 and 100 mg a.i./kg (21.62 and 216.2 mg PPP/kg). There were no behavioral changes of bees observed in the control and test item treated groups during the test up to 10-day oral exposure. Based on the results of the range finding test definitive test was carried out with the test concentration of 0.625, 1.25, 2.5 5.0 and 10.0 mg a.i./kg diet (1.35, 2.70, 5.41, 10.81 and 21.62 mg PPP/kg diet) along with control (50% w/v sucrose in Milli-Q water) and one concentration of the toxic standard, Dimethoate technical at 0.75 mg a.i./kg.

There was no mortality of bees observed in the control and at the tested concentrations of 0.625 mg a.i./kg at the end of 10-day oral exposure. The observed mortalities were 13.3, 33.33, 40.0 and 76.67 % at the end of 10-day oral exposure at tested concentrations of 1.25, 2.5, 5.0 and 10.0 mg a.i./kg, respectively.
There were no behavioral changes of bees observed in the control, test item treated groups and toxic standard groups during the test up to end of 10-day oral exposure. The observed mortality of the bees for the toxic standard, Dimethoate technical treated group at the tested concentration of 0.75 mg a.i./kg was 66.67 % at the end of 10-day oral exposure.

Test design:	tested dose and control in three replicates, 10 bees per replicate
Exposure time:	chronic test, 10 days
Tested concentrations, definitive test:	0.625, 1.25, 2.5 5.0 and 10.0 mg a.i./kg diet (1.35, 2.70, 5.41, 10.81 and 21.62 mg PPP/kg diet)
Dates:	start of the study: 27.04.2023 start of the experimental part: 27.04.2023 end of the experimental part: 13.05.2023 end of the study: 23.01.2024
Statistic:	Statistical method of Probit analysis using an in-house developed and validated computer program.

CONCLUSION

There was no mortality of bees observed in the control and at the tested concentrations of 6.25 mg a.i./kg at the end of 10-day oral exposure.

The observed mortalities were 13.33, 33.33, 40.00 and 76.67 % at the end of 10-day oral exposure at tested concentrations of 1.25, 2.5, 5.0 and 10.0 mg a.i./kg, respectively. There were no behavioural changes of bees observed in the control, test item treated groups and toxic standard groups during the test up to end of 10-day oral exposure.

The observed mortality of the bees for the toxic standard, Dimethoate technical treated group at the tested concentration of 0.75 mg a.i./kg was 66.67 % at the end of 10-day oral exposure.

The amount of diet consumed by the bees in different experimental groups when continuous and *ad libitum* feeding provided over a period of 10 days was estimated. While the mean volume of diet consumed in the control group was 38.183 mg/bee/day the consumption of diet in the treat-

ment group ranged from 32.423 to 37.887 mg/bee/day and in the toxic standard was 30.474 mg/bee/day.

Table KCP 10.3.1.2-1: The lethal dose value based on the nominal concentration

Nominal concentration	Endpoints	fiducial limits
Active ingredient as mg a.i./kg	LC_{50} = 5.02	3.654 to 6.907
Active ingredient as ng a.i./bee/day	LDD_{50} = 167.52	124.061 to 226.201
Active ingredient as ug a.i./bee/day	LDD_{50} = 0.16752	0.124061 to 0.226201
Nominal concentration	Endpoints	
Active ingredient as mg a.i./kg	LOEC = 1.25	
Active ingredient as mg a.i./kg	NOEC = 0.625	
Active ingredient as mg a.i./bee/day	LOEDD = 45.8667	
Active ingredient as ug a.i./bee/day	LOEDD = 45866.7	
Active ingredient as mg a.i./bee/day	NOEDD = 23.6792	
Active ingredient as ug a.i./bee/day	NOEDD = 23679.2	

Nominal concentration	Endpoints	fiducial limits
As mg PPP/kg	LC_{50} = 10.85	7.90 to 14.934
As ng PPP/bee/day	LDD_{50} = 362.205	268.240 to 489.08
As ug PPP/bee/day	LDD_{50} = 0.362205	0.268240 to 0.48908
Nominal concentration	Endpoints	
As mg PPP/kg	LOEC = 2.703	
As mg PPP/kg	NOEC = 1.35	
As mg PPP/bee/day	LOEDD = 99.17	
As ug PPP/bee/day	LOEDD = 99170	
As mg PPP/bee/day	NOEDD = 51.20	
As ug PPP/bee/day	NOEDD = 51200	

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

Not relevant. No studies submitted.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Comments of zRMS: The study is acceptable. The validity criteria according OECD 239 of the test were met.

Validity criteria:

VALIDITY OF THE TEST

The current study was carried out to test the effects of H-01-2022 on bee larvae following repeated exposure. The study met the validity criteria stipulated in the guideline.

- No mortality of larvae was observed in control group from day 3 to day 8 (required: mean cumulative larval mortality ≤ 15% across all replicates).
- In the control plate, the adult emergence rate on day 22 was 83.33 to 91.67 % across all replicates (mean: 88.89; required: mean emergence ≥ 70%).
- In the reference substance group, the percentage mortality of larva on day 8 was 33.33 to 75% across all replicates (mean: 55.55%; required: mean cumulative larval mortality ≥ 50%).

Deviation of the study: none

Agreed toxicity endpoints:

(Effective doses (ED) were calculated based on the non-emergence of adult bees on day 22 compared to control and nominal test doses:

Endpoints	ED value (µg a.i/larva)	Confidence limits (µg a.i/larva)
ED_{10}	3.4427	2.87739 to 4.119
ED_{20}	5.9697	4.98948 to 7.14246
ED_{50}	17.109	14.3001 to 20.4706
NOED	1.5	
LOED	4.5	

Endpoints	ED value (µg PPP/larva)	Confidence limits (µg PPP/larva)
ED_{10}	7.444	6.221 to 8.906
ED_{20}	12.907	10.788 to 15.443
ED_{50}	36.99	30.919 to 44.261
NOED	3.243	
LOED	9.73	

Reference: KCP 10.3.1.4/01

Report	H-01-2022: Honeybee (*Apis mellifera* L.) larval toxicity test, repeated exposure Gangadhar RS; 2023; Study Code: AG-G1149
Guideline(s):	Yes, OECD GD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	-
Production date:	PA110522
Expiration date:	05/2022

2. Vehicle and/or positive control:	larval food without test item was used as control positive control: dimethoat

3. Test organism

Species:	honeybee larvae *Apis mellifera*
Source:	Ecotoxicology Laboratory, Eurofins Advinus Agrosciences Services India Private Limited, Bengaluru 560 058, India larvae of honeybees was sourced from in-house maintained hives after ensuring that the colonies are adequately fed, healthy (i.e., as far as possible disease- and parasite-free), with known history and physiological status, colonies are ensured not to be treated with any chemicals such as antibiotics and anti-Varroa treatments etc, within the four weeks preceding the start of the test
Age:	larvae
Acclimation period:	-

Diet:	Following food was provided based on the needs of the larvae at different stages of development: Diet A (Day 1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose. Diet B (Day 3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose. Diet C (from Day 4 to Day 6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose. Diets were prepared in advance and stored subsequently in a fridge at ≤ +5°C (but not frozen) during the whole duration of the test. The larvae were fed the larval diet (control group) or with larval diet treated with the test item or the toxic standard (treatment groups).
Stability in Feed:	The test item was found to be stable and homogenous in Milli-Q water over 4 hours in the room temperature at the concentrations of 0.05 µg ai/µL and 12 µg ai/µL.
Test units:	the larvae were reared in crystal polystyrene grafting cells having an internal diameter of 9 mm and a depth of 8 mm. Initially the grafting cells were sterilized by immersing for 30 min in ethanol and then dried in a laminar-flow hood, each cell was placed into a well of a 48-well plate, the top of the grafting cell was maintained to the level of the plate, e.g., by placing a piece of dental roll wetted with 500µL of the sterilizing solution enhanced with 15% weight/volume glycerol at the bottom of the wells, emergence box (crystal polypropylene box, 11 x 15 x 12 cm), with a cover aerated with perforated was used to hold pupae
4. Environmental conditions:	
Temperature/ relative humidity:	- day 1-7: temperature 34-34.9°C; relative humidity 95-97% - day 8-14: temperature 34.1-34.8°C; relative humidity 79-81% - day 15-22: temperature 34.1-34.9°C; relative humidity 60-62%
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The objective of the study was to determine the repeated exposure effects of the test item, H-01-2022 on mortality and behavior of larvae of honeybees (*Apis mellifera* L.), under laboratory conditions, to determine the Lowest Observed Effect Concentration (LOEC), the No Observed Effect Concentration (NOEC) and EC50, 20, 10 on day 22 (adult emergence). Based on the results of range finding test, in definitive

test, larvae were exposed at the tested concentrations of 1.5, 4.5, 13.5, 40.5 and 100 µg as/larva (3.24, 9.73, 29.189, 87.57 and 216.22 µg PPP/larva) with repeated exposure along with control and one concentration of the toxic standard, Dimethoate at 7.39 µg as/larva from day 3 to day 6.

Both the range finding, and the definitive tests were conducted as follows. Larvae were housed in the laboratory at a temperature of 34.0 to 34.9°C; relative humidity of 96 to 98% throughout the range finding test. For the definitive test, temperature ranged, and relative humidity ranged between 34.0 and 34.9°C and between 95 to 97% from day 1 to day 7, between 34.1 to 34.8°C and 79 to 81% from day 8 to day 14, and between 34.1 to 34.9°C and 60 to 62% from day 15 to day 22. Plates were kept under dark conditions for the duration of the test. 48-well plates with larvae were placed into a hermetic desiccator with a dish filled with potassium sulphate (K2SO4) saturated solution to keep a water saturated atmosphere (at a relative humidity of 95% ± 5%) which is adequate for larvae from day 1 to day 8. Then, the desiccator was placed into an incubator equipped with a forced air circulation system to homogenize temperature around the desiccator, and as close as possible within that range for the duration of the test. To ensure that all larvae are alive before the first administration of the treated diet on day 3, dead and non-suitable larvae (e.g., too small) were replaced on day 3. Larvae from three colonies was allocated on the same plate to each treatment level. 12 well-fed larvae were selected from each of the three colonies and each treatment group was treated with respective diets. On day 3, larvae were fed with 20 µL of untreated diet B for control, diet B containing test item for the test item groups, and diet B containing reference substance for the reference substance group. On day 4 to 6, the same feeding scheme was applied but using diet C and changing volumes of diet (30, 40 and 50 µL of diet C were used for each larva on days 4, 5 and 6, respectively). The test solution was mixed with the diet just before administration on each day of application. Different micropipette tips were used to administer each treatment (containing the diet) to avoid contamination. On day 8 (pre-pupae stage), well-plates were transferred into a hermetic container at a relative humidity of 80% ± 5% adequate for pupae (to achieve the humidity, a dish filled with a saturated NaCl solution was used). The pieces of dental roll from the wells were removed. Then the container was placed into an incubator at 34-35 ºC. On day 15 (pupae stage), each plate was transferred into an emergence box (crystal polypropylene box, 11 x 15 x 12 cm), with a cover aerated e.g., with perforated. The emerging bees was fed with sucrose solution dispensed ad libitum, using syringes. Then boxes were transferred into an incubator at 34-35°C with a relative humidity within the range 50 - 80%.

Following the first chemical exposure on day 3, mortalities of larvae at the time of feeding from day 4 to day 8 and on day 15 was checked and recorded. An immobile larva or a larva which does not react to the contact of the grafting tool or paintbrush, or which does not show signs of respiration under a stereomicroscope was recorded as dead. Qualitatively, presence of uneaten food was recorded on day 8. On day 15, larvae that have not transformed into pupae were recorded as dead and removed. On day 22, hatched adults that show a normal development, i.e., alive adult bees and dead adults which have left their cell, were recorded. Number of emerged bees and non-emerged bees (pupal mortality) was counted. At the feeding time, the dead larvae were removed systematically for sanitary reasons. Other observations, e.g., larval appearance and size, behaviour, morphological differences, and any other adverse effects after emergence (in comparison with controls) was qualitatively recorded.

Test design:	tested concentrations and control in one replicate; 36 larvae per replicate
Exposure time:	chronic test, exposition: 4 days (from D3 to D6), duration of the test: 22 days
Tested concentrations, definitive test:	1.5, 4.5, 13.5, 40.5 and 100 µg as/larva (3.24, 9.73, 29.189, 87.57 and 216.22 µg PPP/larva)

Dates:	start of the study 26.04.2023 start of the experimental part: 26.04.2023 end of the experimental part: 21.06.2023 end of the study: 24.01.2024
Statistic:	SYSTAT Statistical package, Cochran's Armitage Linear Trend initially and Fisher Exact Test (two tail)
Validity of the test:	No mortality of larvae was observed in control group from day 3 to day 8 (required: mean cumulative larval mortality ≤ 15% across all replicates). In the control plate, the adult emergence rate on day 22 was 83.33 to 91.67 % across all replicates (mean: 88.89; required: mean emergence ≥ 70%). In the reference substance group, the percentage mortality of larva on day 8 was 33.33 to 75% across all replicates (mean: 55.55%; required: mean cumulative larval mortality ≥ 50%).

RESULTS

There was no mortality of larvae in the control group and at the tested concentrations of 1.5 µg as/larva (3.24 µg PPP/larva) after up to Day 8 of exposure. There were 2.78, 11.1, 69.45 and 100 % mortality of larvae at the tested concentrations of 4.5, 13.5, 40.5 and 100 µg as/larva (9.73, 29.189, 87.57 and 216.22 µg PPP/larva) on Day 8 of exposure, respectively. The observed mortality of larvae for the toxic standard, Dimethoate technical treated group at the tested concentration of 7.39 µg as/larva was 55.55 % on Day 8 of exposure. Uneaten food was approximately 5, 10 and 80 % gradient at 13.5, 40.5 and 100 µg as/larva respectively (29.189, 87.57 and 216.22 µg PPP/larva) respectively; there was no uneaten food was observed at control and at 1.5 and 4.5 µg as/larva (3.24 and 9.730 µg PPP/larva); and there was approximately 65% uneaten food was observed at toxic standard group. Food appeared with no changes in color and texture from the start of test (thick creamy fluid) and no fungal growth at the end of test on day 8.

There was 11.11 % mean mortality of pupae in the control group.

There was no mortality in the tested concentrations of 1.5 µg as/larva (3.24 µg PPP/larva) when compared with number of larvae entering pre-pupa stage on day 8. The observed mean pupal mortality were 33.33, 52.78, 94.44 and 100 % at the tested concentrations of 4.5, 13.5, 40.5 and 100 µg as/larva (9.73, 29.189, 87.57 and 216.22 µg PPP/larva) when compared with number of larvae entering pre-pupa stage on day 8, respectively. The observed mortality of pupae for the toxic standard, Dimethoate technical treated group at the tested concentration of 7.39 µg as/larva was 83.33% when compared with the number of larvae entering pre-pupa stage on day 8.

The test item was found to be stable and homogenous in the 4 h at room temperature, at the fortification levels of 0.051 µg ai/µL and 12.025 µg ai/µL.

The analyzed concentration of test item samples showed that the per cent recovery was 93.636 (% RSD was 0.971) for the nominal concentration of 0.110 µg as/µL correspond to 3.24 µg PPP/larva and 94.113 (% RSD was 0.221) for the nominal concentration of 7.140 µg as/µL correspond to 216.22 µg PPP/larva. confirming that the concentration of the test item was within the acceptable limit (80 to 120% of the nominal concentration with an RSD of < 20%), and the suspension was found to be homogenous. No measurable concentration of test item was recorded in the control samples.

The reduction in adult emergence was 0, 13.89, 41.67, 83.33 and 88.89 % at the tested concentrations of

1.5, 4.5, 13.5, 40.5 and 100 µg as/larva (3.24, 9.73, 29.189, 87.57 and 216.22 µg PPP/larva) on Day 22 of exposure when compared with the control. The observed reduction in adult emergence for the toxic standard, Dimethoate technical treated group at the tested concentration of 7.39 µg as/larva was 69.44% on Day 22 of exposure when compared with the vehicle control.

CONCLUSION

The Effective concentration value for reduction in adult emergence based on the nominal concentration on day 22 of the test item exposure are presented below.

Endpoint (nominal concentration)	µg PPP/larva	µg as/larva
EC_{50}	**36.99**	**17.109**
EC_{20}	12.907	5.9697
EC_{10}	7.444	3.4427
LOEC	9.73	4.5
NOEC	**3.243**	**1.5**
EC_{10}	7.444	3.4427
EC_{20}	12.907	5.9697
EC_{50}	**36.99**	**17.109**
NOEC	**3.243**	**1.5**
LOEC	9.73	4.5

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods

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

Comments of zRMS:	The study is acceptable. The validity criteria according to SETAC; ESCORT I, ESCORT II; IOBC/BART/EPPO of the test were met. **Validity criteria:**

Mortality in control group	Mortality should not exceed 5 out of 40 wasps (≤13%) after 48 hours (actual value was 0.00%, therefore, the validity criterion was met).
Parasitisation in control group	Mean number of parasitized aphids (mummies) per female to be ≥ 5 (actual value was 32.40, therefore, the validity criterion was met). No more than two wasps producing zero mummies (actual value was zero, therefore, the validity criterion was met).

Deviation of the study: none

Agreed toxicity endpoints:

Table 1a. Mortality endpoints for *Aphidius rhopalosiphi*.

	H-01-2022						ROGOR L40 ST
	T1 Control	**T2 55.56 mL f.p./ha**	**T3 166.67 mL f.p./ha**	**T4 500 mL f.p./ha**	**T5 1500 mL f.p./ha**	**T6 4500 mL f.p./ha**	**T7 0.3 mL mL f.p./ha**
	Deionised water	**27.78 g a.i./ha**	**83.33 g a.i./ha**	**250 a.i./ha**	**750 g a.i./ha**	**2250 g a.i./ha**	**0.12 g a.i./ha**
Mortality (2 hours) [mean %]	0.00	0.00	0.00	0.00	0.00	2.50	35.00
Mortality (24 hours) [mean %]	0.00	0.00	0.00	0.00	2.50	7.50	67.50
Mortality (48 hours) [mean %]	0.00	0.00	0.00	0.00	2.50	7.50	87.50
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.	***
Endpoints	**mL test item/ha**					**g a.i./ha**	
48-h LR_{10} [95% confidence intervals]	>4500 [95%-CLs n.d.]					> 2250 [95%-CLs n.d.]	
48-h LR_{20} [95% confidence intervals]	>4500 [95%-CLs n.d.]					> 2250 [95%-CLs n.d.]	
48-h LR_{50} [95% confidence intervals]	>4500 [95%-CLs n.d.]					> 2250 [95%-CLs n.d.]	
48-h NOER (Mortality)	≥4500					≥2250	
48-h LOER (Mortality)	>4500					>2250	

f.p.: formulated product
a.i.: active ingredient terbuthylazine nominal content 500 g/L
-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact test with Bonferroni correction, α≤0.001 ***, 0.01 **, 0.05 *
95%-CLs, Confidence Limits
n.d.: not determined due to mathematical reasons

Table 1b. Reproduction endpoints for *Aphidius rhopalosiphi*.

	H-01-2022					
	T1 Control	**T2 55.56 mL f.p./ha**	**T3 166.67 mL f.p./ha**	**T4 500 mL f.p./ha**	**T5 1500 mL f.p./ha**	**T6 4500 mL f.p./ha**
	Deionised water	**27.78 g a.i./ha**	**83.33 g a.i./ha**	**250 a.i./ha**	**750 g a.i./ha**	**2250 g a.i./ha**
Reproduction [mean mummies/female]	32.40	31.40	31.07	31.80	28.27	27.20
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Effect on reproduction [%R]	-	3.09	4.12	1.85	12.76	16.05
Endpoints		**mL test item/ha**			**g a.i./ha**	
12-d ER_{10} [95% confidence intervals]		1666.58 [95%-CLs n.d.]			833.29 [95%-CLs n.d.]	
12-d ER_{20} [95% confidence intervals]		7356.31 [95%-CLs n.d.]			3678.16 [95%-CLs n.d.]	
12-d ER_{50} [95% confidence intervals]		>4500 [95%-CLs n.d.]			> 2250 [95%-CLs n.d.]	
12-d NOER (Reproduction)		≥4500			≥2250	
12-d LOER (Reproduction)		>4500			>2250	

f.p.: formulated product
a.i.: active ingredient terbuthylazine nominal content 500 g/L
n.s., not significantly different compared to the control
a, sequentially-rejective U-test after Bonferroni-Holm correction α≤0.001 ***, 0.01 **, 0.05 *

Reference:	KCP 10.3.2.1/01
Report	Effects of H-01-2022 (terbuthylazine 500 g/L) on parasitoid *Aphidius rhopalosiphi* in the laboratory – Standard laboratory test*;* Mautino G; 2023; Study Code: 1013.H.SAG23/r
Guideline(s):	Yes, SETAC; ESCORT I, ESCORT II; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	liquid
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024

2. Vehicle and/or positive control: vehicle: distilled water
positive control: dimethoate 400 g /L EC

3. Test organism

Species:	Parasitoids (Hymenoptera, Braconidae) *Aphidius rhopalosiphi*
Source:	Katz Biotech AG, Baruth, Germany
Age:	adults, not older than 48 hours
Acclimation period:	2 days under test conditions
Diet:	during the acclimation period and the test, a solution of 30% of honey in 100 mL of water was prepared and put on a cotton wool pad and given ad libitum to the insects, during the mortality assessment it was put on a small plastic tube that was connected with the exposure units at the beginning of the experiment; for the reproduction assessment a solution of 30% (by volume) of honey in water was put on a cotton wool
Test units:	Hatching chambers: cardboard cube (about 35 × 35 cm) with a frontal plastic tube Martality: two treated round glass plates (11.4 cm Ø) fitted onto a round stainless-steel frame (12.5 cm Ø) which had four ventilated holes (1.5 cm outer Ø). Three holes were covered with fine stainless-steel mesh and one was left open to introduce the wasps, a plastic tubing system connected to a pulling pump was set up for the air circulation in the test units Reproduction: untreated pots (15.0 cm Ø) with barley seedlings (Hordeum vulgare; 30 seeds per pot) infested with ≥100 host aphids of all development stages (*Rhopalosiphum padi*; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (22 cm high and 12.5 cm Ø), the cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction, after the introduction of the insects, this hole was plugged up with a cotton wool, after the adult wasps were removed, the polyacrylic cylinders were left on the pots
Introduction of Individuals:	after drying of the test units, 30-40 minutes after the application, for the mortality assessment wasp females were identified by observing their pointed abdomens, transfer was done using an aspirator, following the spraying scheme; only live (alive and apparently unaffected) wasps were introduced, for the reproduction assessment wasp females were selected impartially and transferred using a mouth aspirator, moribund insects were not included in this assessment

4. Environmental conditions:

Temperature:	19.28 ± 0.510 °C (18.55 – 20.04 °C)
Relative humidity:	73.5 ± 2.7% (70.1 – 79.7%) RH

Photoperiod:	900 – 1000 lux (mortality) 10800 – 12580 lux (reproduction) light : dark 16 h:8 h

STUDY DESIGN AND METHOD

The aim of the study was to determine the 48-hour LR_{50} of the test item H-01-2022 (terbuthylazine 500 g/L) to *Aphidius rhopalosiphi* and to evaluate the effects on reproduction (fecundity), following their exposure to the test item application on glass discs. The study consisted of 7 treatments (5 rates of the test item, 1 control group, 1 reference item) with 4 replicates, each containing 10 parasitoids. The parasitoids were exposed on glass discs previously treated with test item and observed for 2, 24 and 48 hours. At 48 hours the observations consisted of an evaluation of percent mortality. The number of alive, affected, moribund and dead parasitoids were recorded. Moribund wasps were counted as dead for the LR_{50} calculation. A minimum of 15 survived females per treatment, except the reference treatment, were removed and their reproductive capacity assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult females were removed after 24 hours and the aphid-infested plants left for a further 12 days before the number of aphid mummies that had developed was assessed. The mean number of parasitized aphids per female (i.e., mummies), obtained from 15 replicates per treatment, was counted. Mummies were produced by female within a 24-hour parasitisation period.

Test design:	4 replicates per treatment for the mortality assessment; 15 replicates per treatment for the reproduction assessment; 10 adults (minimum 5 females) per replicate for the mortality assessment and 1 female per replicate for the reproduction assessment (minimum 15 per treatment)
Exposure time:	mortality phase: 48 hours parasitisation period was 24 hours, all treatment groups were evaluated 12 days after parasitisation
Tested concentrations, definitive test:	55.56, 166.67, 500, 1500 and 4500 ml/ha (27.78, 83.33, 250, 750 and 2250 g as/ha), volume of application was 200 L/ha
Dates:	start of the study: 20.02.2023 start of the experimental part: 03.04.2023 end of the experimental part: 19.04.2023 end of the study: 23.08.2023
Statistic:	Software used for statistical analysis was “ToxRatPro” Solutions GmbH, version 3.3.0. Mortality data were processed using the Fisher's Exact test with Bonferroni correction ($\alpha \leq 0.05$) and at least the LR_{50} calculated where possible. Reproduction data were analysed by the sequentially-rejective U-test after Bonferroni-Holm correction ($\alpha \leq 0.05$) and at least the ER_{50} calculated where possible.

RESULTS

The following validity criteria were met: mortality should not exceed 5 out of 40 wasps (≤13%) after 48 hours, mean number of parasitized aphids (mummies) per female ≥ 5 and no more than two wasps pro-

ducing zero values of mummies.

For test item H-01-2022, mean mortality at 2 hours ranged from 0.00% in all the treatments, except in 4500 mL test item/ha where was equal to 2.50%. The control group and reference item showed a mortality of 0.00% and 35.00%, respectively. Mean mortality at 24 hours ranged from 7.50% in treatment 4500 mL test item/ha to 0.00% in treatments 55.56 mL test item/ha, 166.67 mL test item/ha and 500 mL test item/ha. The control group and reference item showed a mortality value of 0.00% and 67.50%, respectively. After 48 hours of exposure, mortality ranged from 7.50% in treatment 4500 mL test item/ha to 0.00% in treatments 55.56 mL test item/ha, 166.67 mL test item/ha and 500 mL test item/ha, in comparison to the control group (0.00% mortality). The 48-h mortality of reference item ROGOR L 40 ST was significantly (87.50%) different from the control. No significant differences were noticed among the treatments and the control group.

Table KCP 10.3.2.1-1: The effects of H-01-2022 on mortality of *Aphidius rhopalosiphi*

	H-01-2022						**ROGOR L40 ST**
	Control	**55.56 mL f.p./ha**	**166.67 mL f.p./ha**	**500 mL f.p./ha**	**1500 mL f.p./ha**	**4500 mL f.p./ha**	**0.3 mL mL f.p./ha**
	Deionised water	**27.78 g as/ha**	**83.33 g as/ha**	**250 as/ha**	**750 g as/ha**	**2250 g as/ha**	**0.12 g as/ha**
Mortality (2 hours) [mean %]	0.00	0.00	0.00	0.00	0.00	2.50	35.00
Mortality (24 hours) [mean %]	0.00	0.00	0.00	0.00	2.50	7.50	67.50
Mortality (48 hours) [mean %]	0.00	0.00	0.00	0.00	2.50	7.50	87.50

At the end of the mortality assessments (48 hours of exposure), the surviving females were evaluated for reproduction by comparing the parasitisation level in the test-item treatment groups with that one of the control group. A reproductive evaluation was performed after 12 days and the mean number of mummies ranged from 27.20 in treatment 4500 mL test item/ha to 31.80 in treatment 500 mL test item/ha. None significant differences were observed for all the treatments, in comparison to the control group (mean of 32.40 mummies per female).

Table KCP 10.3.2.1-2: The effects of H-01-2022 on fecundity of *Aphidius rhopalosiphi*

	H-01-2022					
	Control	**55.56 mL f.p./ha**	**166.67 mL f.p./ha**	**500 mL f.p./ha**	**1500 mL f.p./ha**	**4500 mL f.p./ha**
	Deionised water	**27.78 g as/ha**	**83.33 g as/ha**	**250 as/ha**	**750 g as/ha**	**2250 g as/ha**
Reproduction [mean mummies/female]	32.40	31.40	31.07	31.80	28.27	27.20
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Effect on reproduction [%R]	-	3.09	4.12	1.85	12.76	16.05

CONCLUSION

No significant differences were noticed among the treatments and the control group; therefore, it can be assumed a 48-hours NOER (mortality) value ≥ 4500 mL test item/ha (treatment 2250 g as/ha) and a 48-h LOER (mortality) > 4500 mL test item/ha, corresponding to 2250 g as/ha. The estimated 48-h LR_{50} of H-01-2022 was > 4500 mL test item/ha (95% confidence intervals not determined), corresponding to 2250 g as/ha (95% confidence intervals not determined). None significant differences were observed for all the treatments, in comparison to the control group (mean of 32.40 mummies per female). Therefore, it can be assumed a 12-day NOER (reproduction) value ≥ 4500 mL test item/ha and a LOER value > 4500 mL test item/ha, corresponding to 2250 g as/ha. The calculated 12-d ER_{10} value for test item H-01-2022 was 1666.58 mL test item/ha (95% confidence intervals not determined), the calculated 12-d ER20 value was 7356.31 mL test item/ha (95% confidence intervals not determined) and the estimated 12-d ER value was > 4500 mL test item/ha (95% confidence intervals not determined). Values correspond to 833.29 g as/ha, 3678.16 g as/ha and 2250 g as/ha, respectively.

Table KCP 10.3.2.1-3: H-01-2022 - Mortality and reproduction endpoints for *Aphidius rhopalosiphi*

Endpoints	mL test item/ha	g as/ha
Mortality		
48-h LR_{10}	>4500	> 2250
48-h LR_{20}	>4500	> 2250
48-h LR_{50}	>4500	> 2250
48-h NOER	≥4500	≥2250
48-h LOER	>4500	>2250
Reproduction		
12-d ER_{10}	1666.58	833.29
12-d ER_{20}	7356.31	3678.16
12-d ER_{50}	>4500	> 2250
12-d NOER	≥4500	≥2250
12-d LOER	>4500	>2250

Comments of zRMS:	The study is acceptable. The validity criteria according to IOBC, BART, EPPO of the test were met. Validity criteria: Mortality in control check: Mortality in the water control to be ≤ 20% on day 7 (actual mortality was 6.00%, so the validity criterion was met). Reproduction in control check: Mean cumulative number of eggs per female in the water control to be ≥ 4 (actual value was 10.02, so this validity criterion was met). Mortality in reference: Corrected mortality to be between 50% and 100% in the toxic reference treatment on day 7 (actual value was 84.04%, so the validity criterion was met). Deviation of the study: none **Agreed toxicity endpoints:**

Table 1a. Mortality endpoints for predatory mite *Typhlodromus pyri*.

	H-01-2022						ROGOR L40 ST
	T1 Control	T2 375 mL f.p./ha	T3 750 mL f.p./ha	T4 1500 mL f.p./ha	T5 3000 mL f.p./ha	T6 6000 mL f.p./ha	T7 ROGOR L 40 ST at 15 mL test item/ha
	Deionised water	187.5 g a.i./ha	375g a.i./ha	750 a.i./ha	1500 g a.i./ha	3000 g a.i./ha	6 g a.i./ha
Mortality (day 3) [mean %]	3.00	5.00	4.00	6.00	7.00	24.00	57.00
Mortality (day 7) [mean %]	6.00	11.00	12.00	16.00	18.00	36.00	85.00
Corrected mortality [a] (48 hours) [%]	-	5.32	6.38	10.64	12.77	31.91	84.04
Significance [b]	-	n.s.	n.s.	*	**	***	***

Endpoints	mL f.p./ha	g a.i./ha
7-day LR_{10} [95% confidence intervals]	1242.95 [738.32 – 1758.73]	621.48 [369.16 – 879.37]
7-day LR_{20} [95% confidence intervals]	3500.06 [2481.43 – 5782.11]	1750.03 [1240.72 – 2891.06]
7-day LR_{50} [95% confidence intervals]	>6000 [95%-CLs n.d.]	>3000 [95%-CLs n.d.]
7-day NOER (Mortality)	≤750	≤375
7-day LOER (Mortality)	1500	750

f.p.: formulated product
a.i.: active ingredient terbuthylazine nominal content 500 g/L
-, not applicable
n.s., not significantly different compared to the control
a, mean mortality corrected by Schneider-Orelli's formula
b, Chi^2 Table test with or without (reference item) Bonferroni Correction, α≤0.001 ***, 0.01 **, 0.05 *
95%-CLs, Confidence Limits
n.d.: not determined due to mathematical reasons

Table 1b. Fecundity endpoints for predatory mite *Typhlodromus pyri*.

	H-01-2022					
	T1 Control	**T2 375 mL f.p./ha**	**T3 750 mL f.p./ha**	**T4 1500 mL f.p./ha**	**T5 3000 mL f.p./ha**	**T6 6000 mL f.p./ha**
	Deionised water	**187.5 g a.i./ha**	**375g a.i./ha**	**750 a.i./ha**	**1500 g a.i./ha**	**3000 g a.i./ha**
Reproduction [mean eggs/female]	10.02	10.93	9.89	8.90	8.68	7.90
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	*
Effect on reproduction [%Pr]	-	-9.04	1.29	11.20	13.33	21.19
Endpoints		**mL f.p./ha**			**g a.i./ha**	
14-day ER_{10} [95% confidence intervals]		1597.62 [241.21 – U.L. n.d.]			798.81 [120.61 – U.L. n.d.]	
14-day ER_{20} [95% confidence intervals]		4054.92 [523.77 – U.L. n.d.]			2027.46 [261.89 – U.L. n.d.]	
14-day ER_{50}		>6000 [95%-CLs n.d.]			>3000 [95%-CLs n.d.]	
14-day NOER (Reproduction)		3000			1500	
14-day LOER (Reproduction)		6000			3000	

f.p.: formulated product
a.i.: active ingredient terbuthylazine nominal content 500 g/L
-, not applicable
n.s., not significantly different compared to the control
a, Williams' t-test $\alpha \leq 0.05$
U.L. n.d., Upper Limit not determined due to mathematical reasons
95%-CLs, Confidence Limits
n.d.: not determined due to mathematical reasons

Reference:	KCP 10.3.2.1/02
Report	Effects of H-01-2022 (terbuthylazine 500 g/L) on predator mite *Typhlodromus pyri* in the laboratory – Standard laboratory test; Mautino G; 2023; Study Code: 1013.H.SAG23/r
Guideline(s):	Yes, IOBC, BART, EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	liquid
Batch no.:	PA110522
Production date:	05.2022

Expiration date:	05.2024
2. Vehicle and/or positive control:	vehicle: deionised water positive control: dimetholate 400 g /L EC
3. Test organism	
Species:	Acari, Phytoseiidae *Typhlodromus pyri* Scheuten
Source:	Katz Biotech AG, Baruth, Germany
Age:	protonymphs ≤ 24 hours old
Acclimation period:	1 day under test conditions
Diet:	larvae fed with 100% apple pollen
Test units:	one glass disc (45-mm Ø) placed in a glass petri dish lid (54-mm Ø), with a central hole (6-mm Ø), located on a grid immersed in water, all systems were contained within a plastic container (250 × 250 × 80 mm^3)
4. Environmental conditions:	
Temperature:	24.50 ± 0.653 °C (23.70 – 25.89 °C)
Relative humidity:	67.6 ± 5.3% (60.1 – 76.4) RH
Photoperiod:	daily cycle 16 h day/8 h night

STUDY DESIGN AND METHOD

The aim of the study was to determine the 7-day LR_{50} of the test item H-01-2022 (terbuthylazine 500 g/L) by assessing *Typhlodromus pyri* mortality and reproduction (fecundity) subsequent to their exposure to the test item applied once on glass discs, compared to a water-treated control and to a reference item. The study comprised of 7 treatments (5 rates of the test item, 1 control group, 1 reference item), with 5 replicates, each containing 20 individuals. The mites were exposed on glass discs previously treated with test item. Exposure time was 7 days for mortality assessments, plus an additional 7 days for fecundity assessments. Observations of mortality were recorded after 3 and 7 days of the exposure. Juvenile mortality was assessed up to day 7, by observing the number of live, affected, escaped, and dead mites. Test organisms were counted as dead mites when they were motionless after touching them with a fine hair brush or when they had an abnormal appearance (e.g., shrivelled up or dried out). Affected, trapped, and escaped mites were considered dead for the LR_{50} calculation. At 14 days after initial exposure, the effect on mite fecundity was also evaluated. The surviving individuals were transferred to fresh test units and the sex ratio calculated. If at day 7 the sex-ratio was less than 1 male: 5 females; males originating from another replicate from the same treatment were added until the appropriate sex-ratio was reached. Number of eggs laid and number of juveniles per female were counted and removed afterwards, on 3 assessments from day 7 on with maximum interval of 3 days up to 14 (inclusive). Fecundity results were recorded considering live females, live males, eggs and nymphs.

Test design:	tested concentrations, reference item and control in 5 replications, number of mites: 20 mites per replicate, 100 mites per treatment
Exposure time:	14 days (7 days of mortality phase + 7 days of fecundity test)

Tested concentrations, definitive test:	375, 750, 1500, 3000 and 6000 mL/ha (187.5, 375, 750. 1500 and 3000 g as/ha), volume of application was 200 L/ha
Dates:	start of the study: 06.03.2023 start of the experimental part: 22.05.2023 end of the experimental part: 05.06.2023 end of the study: 23.08.2023
Statistic:	Software used for statistical analysis was "ToxRatPro", Solutions GmbH, version 3.3.0. Mortality data were processed using the Chi2 Table test with or without (reference item) Bonferroni Correction, $\alpha \leq 0.05$ and at least LR_{50} calculated where possible. Mortality was corrected by the control mortality, using the Schneider-Orelli formula. Reproduction data were analysed by the Williams t-test, $\alpha \leq 0.05$ and at least ER_{50} calculated where possible.

RESULTS

The following validity criteria were met: mortality in the water control ≤ 20% on day 7, mean cumulative number of eggs per female in the water control ≥ 4 and corrected mortality between 50% and 100% in the toxic reference treatment on day 7.

For test item H-01-2022, mean mortality at 3 days ranged from 4.00% in treatment 750 mL test item/ha to 24.00% in treatment 6000 mL test item/ha. The control group and reference item showed a mortality of 3.00% and 57.00%, respectively. Mean mortality at day 7 ranged from 11.00% in treatment 375 mL test item/ha to 36.00% in treatment 6000 mL test item/ha. Significant differences were noticed from treatment 1500 mL test item/ha to 6000 mL test item/ha, in comparison to the control group (6.00% mortality). Reference item ROGOR L 40 ST was significantly different from the control, showing 85.00% of mortality (corrected value: 84.04%).

Table KCP 10.3.2.1-4: The effects of H-01-2022 on mortality of *Typhlodromus pyri*

	H-01-2022						**ROGOR L40 ST**
	Control	**375 mL f.p./ha**	**750 mL f.p./ha**	**1500 mL f.p./ha**	**3000 mL f.p./ha**	**6000 mL f.p./ha**	**15 mL mL f.p./ha**
	Deionised water	**187.5 g as/ha**	**375 g as/ha**	**750 g as/ha**	**1500 g as/ha**	**3000 g as/ha**	**6 g as/ha**
Mortality (day 3) [mean %]	3.00	5.00	4.00	6.00	7.00	24.00	57.00
Mortality (day 7) [mean %]	6.00	11.00	12.00	16.00	18.00	36.00	85.00
Corrected mortality (48 hours) [mean %]	0.00	5.32	6.38	10.64	12.77	31.91	84.04

At the end of the mortality assessments (7 days of exposure) the effect on mite fecundity from test item H-01-2022 was also evaluated. Overall, 3 assessments were performed starting from day 7 up to day 14 (inclusive), with a maximum interval of 3 days each other. The mean number of eggs ranged from 7.90 in treatment 6000 mL test item/ha to 10.93 in treatment 375 mL test item/ha. Significant differences were

observed for treatment 6000 mL test item/ha, in comparison to the control group (10.02 mean eggs/female).

Table KCP 10.3.2.1-5: The effects of H-01-2022 on reproduction of *Typhlodromus pyri*

	H-01-2022					
	Control	**375 mL f.p./ha**	**750 mL f.p./ha**	**1500 mL f.p./ha**	**3000 mL f.p./ha**	**6000 mL f.p./ha**
	Deionised water	**187.5 g as/ha**	**375 g as/ha**	**750 g as/ha**	**1500 g as/ha**	**3000 g as/ha**
Reproduction [mean eggs/female]	10.20	10.93	9.89	8.90	8.68	7.90
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Effect on reproduction [%Pr]	-	-9.04	1.29	11.20	13.33	21.19

CONCLUSION

The estimated 7-day NOER (mortality) value corresponded to ≤750 mL test item/ha (375 g as/ha) and 7-day LOER (mortality) matched with the rate of 1500 mL test item/ha (750 as/ha). The calculated 7-day LR_{10} for H-01-2022 was 1242.95 mL test item/ha (95% confidence intervals: 738.32 – 1758.73 mL test item/ha), the calculated 7-day LR_{20} was 3500.06 mL test item/ha (95% confidence intervals: 2481.43 – 5782.11 mL test item/ha) and the estimated 7-day LR50 was >6000 mL test item/ha (95% confidence intervals not determined). In terms of active substance, the calculated 7-day LR_{10} of H-01-2022 was 621.48 g as/ha (95% confidence intervals: 369.16 – 879.37 g as/ha), the calculated LR_{20} was 1750.03 g as/ha (95% confidence intervals: 1240.72 – 2891.06 g as/ha) and the estimated 7-day LR_{50} was 3000 g as/ha (95% confidence intervals not determined).
For reproduction, significant differences were observed for treatment 6000 mL test item/ha, in comparison to the control group. The 14-day NOER (reproduction) value was 3000 mL test item/ha (1500 g as/ha) and 14-day LOER (reproduction) value matched with the rate of 6000 mL test item/ha (3000 g as/ha). The calculated 14-d ER_{10} for test item H-01-2022 was 1597.62 mL test item/ha (95% confidence intervals: 241.21 mL test item/ha – U.L. not determined), the calculated 14-d ER_{20} value was 4054.92 mL test item/ha (95% confidence intervals: 523.77 mL test item/ha – U.L. not determined). The estimated 14-d ER_{50} value was >6000 mL test item/ha (95% confidence intervals not determined). In terms of active substance, the calculated 14-d ER_{10} correspond to 798.81 g as/ha (95% confidence intervals: 120.61 – U.L. not determined), the calculated 14-d ER_{20} to 2027.46 g as/ha (95% confidence intervals: 261.89 – U.L. not determined) and the estimated 14-d ER_{50} >3000 g as/ha (95% confidence intervals not determined).

Table KCP 10.3.2.1-6: H-01-2022 - Mortality and reproduction endpoints for *Typhlodromus pyri*

Endpoints	**mL test item/ha**	**g as/ha**
Mortality		
7d LR_{10}	1242.95	621.48
7d LR_{20}	3500.06	1750.03
7d LR_{50}	>6000	>3000
7d NOER	≤750	≤375
7d LOER	1500	750
Reproduction		
14-d ER_{10}	1597.62	798.81
14-d ER_{20}	4054.92	2027.46

14-d ER_{50}	>6000	> 3000
14-d NOER	3000	1500
14-d LOER	6000	3000

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

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study is acceptable. The validity criteria according to OECD 222 of the test were met. The study for formulation of **H-01-2022** for *earthworms* with risk assessment may require supplementation. The toxicity endpoints were based on nominal concentration. At the end on the study concentration of substances active was reported and the concentration falls under 80% of nominal at the end of the study. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. However, in this case a higher level of risk assessment for earthworms is required and therefore Member States should consider whether such an addition is necessary in their case. The assessment conclusions will not change following this supplement and a higher tier risk assessment was required anyway. In Poland is not needed. It should be considered at MSs level. Validity criteria:

VALIDITY OF THE TEST

The present experiment was considered valid since, it satisfies the validity criteria given in the guideline.

- Each replicate in control group (containing 10 adults) produced minimum of 65 juveniles by the end of the test (acceptable criterion: each replicate containing 10 adults to have produced ≥ 30 juveniles by the end of the test).
- The coefficient of variation of reproduction in control is 3.0 % (acceptable criterion: the coefficient of variation of reproduction to be ≤ 30 %).
- There was no mortality of the earthworms in the control during the experimental period of 28 days (acceptable criterion: adult mortality over the initial 4 weeks of the test to be ≤ 10 %).
- Reference substance (carbendazim) group exhibited statistically significant reduction in juvenile production at 3 mg a.i./kg dry soil as compared with the control. Hence the test has met the validity acceptance criteria that significant effects should be observed between 1 and 5 mg a.i./kg dry soil in a test. This result infers that the obtained results during this test are valid and hence test is acceptable.

Deviation of the study: none

Agreed toxicity endpoints:

Parameter	Endpoints	Endpoints values as test item (mg test item/kg dry soil)	Endpoints values as active substance (mg as /kg dry soil)
Adult earthworms	**LC50**	> 110.09	> 50.92
Juvenile production	**NOEC**	5.83	2.70
	LOEC	10.49	4.85
	EC_{10}	12.60	5.83
	EC20	13.85	6.41
	EC50	26.48	12.25

Reference:	KCP 10.4.1.1/01
Report	H-01-2022: Earthworm reproduction test (*Eisenia fetida*); Vishala N.; 2023; Study Code: AG-G1153
Guideline(s):	Yes, OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05/2022
Expiration date:	05/2024

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

3. Test organism

Species:	earthworm *Eisenia fetida*
Source:	The earthworm, *Eisenia fetida* bred at Eurofins Advinus Limited were procured from (initial source): Karthik Vermicompost Limited Hebbal, Near GKVK Bengaluru, India
Age:	about 8.5 months old
Body weight:	347.8 - 445.4 mg
Acclimation period:	24 hours 10 minutes
Diet:	air-dried, finely ground and pasteurized cow manure, during test, food was provided one day after adding the earthworms and applying the test item to the artificial soil, thereafter food was provided once a week during the first 4-week test, maximum 5 g of food was spread on the soil surface of each container and moistened with deionized water, when food remained uneaten, the ration was reduced so as to avoid fungal growth or moulding, during second 4-week of test period, food was provided to each test container only once on Day 28
Test units:	HDPE plastic containers having cross sectional area of approximately 200 cm^2 of about one litre capacity were used as test vessels covered with perforated plastic films used to cover the test containers to permit gaseous exchange between the substrate and the atmosphere and for access to light

4. Environmental conditions:

Temperature:	19.8 to 20.1°C
Soil:	5% of sphagnum peat, 20% of kaolin clay and 75% industrial sand on dry weight basis (mass/mass)

Stability of the test compound:	the solution stability of sample solutions corresponding to low dose and high dose was measured by re-injection(triplicates) of the processed solution against freshly prepared standards, the stability was evaluated for the solutions stored at ambient temperature after 24 hours
pH:	6.27
Soil moisture content:	40-60 % of maximum water holding capacity
Photoperiod:	light-dark cycle: 16h : 8h, 564 – 573 lux

STUDY DESIGN AND METHOD

The purpose of the study was to assess the toxicity of test item, H-01-2022 on the reproductive output of the earthworm, *Eisenia fetida* in artificial soil. Based on WHC max (52.2%) of the test substrate the volume of 783 mL (for 3 kg dry test substrate) and 1566 mL (for 6 kg dry test substrate) of deionized water (equivalent to 50% of WHC max) was used for preparation of test medium. The dry artificial soil was premoistened using half of the volume of deionized water that is required to achieve 40-60% of the maximum water holding capacity before mixing test item with it. The same procedure was followed for control and reference substance group. Based on the results of range finding test, the test concentrations 1, 1.8, 3.24, 5.83, 10.49, 18.88, 33.98, 61.16 and 110.09 mg test item/kg dry soil was selected for the definitive test along with the control and reference substance. Adult earthworms were exposed for 28 days to the range of concentrations of H-01-2022 mixed with artificial soil. All the earthworms were weighed at the start of the test (Day 0) and all the live earthworms were weighed at the end of the test (Day 28). All the earthworms were washed with deionized water before weighing. Moisture content of the soil components or artificial soil was determined by drying sub samples at 105 °C and re-weighing after drying of the samples. The effects of the test item on the mortality and growth of adult earthworms were examined after 4 weeks of exposure. The test medium was spread on a stainless-steel plate. The earthworms were examined and the number of surviving earthworms was recorded. Pathological and behavioral symptoms of the earthworms were recorded on Day 28 (adult earthworms) and Day 56 (juveniles). At the end of the second 4-week period (Day 56), the number of juveniles hatched from the cocoons in the test soil and cocoon numbers were counted and recorded. The reproductive output of the worms exposed to the test item is compared to that of the control in order to determine the no observed effect concentration (NOEC) as well as EC_{10}, EC_{20} and EC_{50}.

Test design:	control in 8 replicates with 10 earthworms for each replication; tested concentrations in 4 replicates with 10 earthworms for each replication
Exposure time:	56 days
Tested concentrations, definitive test:	1, 1.8, 3.24, 5.83, 10.49, 18.88, 33.98, 61.16, 110.09 mg/kg dry weight of soil
Dates:	start of the study: 20.04.2023 start of the experimental part: 20.04.2023 end of the experimental part: 05.07.2023 end of the study: 06.10.2023

Statistic:	The statistical analysis of the earthworm bodyweight and juvenile production data was carried out using validated copies of SYSTAT Statistical package Ver.12.0. The body weight of adult earthworms at the end of first 4 week test and juvenile production data collected at the end of second 4 week test was tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modelling by treatment groups. Comparison of means between treatment groups and control group was done using Dunnet test. All analyses and comparisons were evaluated at the 5% ($p \leq 0.05$) level. Regression analysis was made for juvenile production data. The EC10, EC20 and EC50 value evaluations together with relevant fiducial limits were calculated using four parameter method (GraphPad Prism 8.0).
Validity of the test:	Each replicate in control group (containing 10 adults) produced minimum of 65 juveniles by the end of the test (acceptable criterion: each replicate containing 10 adults to have produced ≥ 30 juveniles by the end of the test). The coefficient of variation of reproduction in control is 3.0 % (acceptable criterion: the coefficient of variation of reproduction to be ≤ 30 %). There was no mortality of the earthworms in the control during the experimental period of 28 days (acceptable criterion: adult mortality over the initial 4 weeks of the test to be ≤ 10 %). Reference substance (carbendazim) group exhibited statistically significant reduction in juvenile production at 3 mg a.i./kg dry soil as compared with the control. Hence the test has met the validity acceptance criteria that significant effects should be observed between 1 and 5 mg a.i./kg dry soil in a test. This result infers that the obtained results during this test are valid and hence test is acceptable.

CONCLUSION

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

Table KCP 10.4.1.1-1: Earthworm reproduction test – final results

Parameter	Endpoints	Endpoints values as test item (mg test item/kg dry soil)	Endpoints values as active substance (mg as /kg dry soil)
Adult earthworms	**LC50**	> 110.09	> 50.92
Juvenile production	**NOEC**	5.83	2.70
	LOEC	10.49	4.85

	EC_{10}	12.60	5.83
	EC20	13.85	6.41
	EC50	26.48	12.25

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:

The study is accepted. The validity criteria according to OECD 226 of the test were met.

The validity criteria were met:
- control mean mortality to be ≤ 20% at the end of the test (actual value was 2.50%, so the validity criterion was met),
- the mean number of juveniles per test unit to be ≥ 50 at the end of the test (actual value was 205.50, so this validity criterion was met),
- the coefficient of variation of reproduction in control to be ≤ 30% at the end of the test (actual value was 12.07%, so the validity criterion was met).

Deviation of the study: none

Agreed toxicity endpoints:

***Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for mortality**

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.*
LC_{10} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
LC_{20} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
LC_{50} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
NOEC (Mortality)	≥2238	≥1000
LOEC (Mortality)	>2238	>1000

***Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for reproduction**

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.*
EC_{10} [95% confidence intervals]	1604.90 [95%-CLs n.d.]	717.11 [95%-CLs n.d.]
EC_{20} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
EC_{50} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
NOEC (Reproduction)	≥2238	≥1000
LOEC (Reproduction)	>2238	>1000

Reference: KCP 10.4.2.1/01

Report	Predatory mite *Hypoaspis (Geolaelaps) aculeifer* reproduction test in soil with H-01-2022 (terbuthylazine 500 g/L); Mautino G.; 2023; Study Code: 1015.H.SAG23/r
Guideline(s):	Yes, OECD 226
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	liquid
Batch no.:	PA110522
Production date:	05/2022
Expiration date:	05/2024

2. Vehicle and/or positive control:	vehicle: deionized water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L)

3. Test organism

Species:	Predatory mite (Acari, Laelapidae), Hypoaspis (Geolaelaps) aculeifer Canestrini
Source:	Katz Biotech AG, Baruth, Germany
Age:	28-35 days after the start of the egg-laying period
Sex:	female
Diet:	during all the tests, cheese mites (*Tyrophagus putrescentiae*, Schrank) were provided ad libitum, three times a week, as food source
Test units:	inert plastic (non-toxic) box (diameter: 4.6 cm), partly transparent, with a cross-sectional area that allows the actual soil depth within it of 1.5 cm with polyethylene lid and 20 g of soil/treatment

4. Environmental conditions:

Temperature:	19.18 ± 0.154°C (18.95 – 19.46 °C)

Soil:	artificial soil; Sphagnum-peat 5%, Kaolin clay 7%, Quartz-sand 88%, $CaCO_3$ 0%
Stability of test compound:	the content of H-01-2022 (nominally 500 g/L terbuthylazine) active ingredient was determined in soil samples collected during the trial at the beginning, during and at the end of the test
WHC:	46.38%
pH:	5.86
Humidity:	72.9 ± 1.5% (69.8 – 76.1%) RH
Photoperiod:	photoperiod: 16 h light: 8 h dark, approximately 640 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the effect of H-01-2022 (terbuthylazine 500 g/L) on the vitality and reproduction of predatory mite under laboratory conditions in an artificial soil substrate. All the experimental procedures were designed by following the OECD Guidelines for testing of chemicals no. 226 (2016). For this reason, Hypoaspis (Geolaelaps) aculeifer has been selected to investigate the effect of the test item applied alone on this non-target organism following its application into the soil.

Test design:	9 treatment groups (8 concentrations of the test item; 1 control), consisting of 4 replicates for the test item treatments and for the reference item and 8 replicates for the control group; each replicate contained 10 adult female mites
Exposure time:	14 days
Tested concentrations, definitive test:	36.56 mg/kg soil d.w. (16.33 mg as/kg soil d.w.) 65.80 mg/kg soil d.w. (29.40 mg as/kg soil d.w.) 118.44 mg/kg soil d.w. (52.92 mg as/kg soil d.w.) 213.19 mg/kg soil d.w. (95.26 mg as/kg soil d.w.) 383.74 mg/kg soil d.w. (171.47 mg as/kg soil d.w.) 690.74 mg/kg soil d.w. (308.64 mg as/kg soil d.w.) 1243.33 mg/kg soil d.w. (555.56 mg as/kg soil d.w.) 2238 mg/kg soil d.w. (1000 mg as/kg soil d.w.)
Dates:	start of the study: 28.02.2023 start of the experimental part: 27.04.2023 end of the experimental part: 07.09.2023 end of the study: 08.09.2023
Statistic:	Software used for statistical analysis was “ToxRatPro” Solutions GmbH, version 3.3.0. Mortality data were processed using the Chi2 Table test with Bonferroni Correction, $\alpha \leq 0.05$ and at least the LC50 calculated where possible. Reproduction data were analysed with a Dunnett's t-test $\alpha=0.05$ and at least the EC50 calculated where possible. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for mortality and reproduction were determined, where possible.

Validity of the test:

The validity criteria were met:
- control mean mortality to be ≤ 20% at the end of the test (actual value was 2.50%, so the validity criterion was met),
- the mean number of juveniles per test unit to be ≥ 50 at the end of the test (actual value was 205.50, so this validity criterion was met),
- the coefficient of variation of reproduction in control to be ≤ 30% at the end of the test (actual value was 12.07%, so the validity criterion was met).

RESULTS

All study validity criteria were met. The results are described in section 10 of this report and summarized in the following table.

Table KCP 10.4.2-1: Mortality of soil mite *Hypoaspis (Geolaelaps) aculeifer*

	H-01-2022								
	T1 Control	T2 36.56 mg test item/kg soil d.w.	T3 65.80 mg test item/kg soil d.w.	T4 118.44 mg test item/kg soil d.w.	T5 213.19 mg test item/kg soil d.w.	T6 383.74 mg test item/kg soil d.w.	T7 690.74 mg test item/kg soil d.w.	T8 1243.33 mg test item/kg soil d.w.	T9 2238 mg test item/kg soil d.w.
	Deionised water	16.33 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.92 mg a.i./kg soil d.w.	95.26 mg a.i./kg soil d.w.	171.47 mg a.i./kg soil d.w.	308.64 mg a.i./kg soil d.w.	555.56 mg a.i./kg soil d.w.	1000 mg a.i./kg soil d.w.
Mortality [mean %]	2.50	0.00	2.50	2.50	0.00	2.50	2.50	5.00	5.00
Corrected mortality [mean %]	-	-2.56	0.00	0.00	-2.56	0.00	0.00	2.56	2.56
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Mean adult mortality after 14 days was ranged between 0.00% in 36.56 mg test item/kg soil d.w. to 5.00% in 1243.33 mg test item/kg soil d.w. and 2238 mg test item/kg soil d.w.; compared to 2.50% in the control group. No significant difference in terms of survival was observed in comparison to the control group. Concerning morphological alterations, no effects were noticed as consequence of the treatment.

Table KCP 10.4.2-2: Reproduction of soil mite *Hypoaspis (Geolaelaps) aculeifer*

	H-01-2022								
	T1 Control	T2 36.56 mg test item/kg soil d.w.	T3 65.80 mg test item/kg soil d.w.	T4 118.44 mg test item/kg soil d.w.	T5 213.19 mg test item/kg soil d.w.	T6 383.74 mg test item/kg soil d.w.	T7 690.74 mg test item/kg soil d.w.	T8 1243.33 mg test item/kg soil d.w.	T9 2238 mg test item/kg soil d.w.
	Deionised water	16.33 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.92 mg a.i./kg soil d.w.	95.26 mg a.i./kg soil d.w.	171.47 mg a.i./kg soil d.w.	308.64 mg a.i./kg soil d.w.	555.56 mg a.i./kg soil d.w.	1000 mg a.i./kg soil d.w.
Reproduction [mean juveniles]	205.50	196.75	197.00	192.25	190.50	190.00	189.00	185.75	185.50
Effect on reproduction [%R]	-	4.3	4.1	6.4	7.3	7.5	8.0	9.6	9.7
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

In the control group, the number of juvenile worms per replicate ranged from 166 to 247, with a CV of 12.07%. The mean number of juvenile worms was 205.50 in the control group and in the treated group ranged between 185.50 in 2238 mg test item/kg soil d.w. to 197.00 in 65.80 mg test item /kg soil d.w. The effect on fecundity ranged between 4.1% in 65.80 mg test item /kg soil d.w. to 9.7% in 2238 mg test item/kg soil d.w. No significant difference in terms of fecundity was observed in comparison to the control group.

CONCLUSION

The NOEC and LOEC (mortality) values were estimated to be ≥2238 mg test item/kg soil d.w. (≥1000 mg a.i./kg soil d.w.) and >2238 mg test item/kg soil d.w. (>1000 mg a.i./kg soil d.w.) respectively. The LC_{10} value could not be determined, but was estimated to be >2238 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to >1000 mg a.i./kg soil d.w. The LC_{20} value could not be determined, but was estimated to be >2238 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to >1000 mg a.i./kg soil d.w. The LC_{50} value could not be determined, but was estimated to be >2238 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to >1000 mg a.i./kg soil d.w.

Table KCP 10.4.2-3: *Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for mortality

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.*
LC_{10} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
LC_{20} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
LC_{50} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
NOEC (Mortality)	≥2238	≥1000
LOEC (Mortality)	>2238	>1000

The NOEC and LOEC (reproduction) values were estimated to be ≥2238 mg test item/kg soil d.w. (≥1000 mg a.i./kg soil d.w.) and >2238 mg test item/kg soil d.w. (>1000 mg a.i./kg soil d.w.) respectively. The EC_{10} value observed was of 1604.90 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to 717.11 mg a.i./kg soil d.w. The EC_{20} value could not be determined, but was estimated

to be >2238 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to >1000 mg a.i./kg soil d.w. The EC_{50} value could not be determined, but was estimated to be >2238 mg test item/kg soil d.w. (95% confidence limits not determined) corresponding to >1000 mg a.i./kg soil d.w.

Table KCP 10.4.2-4: *Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for reproduction

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.*
EC_{10} [95% confidence intervals]	1604.90 [95%-CLs n.d.]	717.11 [95%-CLs n.d.]
EC_{20} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
EC_{50} [95% confidence intervals]	>2238 [95%-CLs n.d.]	>1000 [95%-CLs n.d.]
NOEC (Reproduction)	≥2238	≥1000
LOEC (Reproduction)	>2238	>1000

Comments of zRMS:

The study is acceptable. The validity criteria according to OECD 232 of the test were met.

The results of active ingredient analysis of test concentrations of 16.3, 95.3 and 1000 mg/kg dry soil or PPP/kg dry soil were as follows.

Sampling day	% Recovery of test item Mean ± SD (%RSD)		
	16.3 mg/kg dry soil or PPP/kg dry soil	95.3 mg/kg dry soil or PPP/kg dry soil	1000 mg/kg dry soil or PPP/kg dry soil
Treatment start (Day 0)	107.055 ± 0.903 (0.843)	109.466 ± 1.397 (1.276)	111.596 ± 0.855 (0.766)
Treatment end (Day 28)	100.790 ± 0.856 (0.849)	118.298 ± 0.669 (0.566)	115.040 ± 1.002 (0.871)

Validity criteria:

VALIDITY OF THE TEST

The present experiment was considered valid since, it satisfies the validity criteria given in the guideline.

- No mortality of adult collembola was observed in control group which is within allowed 20% at the end of the test.
- Each control replicate (containing 10 adults) produced minimum of 117 juveniles by the end of the test (acceptable criterion: each replicate containing 10 adults to have produced at least 100 juveniles by the end of the test).
- The coefficient of variation calculated for the number of juveniles is 3.5 % (acceptable criterion: the coefficient of variation for the number of juveniles to be ≤ 30 %).

Deviation of the study: none

Agreed toxicity endpoints:

The day 28 juvenile production NOEC, LOEC and Effective Concentrations value based on the nominal concentration of the test item are presented below:

Endpoint	Endpoint value (mg test item/kg dry soil) or (mg PPP/kg dry soil)	Endpoint value (mg a.i./kg dry soil)
NOEC	95.3	44.076
LOEC	171.5	79.319
EC_{10}	295.7394 (153.723 – 319984.262)	136.779 (71.097 - 147992.721)
EC_{20}	396.259 (205.972 – 428744.021)	183.27 (95.262 - 198294.11)
EC_{50}	693.499 (360.4749 – 750352.194)	320.743 (166.72 - 347037.89)

Reference:	KCP 10.4.2.1/02
Report	H-01-2022: *Folsomia candida*, collembolan reproduction test in soil; Mautino G.; 2023; Study Code: AG-G1154
Guideline(s):	Yes, OECD 232
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05/2022
Expiration date:	05/2024

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

3. Test organism

Species:	Collembola (*Folsomia candida*)
Source:	Ecotoxicology Laboratory, Eurofins Advinus Agrosciences Services India Private Limited, Bengaluru 560 058, India

Age:	9-12-day-old juveniles
Diet:	granulated dried baker's yeast, commercially available for household was used as feed, a sufficient amount of 2-10 mg was added to each container at the beginning of the test and after about 2 weeks of the start of test
Test units:	glass beaker (250 mL) was used, so that a moist substrate depth of about 2-4 cm was achieved when 30 g dry weight of substrate was added, perforated transparent lids was used to cover the test containers to permit gaseous exchange between the substrate and the atmosphere and access to light, test containers were positioned randomly in the incubator and re-positioned every once a week

4. Environmental conditions:

Temperature:	19.6 - 20.2°C
Soil:	artificial soil prepared by mixing 5% of sphagnum peat, 20% of kaolin clay and 75% industrial sand on dry weight basis (mass/mass)
Stability of test compound:	the test item treated groups (16.3, 95.3 and 1000 mg/kg dry soil or PPP/kg dry soil) was analyzed at the beginning of the test (day 0) and at end of the test (day 28)
pH:	6.31
WHC:	52.2%
Photoperiod:	photoperiod: 16 h light: 8 h dark, 540 - 582 lux

STUDY DESIGN AND METHOD

The toxic effect of test item, H-01-2022 was studied on the reproduction of collembola, Folsomia candida by artificial soil test. A range finding test was carried out to determine test concentrations for the definitive test. Based on the results of range finding test, the definitive test was carried out using 16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg test item/kg dry soil along with a control (deionized water) and reference substance (Boric Acid) at 100 mg/kg dry soil or PPP/kg dry soil. Synchronous juvenile (F. candida) collembola are exposed to range of concentrations of the test item mixed into a modified OECD artificial soil using a 5% peat. To extract and count the adult and juvenile collembola, the test container was filled with water mixed with blank ink up to a optimum level within the test container. Mortality assessment was made on Day 28 after the start of the exposure. Total number of juveniles produced by parent animals and the survival of parent animals are assessed after 4 weeks. The toxic effect of the test item on adult mortality and reproductive output was expressed as LCx/ECx and the NOEC/LOEC by comparing with controls.

Test design:	each test item treated group had 4 replicates; control and reference substance had 8 replicates and 10 collembolas per replicate
Exposure time:	28 days
Tested concentrations, definitive test:	16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg test item/kg dry soil

Dates:	start of the study: 21.04.2023 start of the experimental part: 21.04.2023 end of the experimental part: 31.05.2023 end of the study: 29.12.2023
Statistic:	The statistical analysis of the collembola juvenile production data was carried out using licensed copies of SYSTAT Statistical package Ver.12.0. The juvenile production data collected at the end test was tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modelling by treatment groups. Comparison of means between treatment groups and groups was done using Dunnet test. All analyses and comparisons were evaluated at the 5% ($p \leq 0.05$) level. Regression analysis was made for juvenile production data. The EC_{10}, EC_{20} and EC_{50} value evaluations together with relevant fiducial limits were calculated using probit method (Finney, 1981).
Validity of the test:	The validity criteria were met: - no mortality of adult collembola was observed in control group which is within allowed 20% at the end of the test; - each control replicate (containing 10 adults) produced minimum of 117 juveniles by the end of the test (acceptable criterion: each replicate containing 10 adults to have produced at least 100 juveniles by the end of the test), - the coefficient of variation calculated for the number of juveniles is 3.5 % (acceptable criterion: the coefficient of variation for the number of juveniles to be ≤ 30 %).

RESULTS

All study validity criteria were met.

There was no mortality of collembolas in control groups and neither in test item treated group on Day 28 after start of exposure.

Table KCP 10.4.2.1-5: Mortality of *Folsomia candida*

Group	Treatment (mg/kg dry soil or PPP/kg dry soil)	Adult Collembola Observations on Day 28 (at the end test)								Number of collembola per replicate at start of test	Mortality of collembola in a group	% Mortality of collembola
		R1	R2	R3	R4	R5	R6	R7	R8			
G1	Control	N (10)	N (10)	N (10)	N (10)	N (10)	N (10)	N (10)	N (10)	10	0	0
G2	16.3	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G3	29.4	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G4	52.9	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G5	95.3	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G6	171.5	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G7	308.7	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G8	555.6	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G9	1000	N (10)	N (10)	N (10)	N (10)	-	-	-	-	10	0	0
G10	Reference Substance 100	N (9), M (1)	N (8), M (2)	N (7), M (3)	N (7), M (3)	N (9), M (1)	N (10)	N (8), M (2)	N (9), M (1)	10	13	16.25

Toxic signs: N: Normal; M: Mortality; Number in the parentheses denotes the total number of affected collembolas.

The percent reduction in the production of juveniles in the test item treated group was 0, 0, 0, 0, 4.5, 12.5, 24.4 and 79.1 % at the tested concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg test item/kg dry soil, when compared with control group. There is no significant change in control group. Juvenile production at 171.5, 308.7, 555.6 and 1000 mg test item/kg dry soil was found to be significantly different from control group. Reference substance (Boric Acid) group at 100 mg/kg dry soil or PPP/kg dry soil exhibited significant reduction in juvenile production as compared with the control. The mean number of juveniles recovered in reference substance was 52.9 and the per cent reduction in the juvenile production of collembolas was 56.7%. The percent mortality of adult collembolas at 100 mg/kg dry soil or PPP/kg dry soil reference substance was 16.25%. This result infers that the obtained results during this test are valid and hence test is acceptable.

Table KCP 10.4.2.1-6: Reproduction of *Folsomia candida*

Group	Treatment (mg/kg dry soil or PPP/kg dry soil)	Observations of Juveniles on Day 28 (at the end)								No. of juveniles produced in a group			Change in juveniles' production (% of control)	% Reduction in juveniles' production (% of control)
		R1	R2	R3	R4	R5	R6	R7	R8	Mean	SD	% CV		
G1	Control	123	120	118	117	119	128	127	126	122.3	4.3	3.5	-	NA
G2	16.3	132	133	128	113	-	-	-	-	126.5	9.3	7.4	103.4	-3.4
G3	29.4	122	125	136	127	-	-	-	-	127.5	6.0	4.7	104.3	-4.3
G4	52.9	132	124	130	126	-	-	-	-	128.0	3.7	2.9	104.7	-4.7
G5	95.3	124	138	112	132	-	-	-	-	126.5	11.2	8.9	103.4	-3.4
G6	171.5	115	104	119	129	-	-	-	-	116.8	10.3	8.8	95.5	4.5
G7	308.7	108	105	115	100	-	-	-	-	107.0	6.3	5.9	87.5	12.5
G8	555.6	98	89	88	95	-	-	-	-	92.5	4.8	5.2	75.6	24.4
G9	1000	23	28	24	27	-	-	-	-	25.5	2.4	9.4	20.9	79.1
G10	Reference Substance 100	53	52	46	59	69	61	45	38	52.9	10.0	18.9	43.3	56.7

SD: Standard Deviation; CV: Coefficient of variation; Toxic signs: N: Normal.
Comparison: Control vs Test item groups; Control Vs Reference substance.

CONCLUSION

The day 28 juvenile production NOEC, LOEC and Effective Concentrations value based on the nominal concentration of the test item are presented below.

Table KCP 10.4.2-7: *Folsomia candida* test– final results for reproduction

Endpoint	Endpoint value (mg test item/kg dry soil) or (mg PPP/kg dry soil)	Endpoint value (mg a.i./kg dry soil)
NOEC	95.3	44.076
LOEC	171.5	79.319
EC_{10}	295.7394 (153.723 – 319984.262)	136.779 (71.097 - 147992.721)
EC_{20}	396.259 (205.972 - 428744.021)	183.27 (95.262 - 198294.11)
EC_{50}	693.499 (360.4749 – 750352.194)	320.743 (166.72 - 347037.89)

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is acceptable. The validity criteria according to OECD 216 of the test were met.

Validity criteria:

Validity of the Test

The test results were evaluated based on the difference of nitrate concentration between treated and control samples and the difference should be within ± 25% (average value). The coefficient of variation (CV) between results of nitrate concentration in control's replicate samples were less than ± 15 %.

Deviation of the study: none

Agreed toxicity endpoints:

Nitrogen transformation (deviation from the control) – final results

Time interval [d]	1PEC 2.4 mg test item/kg soil (1.11 mg as/kg soil),	5PEC 12 mg test item/kg soil (5.55 mg as/kg soil)
0-7	-183.93	-258.93
0-14	20.59	18.07
0-28	4.90	9.79

Reference: KCP 10.5/01

Report: Soil microorganisms: nitrogen transformation test of H-01-2022; Kiran YC; 2023; Study Code: AG-G1155

Guideline(s): Yes, OECD 216

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name): H-01-2022

Formulation: SC (terbuthylazine 500 g/L)

Description (physical state): light grey liquid

Batch no.: PA110522

Production date: 05.2022

Expiration date: 05.2024

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

3. Test organism

Soil: sand content 59.72%

Source:	collected from Agro-Forestry Division, Dry Land Research Centre, GKVK, Bengaluru, Karnataka state
Soil preparation:	Sampling was done by avoiding during or immediately following long periods (greater than 30 days) of drought or water logging. Soil sampling was done in Agro forestry area from a depth of about 25 cm where ploughing did not occur over longer periods (at least one growing season). Soil sample was collected and transported to test facility in cloth bags under ambient conditions. On receipt at test facility the soil was manually cleared of large objects (e.g. stones, parts of plants, etc.) and then moist sieved without excess drying to a particle size less than or equal to 2 mm. Then soil sample was stored in the dark at 4 ± 2°C. The collected soil sample will be stored for a maximum of three months from the date of collection under aerobic conditions
Stability of the compound:	stability and homogeneity if the test compound was checked by determination of active ingredient content in dose solution
Test units:	containers with sufficient headspace

4. Environmental conditions:

Temperature:	20±2 °C
pH	6.15
Organic carbon content:	1.45 %
Microbial biomass:	4.31 %
Soil moisture:	60% ± 5 % of the maximum water holding capacity
Photoperiod:	darkness

STUDY DESIGN AND METHOD

The objective of the study was to investigate the potential effects of a single exposure of test item on nitrogen transformation activity of soil microorganisms. The effect of the test item on nitrogen transformation activity of soil microorganisms was investigated on a sandy clay loam soil. The test was performed at 20 ± 2°C for 28 days and the recorded maximum and minimum temperatures were 20.5°C and 19.6°C, respectively. The soil treated with test item and the untreated control were incubated and sampled for analysis on 0, 7, 14 and 28 days after dosing of test item and incubation. The test was carried out in the dark at temperature of 20 ± 2°C. The variations between results of replicate control samples were within ± 15%. After 28 days of incubation, the lower treatment group deviated by 4.90% and the higher treatment group deviated by 9.79% from control with respect to nitrate formation rates which was below the threshold value of < 25%. Hence, the experiment was concluded after 28 days incubation.

Test design:	concentrations and control in 3 replicates
Exposure time:	28 days

Tested concentrations, definitive test:	1×PEC – 2.4 mg test item/kg soil (1.11 mg as/kg soil), 5×PEC - 12 mg test item/kg soil (5.55 mg as/kg soil)
Dates:	start of the study 08.03.2023 start of the experimental part: 27.03.2023 end of the experimental part: 28.04.2023 end of the study: 17.08.2023
Statistic:	The statistical analysis of the experimental data was carried out using licensed copies of SYSTAT Statistical Package .The quantitative variable (Nitrate content, mg/kg dry weight of soil/day) was tested using ANOVA. Comparison of means between treatment groups and control group was done using F-test. All analyses and comparisons were evaluated at the 5% ($p < 0.05$) level. Statistically non-significant differences ($p > 0.05$) indicated as nonsignificant (NS).
Validity of the test:	the calculated % variations among the replications of control samples were less than 15% indicating the validity of the test on all the intervals

CONCLUSION

Based on the experiment results, it can be concluded that the test item, H-01-2022 does not have a long-term influence on nitrogen transformation in soil microorganisms.

Table KCP 10.5.-1: Nitrogen transformation (deviation from the control) – final results

Time interval [d]	1PEC 2.4 mg test item/kg soil (1.11 mg as/kg soil),	5PEC 12 mg test item/kg soil (5.55 mg as/kg soil)
0-7	-183.93	-258.93
0-14	20.59	18.07
0-28	4.90	9.79

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study is acceptable. The validity criteria according to OECD 208 of the test were met. **Validity criteria:**

- The seedling emergence was 100% in all the tested plant species against the acceptable criteria of at least 70%.
- The seedlings did not exhibit visible phytotoxic effects and the plants exhibited normal variation in growth and morphology for species.
- The mean survival of emerged control seedlings was 100% for the duration of the study against the acceptable criteria of at least 90% for the duration of the study.
- Environmental conditions for a particular species were identical and growing media contained the same amount of substrate in all the tested plant species from the same source.

Deviation of the study: none

Agreed toxicity endpoints:

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOEC	LOEC
Onion	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2400 (0.2362 to 0.2443)	0.2569 (ND)	0.2750 (ND)	0.12	0.24
	Dry shoot weight	0.2391 (0.2304 to 0.2487)	0.2573 (ND)	0.2769 (ND)	0.06	0.12
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Cabbage	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2271 (ND)	0.2349 (ND)	0.2425 (ND)	0.12	0.24
	Dry shoot weight	0.2267 (ND)	0.2347 (ND)	0.2422 (ND)	0.12	0.24
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Carrot	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2240 (ND)	0.2320 (ND)	0.2398 (ND)	0.12	0.24
	Dry shoot weight	0.1641 (ND)	0.1951 (ND)	0.2320 (ND)	0.12	0.24
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Pea	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	>0.48	>0.48	>0.48	-	-
	Shoot height	>0.48	>0.48	>0.48	>0.48	>0.48
	Dry shoot weight	>0.48	>0.48	>0.48	0.06	0.12
	Phytotoxicity	>0.48	>0.48	>0.48	-	-

Crop	Concentration (L product/ha)	Parameter (%)			
		Seedling Emergence	Seedling Survival	*Reduction in shoot height	*Reduction in dry shoot weight
Perennial ryegrass	Control	100	100	-	-
	0.015	100	100	1.7	0.8
	0.03	100	100	0.5	0.3
	0.06	100	100	1.3	0.7
	0.12	100	100	1.2	0.0
	0.24	100	100	10.9	15.9
	0.48	100	0	NA	NA
Cabbage	Control	100	100	-	-
	0.015	100	100	0.0	0.0
	0.03	100	100	0.0	1.1
	0.06	100	100	0.0	0.0
	0.12	100	100	0.0	0.0
	0.24	100	100	40.9	41.8
	0.48	100	0	NA	NA
Sunflower	Control	100	100	-	-
	0.015	100	100	0.0	0.1
	0.03	100	100	0.0	0.1
	0.06	100	100	0.0	0.1
	0.12	100	100	0.0	0.2
	0.24	100	100	3.6	0.2
	0.48	100	0	NA	NA

***: Reduction in shoot height and dry shoot weight as compared to control**

Reference:	KCP 10.6.2/01
Report	H-01-2022: Seedling emergence and seedling growth test with terrestrial plants; Vishala N.; 2023; Study Code: AG-G1156
Guideline(s):	Yes, OECD 208
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024

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

3. Test plants:	*Pisum sativum* (pea), *Daucus carota* (carrot), *Brassica Oleracea* (cabbage), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Lolium perenne* (perennial ryegrass)
Soil:	natural soil (Red soil), sandy clay loam mixed with vermicompost to attain 1.5 percent organic carbon
Stability of test compound:	stability of test item in the matrix was performed at low dose and high dose level
Watering:	Plants were watered with adequate volume of water every day throughout the study. At any point during watering, water logging situations were not observed (which does not exceed 60% of water holding capacity of the natural soil). Initial top watering was used to stimulate seed germination. After seedling emergence, bottom, and top watering (sprinkled over natural soil) was used.
Organic carbon:	1.47%
Organic matter:	2.51%
pH:	7.18
Salt content as electronic conductivity:	15.20 dSm
Test containers:	plastic pots with a saucer under the pot, the pots were large enough to allow normal growth (diameter: 16 cm, surface area about: 201 cm2).
4. Environmental conditions:	
Temperature:	21.9 - 26.3°C
Relative humidity:	71 - 89%
Photoperiod:	controlled light-dark cycles with 16 hours light and 8 hours dark, 23500 to 28800 Lux
CO_2 concentration:	326 to 366 ppm

STUDY DESIGN AND METHODS

The study was aimed at evaluating the effect of H-01-2022 on seedling emergence and seedling growth on 6 terrestrial plants. Monocotyledonous plants viz., onion, perennial ryegrass and dicotyledonous plants viz., pea, carrot, cabbage and sunflower were used in the test. Seeds of the plants were sown in plastic pots of 16 cm diameter (201 cm2) containing 2 kg natural soil. Ten replicate pots with 2 seeds/pot for all plants except onion and four replicate pots with 5 seeds/pot for onion was maintained in the test. Based on the results of the range finding test, definitive test was carried out at rates of 0.015, 0.03, 0.06, 0.12, 0.24 and 0.48 L product/hectare along with one group treated with de-ionized water as control. Each potted soil surface was uniformly sprayed with 0.8 mL of either control or respective test concentration using calibrated airbrush sprayer with fluid nozzle. The experiment was conducted in a special room with PAR (Photosynthetic Active Radiation) lights and suitable environmental conditions were provided for plant species. During the experiment, all pots were observed for seedling emergence and mortality every day. Visual phytotoxicity of plants was observed on Day 7, 14 and 21 after the emergence of 50% of the control seedlings. At the end of the experiment, on day 21, height of the shoot of all surviving plants and replicate-wise dry weight of plants was recorded.

Test design:	number of rates: 6 + control, 10 replicate pots with 2 seeds/pot for all plants except onion and 4 replicate pots with 5 seeds/pot for onion
Exposure time:	14-21 days after 50% emergence of the seedlings in the control group
Tested concentrations, definitive test:	0.015, 0.03, 0.06, 0.12, 0.24 and 0.48 L/ha
Dates:	start of the study 11.08.2023 start of the experimental part: 12.08.2023 end of the experimental part: 15.09.2023 end of the study: 26.12.2023
Statistic:	A dose-response relationship was established in terms of a regression equation by using four parameter Model. Different models were used for estimating ERx (ER10, ER25 and ER50) and its confidence limits, where possible. The statistical analysis of the shoot height and shoot weight data was evaluated using licensed copies of SYSTAT Statistical package version 13.2 Data was tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's) before performing further analysis. When data was found as normal and homogeneous, ANOVA was performed, when data was found as non-normal or non-homogeneous, data were transformed, and ANOVA was done on transformed data for overall group comparison. Dunnett's test was performed for pairwise comparison. Comparison of two group's mean was done using two-sample t-test. The NOEC and LOEC were based on these analyses. All analyses and comparisons were evaluated at the 5% ($p<0.05$) level. The unit of expression of endpoints was L product/ha.
Validity of the test:	The seedling emergence was 100% in all the tested plant species against the acceptable criteria of at least 70%. The seedlings did not exhibit visible phytotoxic effects and the plants exhibited normal variation in growth and morphology for species. The mean survival of emerged control seedlings was 100% for the duration of the study against the acceptable criteria of at least 90% for the duration of the study. Environmental conditions for a particular species were identical and growing media contained the same amount of substrate in all the tested plant species from the same source.

RESULTS

The following table provides the data on seedling emergence, seedling survival, reduction in shoot height and reduction dry shoot weight.

Table KCP 10.6.2-1: Seedling emergence and seedling growth test results

Crop	Concentration (L product/ha)	Parameter (%)			
		Seedling Emergence	Seedling Survival	*Reduction in shoot height	*Reduction in dry shoot weight
Onion	Control	100	100	-	-
	0.015	100	100	0.0	0.9
	0.03	100	100	0.6	1.6
	0.06	100	100	0.9	1.5
	0.12	100	100	1.0	1.9
	0.24	100	100	10.0	10.5
	0.48	100	0	NA	NA
Carrot	Control	100	100	-	-
	0.015	100	100	0.9	1.3
	0.03	100	100	0.5	1.8
	0.06	100	100	1.8	2.0
	0.12	100	100	0.0	2.1
	0.24	100	100	50.4	55.3
	0.48	100	0	NA	NA
Pea	Control	100	100	-	-
	0.015	100	100	0.0	0.4
	0.03	100	100	0.0	1.9
	0.06	100	100	0.0	0.0
	0.12	100	100	0.0	0.0
	0.24	100	100	0.0	0.0
	0.48	100	100	0.0	0.0

Crop	Concentration (L product/ha)	Parameter (%)			
		Seedling Emergence	Seedling Survival	*Reduction in shoot height	*Reduction in dry shoot weight
Perennial ryegrass	Control	100	100	-	-
	0.015	100	100	1.7	0.8
	0.03	100	100	0.5	0.3
	0.06	100	100	1.3	0.7
	0.12	100	100	1.2	0.0
	0.24	100	100	10.9	15.9
	0.48	100	0	NA	NA
Cabbage	Control	100	100	-	-
	0.015	100	100	0.0	0.0
	0.03	100	100	0.0	1.1
	0.06	100	100	0.0	0.0
	0.12	100	100	0.0	0.0
	0.24	100	100	40.9	41.8
	0.48	100	0	NA	NA
Sunflower	Control	100	100	-	-
	0.015	100	100	0.0	0.1
	0.03	100	100	0.0	0.1
	0.06	100	100	0.0	0.1
	0.12	100	100	0.0	0.2
	0.24	100	100	3.6	0.2
	0.48	100	0	NA	NA

*: Reduction in shoot height and dry shoot weight as compared to control

Seedling emergence: Seedling emergence in the control group was 100% in all the tested plants during definitive test. Seedling survival: All the emerged seeds survived till end of the test at the tested concentrations of 0.015, 0.03, 0.06, 0.12 and 0.24 L product/ha in onion, perennial ryegrass, cabbage, Sunflower and carrot; all the emerged seeds survived till end of the test at the tested concentrations of 0.015, 0.03, 0.06, 0.12, 0.24 and 0.48 L product/ha in pea. There was 100% mortality of all plants on day 21 of all species at the tested concentration of 0.48 L product/ha except pea.

Visual toxicity: Plants in control group of all tested plants exhibited normal growth. Visual phytotoxicity (toxic responses) of stunted growth was observed at 0.24 L product/ha in onion, perennial ryegrass, cabbage and carrot and visual phytotoxicity of chlorosis was observed in sunflower at 0.24 L product/ha on day 7, 14 and 21.

The test item was recoverable at the concentrations of 2.5879 mg as/L and 5175.8375 mg as/L in the deionized water. The active ingredient concentration analysis of all the test concentrations showed that the recovery of test item with the nominal concentration was 82.017 to 115.276 % (RSD was 0.751 to 8.199 %) at the start of test indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

CONCLUSION

The 21-day (after the emergence of 50% of the control seedlings) effective rate (ER_{10}, ER_{25} and ER_{50}), NOEC and LOEC values of seedling emergence, seedling survival, shoot height, and dry shoot weight and Phytotoxicity was calculated based on the nominal concentration of the test item are in table below.

Table KCP 10.6.2-2: Seedling emergence and seedling growth test endpoints

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOEC	LOEC
Onion	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2400 (0.2362 to 0.2443)	0.2569 (ND)	0.2750 (ND)	0.12	0.24
	Dry shoot weight	0.2391 (0.2304 to 0.2487)	0.2573 (ND)	0.2769 (ND)	0.06	0.12
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Cabbage	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2271 (ND)	0.2349 (ND)	0.2425 (ND)	0.12	0.24
	Dry shoot weight	0.2267 (ND)	0.2347 (ND)	0.2422 (ND)	0.12	0.24
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Carrot	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2240 (ND)	0.2320 (ND)	0.2398 (ND)	0.12	0.24
	Dry shoot weight	0.1641 (ND)	0.1951 (ND)	0.2320 (ND)	0.12	0.24
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Pea	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	>0.48	>0.48	>0.48	-	-
	Shoot height	>0.48	>0.48	>0.48	>0.48	>0.48
	Dry shoot weight	>0.48	>0.48	>0.48	0.06	0.12
	Phytotoxicity	>0.48	>0.48	>0.48	-	-

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOEC	LOEC
Perennial ryegrass	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3649 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2385 (0.2311 to 0.2453)	0.2513 (ND)	0.2749 (ND)	0.12	0.24
	Dry shoot weight	0.2366 (ND)	0.2418 (ND)	0.2516 (ND)	0.12	0.24
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-
Sunflower	Seedling emergence	>0.48	>0.48	>0.48	>0.48	>0.48
	Seedling survival	0.3629 (ND)	0.3651 (ND)	0.3685 (ND)	-	-
	Shoot height	0.2464 (ND)	0.2535 (ND)	0.2609 (ND)	0.12	0.24
	Dry shoot weight	0.2684 (ND)	0.2768 (ND)	0.2854 (ND)	0.24	0.48
	Phytotoxicity	0.1812 (ND)	0.1825 (ND)	0.1834 (ND)	-	-

Comments of zRMS:	The study is acceptable. The validity criteria according to OECD 227 of the test were met. Validity criteria: The present experiment was considered valid since the control satisfies the validity criteria given in the guideline as below. • The seedling emergence was 100% in all the tested plant species against the acceptable criteria of at least 70%. • The plants did not exhibit visible phytotoxic effects and the plants exhibited normal variation in growth and morphology for species. • The mean plant survival was 100% for the duration of the study against the acceptable criteria of at least 90% for the duration of the study. • Environmental conditions for a particular species were identical and growing media contained the same amount of substrate in all the tested plant species from the same source. Deviation of the study: none **Agreed toxicity endpoints:**

Endpoints values as test item (L product/ha)

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Onion	Plant mortality	0.2305 (0.08256-0.4454)	0.4683 (0.2983-0.7598)	>0.80	0.20	0.40
	Shoot height	0.6317 (0.5627-ND)	>0.80	>0.80	0.20	0.40
	Dry shoot weight	0.6565 (0.6542-0.6588)	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-
Cabbage	Plant mortality	0.2621 (0.1536-0.3976)	0.4740 (0.3636-0.6020)	>0.80	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-
Carrot	Plant mortality	0.2441 (0.1632-0.3377)	0.3969 (0.3162-0.4874)	0.6452 (0.5450-0.7902)	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.1497 (0.1475-ND)	0.1523 (ND)	0.1535 (ND)	-	-
Pea	Plant mortality	0.2155 (0.1048-0.3589)	0.3803 (0.2608-0.5258)	0.6710 (0.5069-1.044)	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.1497 (0.1475-ND)	0.1523 (ND)	0.1535 (ND)	-	-

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Perennial ryegrass	Plant mortality	0.3079 (0.2638-0.3553)	0.4594 (0.4195-0.5014)	0.6854 (0.6421-0.7339)	0.20	0.40
	Shoot height	0.6234 (0.6191-0.6280)	0.7643 (0.7631-0.7655)	>0.80	0.20	0.40
	Dry shoot weight	0.5723 (0.5203-0.6600)	0.7404 (0.7177-0.7660)	>0.80	0.20	0.40
	Phytotoxicity	0.6040 (ND)	0.6084 (ND)	0.6135 (ND)	-	-
Sunflower	Plant mortality	0.2611 (0.1329-0.4294)	0.5025 (0.3666-0.6836)	>0.80	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-

Note: Values in parentheses are 95% fiducial limits. ND: Not determined. Fiducial limit(s) not obtained to many endpoints due to the nature of data.

Endpoints values as test item (g a.i. /ha)

Crops and Parameter		Endpoints values as test item (g a.i. /ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Onion	Plant mortality	115.25 (41.28-222.7)	234.15 (149.15-379.9)	>400	100	200
	Shoot height	315.85 (281.35-ND)	>400	>400	100	200
	Dry shoot weight	328.25 (327.1-329.4)	>400	>400	100	200
	Phytotoxicity	148.35 (146.1-ND)	152.2 (ND)	153.45 (ND)	-	-
Cabbage	Plant mortality	131.05 (76.8-198.8)	237 (181.8-301)	>400	100	200
	Shoot height	>400	>400	>400	100	200
	Dry shoot weight	>400	>400	>400	100	200
	Phytotoxicity	148.35 (146.1-ND)	152.2 (ND)	153.45 (ND)	-	-
Carrot	Plant mortality	122.05 (81.6-168.85)	198.45 (158.1-243.7)	322.6 (272.5-395.1)	100	200
	Shoot height	>400	>400	>400	100	200
	Dry shoot weight	>400	>400	>400	100	200
	Phytotoxicity	74.85 (73.75-ND)	76.15 (ND)	76.75 (ND)	-	-

Crops and Parameter		Endpoints values as test item (g a.i. /ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Pea	Plant mortality	107.75 (52.4-179.45)	190.15 (130.4-262.9)	335.5 (253.45-522)	100	200
	Shoot height	>400	>400	>400	100	200
	Dry shoot weight	>400	>400	>400	100	200
	Phytotoxicity	74.85 (73.75-ND)	76.15 (ND)	76.75 (ND)	-	-
Perennial ryegrass	Plant mortality	153.95 (131.9-177.65)	229.7 (209.75-250.7)	342.7 (321.05-366.95)	100	200
	Shoot height	311.7 (309.55-314)	382.15 (381.55-382.75)	>400	100	200
	Dry shoot weight	286.15 (260.15-330)	370.2 (358.85-383)	>400	100	200
	Phytotoxicity	302 (ND)	304.2 (ND)	306.75 (ND)	-	-
Sunflower	Plant mortality	130.55 (66.45-214.7)	251.25 (183.3-341.8)	>400	100	200
	Shoot height	>400	>400	>400	100	200
	Dry shoot weight	>400	>400	>400	100	200
	Phytotoxicity	148.35 (146.1-ND)	152.2 (ND)	153.45 (ND)	-	-

Note: Values in parentheses are 95% fiducial limits. ND: Not determined. Fiducial limit(s) not obtained to many endpoints due to the nature of data.

Reference:	KCP 10.6.2/02
Report	H-01-2022: Vegetative Vigour Test; Vishala N.; 2023; Study Code: AG-G1157
Guideline(s):	Yes, OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	H-01-2022
Formulation:	SC (terbuthylazine 500 g/L)
Description (physical state):	-
Batch no.:	PA110522
Production date:	05.2022
Expiration date:	05.2024
2. Vehicle and/or positive control:	vehicle control: water positive control: not relevant
3. Test plants:	*Pisum sativum* (pea), *Daucus carota* (carrot), *Brassica Oleracea* (cabbage), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Lolium perenne* (perennial ryegrass)
Soil:	natural soil (Red soil), sandy clay loam mixed with vermicompost to attain 1.5 percent organic carbon
Stability of test compound:	stability of test item in the matrix was performed at low dose and high dose level
Organic carbon:	1.59%
Organic matter:	2.74%
pH:	7.19
Salt content as electronic conductivity:	15.26 dSm
Test containers:	plastic pots with a saucer under the pot, the pots were large enough to allow normal growth
4. Environmental conditions:	
Temperature:	22.3 - 25.7°C
Relative humidity:	71 - 89%
Photoperiod:	natural light as well as artificial lights, controlled light-dark cycles with 16 h light and 8 h dark, 300 - 400 µE/m2/s (an equivalent range of 22400 to 25700 Lux)
CO_2 concentration:	326 - 384 ppm

STUDY DESIGN AND METHODS

The study was aimed at evaluating the effect of H-01-2022 on plants following deposition of the test item on the leaves and above-ground portions of plants on 6 terrestrial plants. Monocotyledonous plants viz., onion, perennial ryegrass and dicotyledonous plants viz., pea, carrot, cabbage and sunflower were used in

the test. Plants were grown in pots of 16 cm diameter (201 cm2) containing 2 kg natural soil from seed to the 3 true leaf stage. Twenty replicates (pot) each with single plant for each test item treated groups and control for all crop plants were maintained in the test. All plant species were treated at the rate of 0.05, 0.10, 0.20, 0.40 and 0.80 L product/ha along with one group treated with de-ionized water as control. Respective plant and leaf surfaces was uniformly sprayed with 0.8 mL of either control or test concentration using calibrated airbrush sprayer with fluid nozzle.

The experiment was conducted in a quonset greenhouse equipped with automated ventilation and humidity controller. The air temperature, humidity, carbon dioxide and light intensity with 16-h light and 8-h dark period was maintained throughout the experiment period.

During the growing period before the start of the treatment, all pots of a species were observed for emergence and mortality of seeds. After the treatment all pots were observed daily for mortality of plants till the end of the test. Visual phytotoxicity of plants was observed on Day 7, 14 and 21 from the start of the treatment. At the end of the test, on day 21, height of the shoot of all surviving plants and replicate-wise dry weight of plants was recorded.

Test design:	number of rates: 5 + control; each test item treated groups and control groups had twenty replicates (pots) each with single plant.
Exposure time:	21 days after the spraying
Tested concentrations, definitive test:	of 0.015, 0.03, 0.06, 0.12, 0.24 and 0.48 L/ha
Dates:	start of the study 20.09.2023 start of the experimental part: 20.09.2023 end of the experimental part: 01.11.2023 end of the study: 28.12.2023
Statistic:	A dose-response relationship was established in terms of a regression equation by using four parameter Model. Different models were used for estimating ERx (ER10, ER25 and ER50) and its confidence limits, where possible. The statistical analysis of the shoot height and shoot weight data was evaluated using licensed copies of SYSTAT Statistical package version 13.2 Data was tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's) before performing further analysis. When data was found as normal and homogeneous, ANOVA was performed, when data was found as nonnormal or non-homogeneous, data were transformed, and ANOVA was done on transformed data for overall group comparison. Dunnett's test was performed for pairwise comparison. Comparison of two group's mean was done using two sample t-test. The NOEC and LOEC were based on these analyses. All analyses and comparisons were evaluated at the 5% ($p<0.05$) level. The unit of expression of endpoints was L product/ha.

Validity of the test:

The seedling emergence was 100% in all the tested plant species against the acceptable criteria of at least 70%.
The plants did not exhibit visible phytotoxic effects and the plants exhibited normal variation in growth and morphology for species.
The mean plant survival was 100% for the duration of the study against the acceptable criteria of at least 90% for the duration of the study.
Environmental conditions for a particular species were identical and growing media contained the same amount of substrate in all the tested plant species from the same source.

RESULTS

The following table provides the data on treated plant mortality, reduction in shoot height, reduction dry shoot weight and visual phytotoxicity during definitive test.

Table KCP 10.6.2-3: Vegetative vigour test results

Crop	Concentration (L product/ha)	Parameter (%)			
		Plant Mortality	*Reduction in shoot height	*Reduction in dry shoot weight	Visual phytotoxicity
Onion	Control	0	-	-	0
	0.05	0	0	0	0
	0.10	0	0	0	0
	0.20	0	1.9	0	0
	0.40	30	1.9	1.1	100
	0.80	40	20.2	21.8	100
Carrot	Control	0	-	-	0
	0.05	0	0	0	0
	0.10	0	0	0.3	0
	0.20	0	0.4	1.0	100
	0.40	30	1.2	3.3	100
	0.80	60	2.3	4.8	100
Pea	Control	0	-	-	0
	0.05	0	0	0.1	0
	0.10	0	0.1	0.3	0
	0.20	0	0.3	1.1	100
	0.40	35	1.9	4.8	100
	0.80	55	3.9	6.2	100

Crop	Concentration (L product/ha)	Parameter (%)			
		Plant Mortality	*Reduction in shoot height	*Reduction in dry shoot weight	Visual phytotoxicity
Perennial ryegrass	Control	0	-	-	0
	0.05	0	0	0.2	0
	0.10	0	0	0.4	0
	0.20	0	0.1	1.5	0
	0.40	20	1.0	2.2	0
	0.80	60	29.9	31.7	100
Cabbage	Control	0	-	-	0
	0.05	0	0	0.5	0
	0.10	0	0	0.8	0
	0.20	0	0	1.2	0
	0.40	25	0.5	3.1	100
	0.80	45	3.8	4.7	100
Sunflower	Control	0	-	-	0
	0.05	0	0	0	0
	0.10	0	0.1	0.2	0
	0.20	0	0.4	0.6	0
	0.40	25	1.8	2.0	100
	0.80	40	2.1	3.4	100

*: Reduction in shoot height and dry shoot weight as compared to control

There was no mortality of plants observed in the control group of all the crops on day 21.

The mortality of treated plants in onion, carrot, perennial ryegrass, cabbage, pea and sunflower at the test concentrations of 0.05, 0.10 and 0.20 L product/ha was almost comparable with the control group.
Visual toxicity: Plants in control group of all tested plants exhibited normal growth. Seed set and flower formation was observed in pea at the tested concentration 0.05 and 0.10 and in the control group on day 21.
Flower formation was observed in Sunflower at the tested concentration 0.05, 0.10 and 0.20 and in the control group on day 21.
Following table presents visual toxicity observed in test item treated plants.

Table KCP 10.6.2-4: Vegetative vigour test phytotoxicity

Crop plant	Test concentration (L product/ha)	Observation period (Day)	Visual phytotoxicity observed
Perennial ryegrass	0.80	14 and 21	Stunted growth
Onion	0.40	21	Wilting
	0.80	21	Stunted growth, wilting
Carrot	0.20, 0.40 and 0.80	7, 14 and 21	Chlorosis

Pea	0.20, 0.40 and 0.80	21	Chlorosis
Sunflower	0.40 and 0.80	14 and 21	Chlorosis
Cabbage	0.40 and 0.80	14 and 21	Wilting

CONCLUSION

The 21-day effective rate (ER_{10}, ER_{25} and ER_{50}), NOER and LOER values of plant mortality, shoot height, and dry shoot weight and phytotoxicity was calculated based on the nominal concentration of the test item and are provided below.

Table KCP 10.6.2-4: Vegetative vigour test results endpoints

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Onion	Plant mortality	0.2305 (0.08256-0.4454)	0.4683 (0.2983-0.7598)	>0.80	0.20	0.40
	Shoot height	0.6317 (0.5627-ND)	>0.80	>0.80	0.20	0.40
	Dry shoot weight	0.6565 (0.6542-0.6588)	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-
Cabbage	Plant mortality	0.2621 (0.1536-0.3976)	0.4740 (0.3636-0.6020)	>0.80	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-
Carrot	Plant mortality	0.2441 (0.1632-0.3377)	0.3969 (0.3162-0.4874)	0.6452 (0.5450-0.7902)	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.1497 (0.1475-ND)	0.1523 (ND)	0.1535 (ND)	-	-
Pea	Plant mortality	0.2155 (0.1048-0.3589)	0.3803 (0.2608-0.5258)	0.6710 (0.5069-1.044)	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.1497 (0.1475-ND)	0.1523 (ND)	0.1535 (ND)	-	-

Crops and Parameter		Endpoints values as test item (L product/ha)				
		ER_{10}	ER_{25}	ER_{50}	NOER	LOER
Perennial ryegrass	Plant mortality	0.3079 (0.2638-0.3553)	0.4594 (0.4195-0.5014)	0.6854 (0.6421-0.7339)	0.20	0.40
	Shoot height	0.6234 (0.6191-0.6280)	0.7643 (0.7631-0.7655)	>0.80	0.20	0.40
	Dry shoot weight	0.5723 (0.5203-0.6600)	0.7404 (0.7177-0.7660)	>0.80	0.20	0.40
	Phytotoxicity	0.6040 (ND)	0.6084 (ND)	0.6135 (ND)	-	-
Sunflower	Plant mortality	0.2611 (0.1329-0.4294)	0.5025 (0.3666-0.6836)	>0.80	0.20	0.40
	Shoot height	>0.80	>0.80	>0.80	0.20	0.40
	Dry shoot weight	>0.80	>0.80	>0.80	0.20	0.40
	Phytotoxicity	0.2967 (0.2922-ND)	0.3044 (ND)	0.3069 (ND)	-	-

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

Not relevant. No studies submitted. The higher tier tests are not considered essential, because existing laboratory data for formulation are used and deemed to be sufficient for evaluation and risk assessment.

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

Not relevant. No studies submitted.

A 2.8 KCP 10.8 Monitoring data

Not relevant. No studies submitted.