FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 126085 A

Product name: MEPCY

Chemical active substances:

Chlormequat chloride, 345 g/L

Mepiquat chloride, 115 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem Ltd.

Submission date: February 2022

MS Finalisation date: May 2023, August 2023, January 2024, April 2024

Version history

When	What
May 2023	ZRMs evaluated dRR submitted by Applicant.
August 2023	Final version of RR after commenting period
September 2023	Applicant update
January 2024	zRMS assessment after Applicant's update
April 2024	Final version of RR after commenting period (II round)

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. inter-val between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	**Winter wheat**	F	Reduction of height to prevent lodging	Foliar Spray	BBCH 29-32	a) 1 b) 1	NA	a) 2.0 b) 2.0	a) 0.69 chlomequat chloride + 0.23 mepi-quat chloride b) 0.69 chlomequat chloride + 0.23 mepi-quat chloride	200-400			A	A	A	A	A	A	A

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

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

zRMS comments:

All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

In following text, simplified names of active substances were used:
Chlormequat instead Chlormequat chloride
Mepiquat instead mepiquat chloride.

9.1.1 Overall conclusions

9.1.1.1 Not relevant.

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

- **Birds:**

According to the screening and first tier risk assessment for cereals, all the TER_a and TER_{lt} values for Chlormequat and Mepiquat are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) presents no unacceptable acute and long-term risk to birds according to the intended uses on cereals.

- **Mammals:**

According to the first-tier risk assessment for cereals, the TER_a values for the active substance Chlormequat are lower than the Annex VI trigger of 10 for small omnivorous mammal 'mouse'. A refinement of the risk was done and the TER_a were above the trigger showing no risk. The TER_{lt} values for Chlormequat are greater than the Annex VI trigger of 5. According to screening and first-tier assessment for cereals, all the TER_a and TER_{lt} values for Mepiquat are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MEPCY presents no unacceptable acute and long-term risk to birds according to the intended uses on cereals.

9.1.1.3 Effects on aquatic organisms (KCP 10.2)

MEPCY:
Based on FOCUS Step 1 and 2, calculated PEC/RAC ratios for the formulated MEPCY did indicate an acceptable risk for aquatic organisms for all intended uses.

Chlormequat:
For the intended uses on winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate prolonged as characterised by a NOEC for *Daphnia magna* of 2.4 mg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

Mepiquat:
For the intended uses winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate acute as characterised by an EC_{50} for *Daphnia magna* of 68.5 mg/L in connection with an assessment factor of 100) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

9.1.1.4 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) is expected according to the proposed intended uses on cereals.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field HQ value calculated for Chlormequat chloride and Mepiquat chloride for the representative species *Typhlodromus pyri* and *Aphidius rhopalosiphi* are lower than the trigger of 2 for Tier I tests, indicating no risk to non-target arthropods in vegetated off-field areas following application according to the proposed use patterns.

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

- **Earthworms and other non-target soil organisms:**

The acute and chronic TER for Chlormequat and Mepiquat are above the Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that Chlormequat and Mepiquat do not poses acute and long-term risk to earthworms and other soil macro- and mesofauna.

- **Soil microorganisms:**

Risk assessments conducted with relevant PEC_{soil} for Chlormequat and Mepiquat in MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

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

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) shows that Annex VI trigger of 5 is not exceeded, indicating that MEPCY poses a low risk to non-target plants when applied according to the proposed use rates.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

Not relevant.

9.1.3 Consideration of metabolites

Not relevant.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. However, the provision of further data on MEPCY is not considered essential, because endpoints obtained with the active substances are sufficient to evaluate the

risk and new studies should not be conducted in regards of animal welfare (EFSA Journal 2009; 7(12):1438).

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
Coturnix japonica	Chlormequat chloride	Oral 1 d Acute	LD_{50} = 441 mg/kg bw	EFSA Scientific Report (2008) 179, 1-77
Coturnix japonica	Chlormequat chloride	Dietary 8 d Short-term	**LDD_{50} > 310 mg/kg bw/d**	
Coturnix japonica	Chlormequat chloride	Dietary Reproductive toxicity	NOEL = 54.8 mg/kg bw/d	
Colinus virginianus	Mepiquat chloride	Oral 1 d Acute	LD_{50} > 2000 mg/kg bw	EFSA Scientific report (2008) 146, 1-73
Colinus virginianus	Mepiquat chloride	Dietary 8 d Short-term	**LD_{50} > 1326 mg/kg bw**	
Coturnix japonica	Mepiquat chloride	Dietary Reproductive toxicity	**NOED = 100.7 mg/kg bw/d**	

9.2.1.1 Justification for new endpoints

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

According to the Guidance EFSA/2009/1438, where the dietary LD_{50} is lower than the acute LD_{50}, the dietary value should be used in the acute risk assessment. Therefore, LD_{50} > 310 mg/kg bw/d was used in the acute risk assessment as worst-case for Chlormequat. Moreover, where the dietary LC_{50}/10 is lower than the NOAEL, the lowest value should be used in the reproductive risk assessment. Therefore, NOEC of 31 mg/kg bw/d was used in the long-term risk assessment as a worst case for Chlormequat.

According to the Guidance EFSA/2009/1438, where the dietary LD_{50} is lower than the acute LD_{50}, the dietary value should be used in the acute risk assessment. Therefore, LD_{50} > 1326 mg/kg bw/d was used in the acute risk assessment as worst-cases for Mepiquat.

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 ta-

bles.

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

Intended use	**Winter wheat**				
Active substance/product	Chlormequat				
Application rate (g/ha)	1 x 690				
Acute toxicity (mg/kg bw)	> 310				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose". Grass + cereals. 100% cereal shoots	30.5	1.0	21.05	14.7
Cereals BBCH 10-29	Small omnivorous bird “lark”. Combination (invertebrates with interception). 25% crop leaves, 25% weed seeds, 50% ground arthropods	24.0	1.0	16.56	18.7
Cereals BBCH 30-39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves, 25% weed seeds, 50% ground arthropods	12.0	1.0	8.28	37.4
Reprod. toxicity (mg/kg bw/d)	31				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose". Grass + cereals. 100% cereal shoots	16.2	1.0 x 0.53	5.92	5.2
Cereals BBCH 10-29	Small omnivorous bird “lark”. Combination (invertebrates with interception). 25% crop leaves, 25% weed seeds, 50% ground arthropods	10.9	1.0 x 0.53	3.99	7.8
Cereals BBCH 30-39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves, 25% weed seeds, 50% ground arthropods	5.4	1.0 x 0.53	1.97	15.7
Active substance/product	Mepiquat				
Application rate (g/ha)	1 x 230				
Acute toxicity (mg/kg bw)	1326				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Indicator species for screening	158.8	1.0	36.52	36.3

Reprod. toxicity (mg/kg bw/d)	100.7				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Indicator species for screening	64.8	1.0 x 0.53	7.90	12.8

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.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)[1], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}\,(\text{mix}) = \left(\sum_i \frac{X\,(a.s._i)}{LD_{50}\,(a.s._i)} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture
$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation MEPCY addressed in this dossier is 34.5% chlormequat and 11.5% mepiquat, making up a total of 460 g a.s./L product. According to GAP, the maximum application rate is 2 L product/ha, therefore, an application rate of 920 g a.s./ha was considered in the assessment.

Table 9.2-3 shows the calculation of the predicted LD_{50} (mix) of chlormequat and mepiquat when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Table 9.2-3: Avian LD_{50} (mix) for chlormequat and mepiquat when combined as MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat
Content in the formulation MEPCY	34.5%	11.5%
Fraction in the a.s. mixture	0.75	0.25
LD_{50} of a.s. [mg/kg bw]	310	1326
Fraction / LD_{50}	0.00242	0.00019
Sum	0.00261	
1/ sum = predicted LD_{50} (mix)	383.45 mg mix/kg bw	

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions of the formulated product MEPCY.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 9.2-4: Avian "tox per fraction" for the MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat	"mix"
Content in the formulation MEPCY	34.5%	11.5%	46%
Fraction in mixture	0.75	0.25	1.0
LD_{50} (mg/kg bw)	310	1326	383.45
Tox per fraction	413.33	5304.00	383.45
Contribution to predicted toxicity	92.77%	7.23%	

Chlormequat contributes to 92.77% to mixture toxicity, while the mepiquat have an impact on the predicted risk of 7.23%, therefore, the risk assessment for MEPCY can be performed for the most toxic active substance alone, in this case chlormequat, and no further considerations according to Steps 2 - 4 are necessary.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.2-5: Avian NOEL (mix) for chlormequat and mepiquat when combined as MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat
Content in the formulation MEPCY	34.5%	11.5%
Fraction in the a.s. mixture	0.75	0.25
NOEL of a.s. [mg/kg bw]	31	100.7
Fraction / NOEL	0.0242	0.0025
Sum	0.0267	
1/ sum = predicted NOEL (mix)	37.49 mg mix/kg bw	

It is obvious from the comparison of the (low) long- term oral toxicity of the active substances, and their relative proportions of the formulated product MEPCY, that any risk of long-term effects would very much be similar to toxicity of one of the active substances.

Table 9.2-6: Avian "tox per fraction" for the MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat	"mix"
Content in the formulation MEPCY	34.5%	11.5%	46%
Fraction in mixture	0.75	0.25	1.0
NOEL (mg/kg bw)	31	100.7	37.49
Tox per fraction	41.33	402.80	37.49
Contribution to predicted toxicity	90.69%	9.31%	

Chlormequat contributes to 90.69% to mixture toxicity, while the mepiquat have an impact on the predicted risk of 9.31%, therefore, the risk assessment for MEPCY can be performed for the most toxic active substance alone, in this case chlormequat, and no further considerations according to Steps 2 - 4 are necessary.

The risk assessment at screening and Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
Safe use of active substance for birds such as chlormequat chloride and mepiquat chloride were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

Combined acute and log-term risk assessment for birds was accepted by RMS.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since MEPCY 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 109.3 (geometric mean, n = 4, Confirmatory data – Chlormequat (May 2014)), Chlormequat belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	690		

Acute toxicity (mg/kg bw)	=	> 310	quotient	=	2.23
Reprod. toxicity (mg/kg bw/d)	=	31	quotient	=	22.26

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

With a K(f)oc of 702.02 (geometric mean, n = 12, EFSA Scientific Report (2008) 146, 1-73), Mepiquat belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	230			
Acute toxicity (mg/kg bw)	=	1326	quotient	=	0.17
Reprod. toxicity (mg/kg bw/d)	=	100.7	quotient	=	2.28

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

Agree with the presented risk assessment.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Chlormequat amounts from -3.07 to -3.47 (EFSA Scientific Report (2008) 179, 1-77) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Mepiquat amounts from -3.14 to -3.55 (EFSA Scientific Report (2008) 146, 1-73) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Agree with the presented risk assessment.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Agree.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

Agree.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

Agree.

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

Not relevant.

Agree.

9.2.4 Overall conclusions

According to the screening and first tier risk assessment for cereals, all the TER_a and TER_{lt} values for Chlormequat and Mepiquat are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) presents no unacceptable acute and long-term risk to birds according to the intended uses on cereals.

Agree with the presented risk assessment.

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

Effects on mammals of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. However, the provision of further data on the formulation MEPCY is not considered essential, because risk may be reliably assessed using the EU-agreed endpoints only and new studies should not be conducted in regards of animal welfare (EFSA Journal 2009; 7(12):1438).

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
Rabbit	Chlomrequat chloride	Oral 1 d Acute	LD_{50} = 115 mg/kg bw	EFSA Scientific Report (2008) 179, 1-77
Rat	Chlomrequat chloride	Dietary Reproductive toxicity Multigeneration study	NOAEL = 74 mg/kg bw/d reproduction NOAEL = 41 mg/kg bw/d offspring	
Rat	Mepiquat chloride	Oral 1 d Acute	LD_{50} = 200 mg/kg bw	EFSA Scientific Report (2008) 146, 1-73
Rat	Mepiquat chloride	Dietary Reproductive toxicity Two-generation study	NOAED = 155 mg/kg bw/d	

9.3.1.1 Justification for new endpoints

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

9.3.2 Risk assessment for spray applications

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

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

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

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

Intended use	**Winter wheat**				
Active substance/product	Chlormequat				
Application rate (g/ha)	1 x 690				
Acute toxicity (mg/kg bw)	115				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	5.4	1.0	3.73	30.9
Cereals BBCH 10-29	Small omnivorous mammal “mouse”. Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	17.2	1.0	11.87	**9.7**
Cereals BBCH 30-39	Small omnivorous mammal “mouse”. Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	8.6	1.0	5.93	19.4
Reprod. toxicity (mg/kg bw/d)	41				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	1.9	1.0 x 0.53	0.69	59.0

Cereals BBCH 10-29	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	7.8	1.0 x 0.53	2.85	14.4
Cereals BBCH 30-39	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	3.9	1.0 x 0.53	1.43	28.8
Active substance/product	Mepiquat				
Application rate (g/ha)	1 x 230				
Acute toxicity (mg/kg bw)	200				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	5.4	1.0	1.24	161.0
Cereals BBCH 10-29	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	17.2	1.0	3.96	50.6
Cereals BBCH 30-39	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	8.6	1.0	1.98	101.1
Reprod. toxicity (mg/kg bw/d)	155				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Indicator species for screening	48.3	1.0 x 0.53	5.89	26.3

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

The risk assessment at screening and Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of active substance for birds such as chlormequat chloride and mepiquat chloride were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable. TER for first-tier assessment for of the acute risk for mouse for chlormequat chloride is **9.7.** In opinion RMS the acute risk assessment for small omnivorous mammal "mouse" with TER = 9.7 should be accepted without refinement. This value is very close to the trigger value of 10.

Combined acute and log-term risk assessment for birds was accepted by RMS. Refinement for combined acute risk assessment for small omnivorous mammal "mouse" should be performed based on deposition factor 0.8. This approach was accepted by RMS.

The refinement risk assessment for mammals should be considered at MS level.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)[2], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}\,(\text{mix}) = \left(\sum_{i} \frac{X\,(a.s._i)}{LD_{50}\,(a.s._i)} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation MEPCY addressed in this dossier is 34.5% chlormequat and 11.5% mepiquat, making up a total of 460 g a.s./L product. According to GAP, the maximum application rate is 2 L product/ha, therefore, an application rate of 920 g a.s./ha was considered in the assessment.

Table 9.3-3 shows the calculation of the predicted LD_{50} (mix) of chlormequat and mepiquat when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009).

Table 9.3-3: Mammalian LD_{50} (mix) for chlormequat and mepiquat when combined as MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat
Content in the formulation MEPCY	34.5%	11.5%
Fraction in the a.s. mixture	0.75	0.25
LD_{50} of a.s. [mg/kg bw]	115	200
Fraction / LD_{50}	0.00652	0.00125
Sum	0.00777	
1/ sum = predicted LD_{50} (mix)	128.67 mg mix/kg bw	

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 9.3-4: Mammalian "tox per fraction" for MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat	"mix"
Content in the formulation MEPCY	34.5%	11.5%	33.4%
Fraction in mixture	0.75	0.25	1.0
LD_{50} (mg/kg bw)	115	200	128.67
Tox per fraction	153.33	800.00	128.67
Contribution to predicted toxicity	83.92%	16.08%	

Chlormequat contributes to 83.92% to mixture toxicity, while the Mepiquat have an impact on the predicted risk of 16.08%, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.3-5: First-tier assessment of the acute risk for mammals due to the use of MEPCY in cereals

Intended use	Cereals				
Active substance/product	MEPCY				
Application rate (g/ha)	1 x 920				
LD_{50} (mix) (mg/kg bw)	128.67				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	5.4	1.0	4.97	25.9
Cereals BBCH 10-29	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	17.2	1.0	15.82	**8.1**
Cereals BBCH 30-39	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	8.6	1.0	7.91	16.3

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.

According to reults, an unacceptable acute risk is obtained for combined exposure acording to the proposed GAP. Therefore, a refinement is needed.

COMBINED EXPOSURE REFINEMENT

As exposed in section 9.3.2.2, the chlormequat toxicity endpoint in mammals could be refined, based on the geometric mean of endpoints obtained from four rat studies, three mice studies and one study in rab-

bits (see table 9.3-11). As a result, an LD_{50} of 343 mg a.s./kg bw is obtained. Also, a deposition factor of 0.8 corresponding to winter cereals (BBCH stages 29-32) was used.

Table 9.3-6 shows the calculation of the predicted LD_{50} (mix) of chlormequat and mepiquat when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009) after considering the new calculated endpoint.

Table 9.3-6: Mammalian LD_{50} (mix) for chlormequat and mepiquat when combined as MEPCY (step 1 in EFSA GD 2009, Appendix B) – refined chlormequat LD_{50}

	Chlormequat	Mepiquat
Content in the formulation MEPCY	34.5%	11.5%
Fraction in the a.s. mixture	0.75	0.25
LD_{50} of a.s. [mg/kg bw]	343	200
Fraction / LD_{50}	0.00219	0.00125
Sum	0.00344	
1/ sum = predicted LD_{50} (mix)	290.99 mg mix/kg bw	

Table 9.3-7: Mammalian "tox per fraction" for MEPCY (step 1 in EFSA GD 2009, Appendix B) – refined chlormequat LD_{50}

	Chlormequat	Mepiquat	"mix"
Content in the formulation MEPCY	34.5%	11.5%	33.4%
Fraction in mixture	0.75	0.25	1.0
LD_{50} (mg/kg bw)	343	200	290.99
Tox per fraction	457.33	800.00	290.99
Contribution to predicted toxicity	63.63%	36.37%	

Chlormequat contributes to 63.63% to mixture toxicity, while the Mepiquat have an impact on the predicted risk of 36.37%, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.3-8: First-tier assessment of the acute risk for mammals due to the use of MEPCY in cereals – refined chlormequat LD_{50}

Intended use **Active substance/product** **Application rate (g/ha)**	Cereals MEPCY 1 x 920						
Acute toxicity (mg/kg bw) **TER criterion**	290.99 10						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_{90}*× DF*** **(mg/kg food)**	**MAF_{90}**	**PT**	**DDD_{90}** **(mg/kg bw/d)**	**TER_a**
Wood mouse (*Apodemus sylvaticus*)	Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	0.27[1]	64.5[1] ~~× 0.8[2]~~	1.0	1.0	~~12.82~~ 16.02	~~22.7~~ 18.16

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

According to reults, no unacceptable acute risk is obtained for combined exposure acording to the proposed GAP.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.3-9: Mammals NOEL (mix) for chlormequat and mepiquat when combined as MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat
Content in the formulation MEPCY	34.5%	11.5%
Fraction in the a.s. mixture	0.75	0.25
NOEL of a.s. [mg/kg bw]	41	155
Fraction / NOEL	0.0183	0.0016
Sum	0.0199	
1/ sum = predicted NOEL (mix)	50.24 mg mix/kg bw	

It is obvious from the comparison of the (low) long- term oral toxicity of the active substances, and their relative proportions of the formulated product MEPCY, that any risk of long-term effects would very much be similar to toxicity of one of the active substances.

Table 9.3-10: Mammals "tox per fraction" for the MEPCY (step 1 in EFSA GD 2009, Appendix B)

	Chlormequat	Mepiquat	"mix"
Content in the formulation MEPCY	34.5%	11.5%	46%
Fraction in mixture	0.75	0.25	1.0
NOEL (mg/kg bw)	41	155	50.24
Tox per fraction	54.67	620.00	50.24
Contribution to predicted toxicity	91.90%	8.10%	

Chlormequat contributes to 91.90% to mixture toxicity, while the mepiquat have an impact on the predicted risk of 8.10%, therefore, the risk assessment for MEPCY can be performed for the most toxic active substance alone, in this case chlormequat, and no further considerations according to Steps 2 - 4 are necessary.

Combined acute and log-term risk assessment for birds was ~~accepted~~ corrected by RMS. Refinement for combined acute risk assessment for small omnivorous mammal "mouse" ~~should be performed~~ was corrected ~~based on deposition factor 0.~~ by zRMS . The RUD 64.5 already includes interception for invertebrates and use of interception for refinement of residues on insects was discussed during expert meeting for chlormequat chloride and was rejected. This approach was accepted by RMS.

TER corrected = 18.16. The risk assessment is accepted. However, the refinement risk assessment for mammals should be considered by MSs level.

9.3.2.2 Higher-tier risk assessment

The Tier I risk assessment showed an unacceptable acute risk for small omnivorous mammal "mouse". A further higher-tier risk assessment was needed, and the following parameters were refined:

Endpoint refinement
In Confirmatory data (March 2014) it is specified:
In reference of the summary table given in the mammal toxicity DAR, the acute oral toxicity of Chlormequat chloride has been determined in four rat studies, three mice studies and on study in rabbits. It is possible to derive a geometric mean of the endpoints in the acute dietary assessment as different studies exist for one species, and furthermore different species have been tested. In accordance with the EFSA Guidance Document (2009), a geometric mean can be derived if the studies are considered to be equivalent in terms of guideline and, in particular, the vehicle/solvent used. A toxicology specialist has advised that all the rat and mouse studies comply with the minimum criteria for the OECD acute oral LD_{50} study (the basics being 5 animals/sex/group and 3 or more dose levels). They all used water as the vehicle. Furthermore, the results between these studies are very similar. It is noted that the study by Munk and Freisberg (1975) is the only value which is noticeable different; however, as this value is within a factor of 2 of any other value, it is not considered to be an outlier. The rabbit study (1975) does not match the minimum requirement and gives the lowest toxicity value. Despite this, the RMS proposes that this study can still be used in the geometric mean calculations; the inclusion of the endpoint produces a lower LD_{50} geometric mean and thus a more conservative risk assessment.

All differences between males and females in the acute oral toxicity studies have been calculated to be <25%, therefore the combined endpoints of males and females have been used in calculations of the geomean below. It is worth noting that some study summaries only stated a combined endpoint. Furthermore, the study on rabbits produces an LD_{50} of 115 mg a.s./kg bw, it is not stated whether this study endpoint is based on combined sexes, reference is only made to the use of mixed breeds. Despite this no other study was considered with rabbits and so this endpoint will be used in the calculations of the geomean.

It has however been noted that there are some discrepancies between the endpoints for combined sexes stated in the summary table in the mammalian toxicity section of the DAR and between the individual study summaries in the DAR. For completeness these studies have been requested again and the endpoints given in the summary table have been clarified as being correct. Therefore, for future reference the endpoints state in the study summaries in the mammal toxicology DAR should not be relied on.

Using a stepwise approach, a geometric mean is first calculated for the acute oral toxicity endpoints derived from the studies with rats and mice, respectively. Next, this value is used to derive the geometric mean of the endpoints determined for the three different species. The LD_{50} geomean of 343 mg/kg b.w. has been calculated based on these values in the summary table.

Table 9.3-11: Calculations of the relevant mammalian toxicity endpoints for the acute risk assessment of Chlormequat chloride (from section B.9.3.7 of the DAR of April 2007)

Species	Experimental LD_{50}	Reference	LD_{50} (geometric mean single species)	LD_{50} (geometric mean all species)
Rat	522	Lowe C.A. (1990) RD#1990/10676	598	343
Rat	534	Suresh T.P. (1991a) Report ST959-AOR		
Rat	883	Munk R., Freisberg K.O.(1975) RD#1975/012		
Rat	520	Hattori K. (1981) RD#1981/10230		
Mouse	629	Suresh T.P. (1991b) Report ST960-AOM	586	
Mouse	589	Munk R., Freisberg K.O.(1975) RD# 1975/0072		
Mouse	544	Hattori K. (1981) RD#1981/10230		
Rabbit	115	Kirsch P. et al. (1975) RD#1975/091	115	

An LD_{50} geomean, of 343 mg a.s./kg bw will be used in the acute mammal toxicity risk assessment.

Deposition factor

MEPCY will be applied directly to crop. Since weeds, weed seeds and ground arthropods will be covered by the crop, an interception by the crop has to be taken into account. For winter cereals, BBCH stages 29-32 corresponds with the tillering and elongation stages, and according to the interception values of FOCUS (2000), for winter cereals at such stages, an interception factor of 20% should be considered as highest worst case. Therefore, for the refinement of the risk a deposition factor of 0.8 should be applied.

Table 9.3-12: Higher-tier assessment of the acute risk for mammals due to the use of MEPCY in winter wheat – refined LD_{50}

Intended use	**Winter wheat**
Active substance/product	Chlormequat
Application rate (g/ha)	1 x 690
Acute toxicity (mg/kg bw)	343
TER criterion	10

Focal species	Food category, % in diet	FIR/bw	RUD_{90}*× DF* (mg/kg food)	MAF_{90}	PT	DDD_{90} (mg/kg bw/d)	TER_a
Wood mouse (*Apodemus sylvaticus*)	Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	0.27[1]	64.5[1] × ~~0.8~~[2]	1.0	1.0	~~9.61~~ 12.02	~~35.7~~ 28.54

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

[1] According to Appendix A of EFSA/2009/1438.

[2] Deposition factor according to FOCUS groundwater guidance.

The RUD 64.5 already includes interception for invertebrates and use of interception for refinement of residues on insects was discussed during expert meeting for chlormequat chloride and was rejected. This approach was accepted by RMS. TER corrected = 28.54. The risk assessment is accepted. However, the refinement risk assessment for mammals should be considered by MSs level.

The use of this conservative geometric mean (343 mg a.s./kg bw) demonstrates an acceptable acute risk to mammals. So it is clear that even without inclusion of the rabbit study in the geometric mean calculation, an acceptable risk would still be demonstrated for mammal.

Refinement risk assessment for small omnivorous mammal "mouse" was not evaluated by RMS. TER for first-tier assessment of the acute risk for mouse is **9.7.** In opinion RMS the acute risk assessment for small omnivorous mammal "mouse" with TER = 9.7 should be accepted without refinement. This value is very close to the trigger value of 10. However, the refinement risk assessment for small omnivorous mammal "mouse" should be considered at MS level.

Updated 2024
The higher tier refinement presented by the Applicant (geomean LD_{50}) is the same step wise approach as presented by the notifier of Chlormequat in the Addendum – Confirmatory Data (March 2014, with post-commenting changes marked in red – May 2014). The geomean LD_{50} approach was discussed in the Addendum and finally considered acceptable, demonstrating an acceptable risk for small omnivorous mammals. Moreover, as a result of the confirmatory data evaluation, the List of Endpoints was amended and the higher tier refinement for mammals was included. Therefore, the higher tier refinement submitted by Applicant in the dRR has already been evaluated an accepted at EU level. However, the refinement risk assessment for small omnivorous mammal "mouse" should be considered at MS level.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc of 109.3 (geometric mean, n = 4, Confirmatory data – Chlormequat (May 2014)), Chlormequat belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	690		
Acute toxicity (mg/kg bw)	=	115	quotient	= 6.00
Reprod. toxicity (mg/kg bw/d)	=	41	quotient	= 16.83

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50 for at least one use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

With a K(f)oc of 702.02 (geometric mean, n = 12, EFSA Scientific Report (2008) 146, 1-73), Mepiquat belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	230		

Acute toxicity (mg/kg bw)	=	200	quotient	=	1.15
Reprod. toxicity (mg/kg bw/d)	=	155	quotient	=	1.48

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

Agree with the presented risk assessment.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Chlormequat amounts to -3.07 to -3.47 (EFSA Scientific Report (2008) 179, 1-77) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Mepiquat amounts from -3.14 to -3.55 (EFSA Scientific Report (2008) 146, 1-73) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Agree with the presented risk assessment.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Agree.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

Agree.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

Agree.

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

Not relevant.

Agree.

9.3.4 Overall conclusions

According to the first-tier risk assessment for cereals, the TER_a values for the active substance

Chlormequat are lower than the Annex VI trigger of 10 for small omnivorous mammal 'mouse'. A refinement of the risk was done and the TER_a were above the trigger showing no risk. The TER_{lt} values for Chlormequat are greater than the Annex VI trigger of 5. According to screening and first-tier assessment for cereals, all the TER_a and TER_{lt} values for Mepiquat are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MEPCY presents no unacceptable acute and long-term risk to birds according to the intended uses on cereals.

No unacceptable acute and long-term risk to mammals according to the intended uses on cereals for Mepcy (SHA 126085 A) could be concluded.

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

No data available.

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

Effects on aquatic organisms of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. 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 – Chlormequat chloride

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Chlormequat chloride	96 h, f	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Scientific Report (2008) 179; 1-77
Oncorhynchus mykiss	Chlormequat chloride	21 d, ss	NOEC = 43.1 mg a.s./L_{nom}	
Aquatic invertebrate				
Daphnia magna	Chlormequat chloride	48 h, s	EC_{50} = 31.7 mg a.s./L $_{nom}$	EFSA Scientific Report (2008) 179; 1-77
Daphnia magna	Chlormequat chloride	21 d, ss	NOEC = 2.4 mg a.s./L $_{nom}$	
Algae				
Pseudokirchneriella subcapitata	Chlormequat chloride	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$ E_bC_{50} > 100 mg a.s./L $_{nom}$	EFSA Scientific Report (2008) 179; 1-77
Higher plant				
Lemna gibba	Chlormequat chloride	7 d, s	E_rC_{50} = 28.0 mg a.s./L $_{mm}$ E_bC_{50} = 5.3 mg a.s./L $_{mm}$	EFSA Scientific Report (2008) 179; 1-77

Species	Substance	Exposure System	Results	Reference
Higher-tier studies (micro- or mesocosm studies)				
No study submitted.				

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 – Mepiquat chloride

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Mepiquat chloride	96 h, s	LC_{50} > 100 mg a.s./L	EFSA Scientific Report (2008) 146, 1-73
Oncorhynchus mykiss	Mepiquat chloride	28 d, f	NOEC = 100 mg a.s./L	
Oncorhynchus mykiss	Mepiquat chloride	95 d, f	NOEC = 100 mg a.s./L	
Aquatic invertebrate				
Daphnia magna	Mepiquat chloride	48 h, s	EC_{50} = 68.5 mg a.s./L	EFSA Scientific Report (2008) 146, 1-73
Daphnia magna	Mepiquat chloride	21 d, ss	NOEC = 12.5 mg a.s./L	
Daphnia magna	Mepiquat chloride	21 d, ss	NOEC = 12.5 mg a.s./L	
Algae				
Anabaena flos-aquae	Mepiquat chloride	96 h, s	E_rC_{50} = 44.8 mg a.s./L E_bC_{50} = 14.4 mg a.s./L	EFSA Scientific Report (2008) 146, 1-73
Higher plant				
Lemna gibba	Mepiquat chloride	14 d, s	E_rC_{50} = 15.41 mg a.s./L E_bC_{50} = 2.6 mg a.s./L	EFSA Scientific Report (2008) 146, 1-73
Higher-tier studies (micro- or mesocosm studies)				
No study submitted.				

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 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL)

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	MEPCY	96 h, s	LC_{50} > 100 mg/L	KCP 10.2.1-01 Saiqa Nazhath, M. (2021). G14214
Aquatic invertebrate				
Daphnia magna	MEPCY	48 h, s	EC_{50} > 100 mg/L	KCP 10.2.1-02 Saiqa Nazhath, M. (2021). G14215

Species	Substance	Exposure System	Results	Reference
Algae				
Pseudokirchneriella subcapitata	MEPCY	72 h, s	E_rC_{50} >100 mg/L E_yC_{50} >100 mg/L	KCP 10.2.1-03 Saiqa Nazhath, M. (2021). G14216
Higher plant				
Lemna gibba	MEPCY	7 d, s	E_rC_{50} >100 mg/L E_yC_{50} >100 mg/L	KCP 10.2.1-04 Kanchana, P. (2023). 13004/2023
Higher-tier studies (micro- or mesocosm studies)				
No study submitted.				

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

9.5.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New endpoints from studies with the MEPCY formulation were included and used in the risk assessment.

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 and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

MEPCY

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MEPCY for each organism group based on drift calculations for winter wheat

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Higher Plant**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000	E_rC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		1000	1000	10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	20.092	0.020	0.020	0.002	0.002

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

CHLORMEQUAT

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Chlormequat for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MEPCY in winter wheat

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher Plant**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint (µg/L)		LC_{50} 100000	NOEC 43100	EC_{50} 31700	NOEC 2400	E_rC_{50} 100000	E_rC_{50} 28000
AF		100	10	100	10	10	10
RAC (µg/L)		1000	4310	317	240	10000	2800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	207.09	0.207	0.048	0.653	0.863	0.021	0.074
Step 2							
S-Europe	61.99	0.062	0.014	0.196	0.258	0.006	0.022
N-Europe	32.95	0.033	0.008	0.104	0.137	0.003	0.012

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

MEPIQUAT

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Mepiquat for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MEPCY in winter wheat

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plant**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint (µg/L)		LC_{50} 100000	NOEC 100000	EC_{50} 68500	NOEC 12500	E_rC_{50} 44800	E_rC_{50} 15410
AF		100	10	100	10	10	10
RAC (µg/L)		1000	10000	685	1250	4480	1541
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	41.72	0.042	0.004	0.061	0.033	0.009	0.027
Step 2							
S-Europe	11.54	0.012	0.001	0.017	0.009	0.003	0.007
N-Europe	6.40	0.006	0.001	0.009	0.005	0.001	0.004

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

Risk assessment for the combinations of a.s. in the formulation

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation. In addition, as demonstrated in the short-term studies here above there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. An evaluation of the risk posed by the intact formulation is therefore relevant only for the acute/short-term assessment. The long-term risk was assessed considering data for the active substances in the formulation and no chronic combined risk assessment has been performed.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ XXX}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Active susbtance / species	Test system	Endpoint (mg a.s./L)
Chlormequat chloride		
Oncorhynchus mykiss	LC_{50} 96h	>100
Daphnia magna	E_rC_{50} 48h	31.7
Pseudokirchneriella subcapitata	E_rC_{50} 72h	>100
Mepiquat chloride		
Oncorhynchus mykiss	LC_{50} 96h	>100
Daphnia magna	E_rC_{50} 48h	68.5
Pseudokirchneriella subcapitata	E_rC_{50} 72h	44.8

The calculated MDR values are between 0.2 and 5 (see Table 9.5-7), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for this organisms. No synergisms or additional toxicity occurs due to the co-formulants.

Table 9.5-7: Summary of results obtained in the studies with the formulated product MEPCY and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of MEPCY ($LC_{50\ MEPCY}$ or $EC_{50\ MEPCY}$) (mg/L)	Measured toxicity of MEPCY (converted to be a.i. based) ($LC_{50\ MEPCY}$ or $EC_{50\ MEPCY}$) (mg a.s./L)	Calculated mixture toxicity[a] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Model deviation ratio (MDR = $EC_{50\ mix\text{-}CA}$ / $EC_{50\ MEPCY}$)
O. mykiss	LC_{50}, acute, 96 h	100	42.279	100.000	2.365
D. magna	EC_{50}, acute, 48 h	100	42.279	36.618	0.866
Algae	E_bC_{50}, 72 h	100	42.279	76.451	1.808

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Chlormequat (345 g/L*)* and Mepiquat (115 g/L) within the formulation.

All the calculated factors are between 0.8-1.2 (see Table 9.5-8), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is similar to the mixture composition at the PEC_{mix}.

Table 9.5-8: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in MEPCY) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated mixture toxicity (a.s. in PEC_{mix})[b] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$ at lower exposure tier	Factors ($EC_{50\ mix\text{-}CA}$ (a.s. in MEPCY)/$EC_{50\ mix\text{-}CA}$ (a.s. in PEC_{mix})) at lower exposure tier
O. mykiss	LC_{50}, acute, 96 h	100.000	100.000	1.000
D. magna	EC_{50}, acute, 48 h	36.618	34.838	1.051
Algae	E_bC_{50}, static, 72 h	76.451	82.877	0.922

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Chlormequat (345 g/L*)* and Mepiquat (115 g/L) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Chlormequat (0.20709 mg/L at Step 1) and Mepiquat (0.04172 mg/L at Step 1).

Table 9.5-9: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in MEPCY) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated toxicity per fraction of MEPCY (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity $(1\text{-}EC_{x\ mix\text{-}CA} \times (1/EC_{x\ mix\text{-}CA} - TU_i))$ [%]
O. mykiss	LC_{50}, acute, 96 h	100.000	Chlormequat: 133.333 Mepiquat: 400.000	Chlormequat: 75.00% Mepiquat: 25.00%
D. magna	EC_{50}, acute, 48 h	36.618	Chlormequat: 42.267 Mepiquat: 274.000	Chlormequat: 86.64% Mepiquat: 13.36%
Algae	E_bC_{50}, static, 72 h	76.451	Chlormequat: 133.333 Mepiquat: 179.200	Chlormequat: 57.34% Mepiquat: 42.7%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ MEPCY}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of of Chlormequat (345 g/L) and Mepiquat (115 g/L) within the formulation.

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'.

Regarding MEPCY, no active substance is clearly driving the acute risk for fish, daphnia and algae. The studies performed with the formulated product do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-9).

Table 9.5-10: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in all crops for fish

Exposure	Lower exposure tier		Higher exposure tier	
	Chlormequat	Mepiquat	Chlormequat	Mepiquat
Exposure tier (FOCUS step)	Step 1	Step 1	Step 2	Step 2
PECsw [mg a.s./L]	0.20709	0.04172	0.06199	0.01154
Relative proportions of the individual mixture components in the environment (pi PEC)	0.832	0.168	0.843	0.157
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.24881		0.07353	
ETRmix = PECmix/ECx PPP	0.006		0.002	
Trigger	0.01			

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for fish.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPPP) and a.s. (ECxa.s.):	Please refer to table 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPPP) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPPP) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPPP).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5-7	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is 0.8 – 1.2.	Please refer to table 9.5-8	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	Low risk demonstrated if $ETR_{mix} < 0.01$		**Low risk**

Table 9.5-11: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in all crops for *Daphnia*

Exposure	Lower exposure tier		Higher exposure tier	
	Chlormequat	**Mepiquat**	**Chlormequat**	**Mepiquat**
Exposure tier (FOCUS step)	Step 1	Step 1	Step 2	Step 2
PECsw [mg a.s./L]	0.20709	0.04172	0.06199	0.01154
Relative proportions of the individual mixture components in the environment (pi PEC)	0.832	0.168	0.843	0.157
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.24881		0.07353	
ETRmix = PECmix/ECx PPP	0.006		0.002	
Trigger	0.01			

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for fish.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPPP) and a.s. (ECxa.s.):	Please refer to table 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPPP) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPPP) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPPP).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5-7	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is 0.8 – 1.2.	Please refer to table 9.5-8	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	Low risk demonstrated if $ETR_{mix} < 0.01$		**Low risk**

Table 9.5-12: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in all crops for alga

Exposure	Lower exposure tier		Higher exposure tier	
	Chlormequat	**Mepiquat**	**Chlormequat**	**Mepiquat**
Exposure tier (FOCUS step)	Step 1	Step 1	Step 2	Step 2
PECsw [mg a.s./L]	0.20709	0.04172	0.06199	0.01154
Relative proportions of the individual mixture components in the environment (pi PEC)	0.832	0.168	0.843	0.157
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.24881		0.07353	
ETRmix = PECmix/ECx PPP	0.006		0.002	
Trigger	0.1			

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for alga.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPPP) and a.s. (ECxa.s.):	Please refer to table 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPPP) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPPP) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPPP).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5-7	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is 0.8 – 1.2.	Please refer to table 9.5-8	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	Low risk demonstrated if $ETR_{mix} < 0.1$		**Low risk**

9.5.3 Overall conclusions

MEPCY:
Based on FOCUS Step 1 and 2, calculated PEC/RAC ratios for the formulated MEPCY did indicate an acceptable risk for aquatic organisms for all intended uses.

Chlormequat:
For the intended uses on winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate prolonged as characterised by a NOEC for *Daphnia magna* of 2.4 mg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

Mepiquat:
For the intended uses winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate acute as characterised by an EC_{50} for *Daphnia magna* of 68.5 mg/L in connection with an assessment factor of 100) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

zRMS comment: The evaluation of the risk for aquatic 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" (EFSA Journal 2013;11(7):3290).

The PEC/RAC ratio was <1 value, indicating an acceptable acute and long term risk assessment risk for all aquatic organism from exposure of a.s.- mepiquat chloride and chlormequat chloride and ppp SHA 126085 A/MEPCY.

Risk assessment for mixture toxicity for PPP Mepcy/SHA 126085 A was accepted by RMS.

The study on the effects of MEPCY (SHA 126085 A) on aquatic plants - *Lemna gibba* was not provided by Applicant. In this case, the Applicant used the available data for PPP to indicate acceptable risk for aquatic organisms such as fish, aquatic invertebrates and algae. However, in opinion RMS this approach may be questioned. The study of effects on *Lemna gibba* shall be required for Mepcy (SHA 126085 A) belongs to plant growth regulators and contains more than 1 substance active.

The risk assessment for *Lemna gibba* with PPP Mepcy (SHA 126085 A) should be considered at MSs level.

Data gap:
The study on the effects of MEPCY (SHA 126085 A) on *Lemna gibba* with risk assessment should be provided by Applicant.

January 2024 updated
To address the current data gap for *Lemna gibba* conducted by Applicant according to the OECD Guidelines. The new study for *Lemna gibba* with formulated product MEPCY has been accepted by zRMS. Toxicity data and risk assessment for *Lemna gibba* was available for the PPP MEPCY and a low risk was demonstrated for this species. Refinement risk assessment is not needed.

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

Effects on bees of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. 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	Chlormequat chloride	Oral	LD_{50} > 80.2 µg/bee	EFSA Scientific Report (2008) 179, 1-77
Apis mellifera	Chlormequat chloride	Contact	LD_{50} > 65.2 µg/bee	
Apis mellifera	Mepiquat chloride	Oral	LD_{50} > 107.4 µg/bee	EFSA Scientific Report (2008) 146, 1-73
Apis mellifera	Mepiquat chloride	Contact	LD_{50} > 100 µg/bee	

Species	Substance	Exposure System	Results	Reference
Apis mellifera	MEPCY	Oral	LD_{50} > 100 µg/bee	KCP 10.3.1.1.1 Likith, N.G. 2020 Report No. G14217
Apis mellifera	MEPCY	Contact	LD_{50} > 100 µg/bee	KCP 10.3.1.1.2 Likith, N.G. 2020 Report No. G14218
Apis mellifera	MEPCY	Chronic, 10 d	LDD_{50} = 63.12 µg/bee/day (19.01 µg Chlormequat /bee and 6.31 µg Mepiquat /bee) NOEDD = 39.62 µg/bee/day (11.93 µg Chlormequat /bee and 3.96 µg Mepiquat/bee)	KCP 10.3.1.2-01 Prabha, K.L., 2023, 11510/2022
Apis mellifera	MEPCY	Larval, repeated exposure	NOED = 70.0 µg/larva (21.07 µg Chlormequat/larva and 7.00 µg Mepiquat/larva) ED_{10} = 71.73 µg/larva (21.59 µg Chlormequat/larva and 7.17 µg Mepiquat/larva)	KCP 10.3.1.3-01 Prabha, K.L., 2023, 11511/2022
Higher-tier studies (tunnel test, field studies)				
Not required.				

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. In addition, new acute toxicity studies were performed with the formulation MEPCY and therefore the resulting endpoints are used in the risk assessment on the product.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of MEPCY in winter wheat

Intended use	**Winter wheat**		
Active substance	Chlormequat		
Application rate (g/ha)	1 x 690 g a.s./ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 80.2	690	8.60
Contact toxicity	> 65.2		10.58
Active substance	Mepiquat		
Application rate (g/ha)	1 x 230 g a.s./ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 107.4	230	2.14
Contact toxicity	> 100		2.30
Product	MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL)		
Application rate (g/ha)	1 x 2 L/ha (2176 g/ha*)		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	2176	21.76
Contact toxicity	> 100		21.76

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* Considering a density of 1.088 g/cm^3

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

First-tier assessments indicate that no unacceptable risk for bees exposed to MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) is expected according to the proposed intended uses on cereals.

zRMS comment:

Bees:
The risk assessment for bees was conducted in accordance with SANCO/10329/2002 rev. 2 final. The acute oral and contact toxicity data are available for the formulation SHA 126085A/MEPCY. Based on the first-tier assessment results, the risk is acceptable (HQ values exceeded 50) for the product. In addition, the chronic study for adult bees and a study effects on honey bee development and other honey bee life stages should be submitted by Applicant. The risk assessment based on this studies should be considered when GD for Bees, 2013 is implemented at EU level. zRMS point out according to commission regulation No 284/2013 chronic and larval studies with formulated product are required for products containing more than 1 active substance.

Final decision should be taken into account at MSs level.

Updated January 2024
To address the current data gap for **bees** were conducted by Applicant according to the OECD Guidelines. The chronic and larval studies with formulated product MEPCY are provided by Applicant for products MEPCY. The chronic studies for bees were accepted by zRMS. The risk assessment based on this studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

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

Effects on non-target arthropods of Chlormequat 34.5% + Mepiquat 11.5% SL were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. ~~However, the provision of further data on the MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) is not considered essential, because active substance toxicity data can be used~~. 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
Laboratory studies – Chlormequat chloride				
Typhlodromus pyri	Chlormequat chloride	Laboratory test glass plates (2D)	LR_{50} > 2250 g a.s./ha	EFSA Scientific Report (2008) 179, 1-77
Aphidius rhopalosiphi	Chlormequat chloride	Laboratory test glass plates (2D)	LR_{50} > 2200 g a.s./ha	

Species	Substance	Exposure System	Results	Reference
Poecilus cupreus (carabid beetle)	STE 24371 W (Chlormequet chloride 720 g/L)	Silica sand 14 days	At 1512 g a.s./ha: 0% mortality -40% feeding	
Poecilus cupreus (carabid beetle)	Stabilan (Chlormequat chloride 465 g/L)	Quartz sand 14 days	At 1395 g a.s./ha: 0% mortality -1.3% feeding	
Poecilus cupreus (carabid beetle)	Stabilan (chlormequat chloride 465 g/L)	Quatrz sand 14 days	At 1395 g a.s./ha: 0% mortality -15% feeding	
Aleochara bilineata (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Sand 5 day survival 10 day hatching	At 1395 g a.s./ha: 0% mortality No significant difference reproduction	
Aleochara bilineata (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Moist sand 55 days	At 1395 g a.s./ha: +4.3% parasitic capacity	
Aleochara bilineata (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Moist quartz sand 4 weeks	At 1395 g a.s./ha: +26% parasitisation	
Chrysoperla carnea (green lacewing)	BAS 062 03 W (Chlormequat chloride 765.8 g/L)	Glass plates 4-5 days after pupation 7 days hatching	At 2297.4 g a.s./ha: Slight reduction mortality No effect reproduction	
Laboratory studies – Mepiquat chloride				
Typhlodromus pyri	BAS 083 52 W (Mepiquat chloride 617.6 g/L)	Laboratory test glass plates (2D)	LR_{50} = 1530 g a.s./ha	ESFSA Scientific Report (2008) 146, 1-73
Aphidius rhopalosiphi	BAS 083 52 W (Mepiquat chloride 617.6 g/L)	Laboratory test glass plates (2D)	LR_{50} = 1366 g a.s./ha	
Laboratory studies – MEPCY				
Typhlodromus pyri	MEPCY	Laboratory test glass plates (2D)	LR_{50} = 8930 g a.s./ha ER_{50} = 8610 g a.s./ha	KCP 10.3.2.1-01 Parkavi, B., 2023; 13006/2023
Aphidius rhopalosiphi	MEPCY	Laboratory test glass plates (2D)	LR_{50} = 8790 g a.s./ha ER_{50} = 8610 g a.s./ha	KCP 10.3.2.1-02 Angayarkanni, V., 2023; 13005/2023
Field or semi-field tests				
Not required.				

9.7.1.1 Justification for new endpoints

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

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 MEPCY in winter wheat

Intended use	**Winter wheat**		
Active substance/product	Chlormequat chloride		
Application rate (g/ha)	1 x 690 g a.s./ha		
MAF	1.0		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 2250	690	0.31
Aphidius rhopalosiphi	> 2200		0.31
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	1395	690	yes
Aleochara bilineata	1395		yes
Chrysoperla carnea	2297.4		yes
Active substance/product	Mepiquat chloride		
Application rate (g/ha)	1 x 230 g a.s./ha		
MAF	1.0		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1530	230	0.15
Aphidius rhopalosiphi	1366		0.17
Active substance/product	**MEPCY**		
Application rate (L/ha)	1 x 2 L f.p./ha		
MAF	1.0		
Test species Tier I	**LR_{50} / ER_{50} (lab.) (L/ha)**	**$PER_{in\text{-}field}$ (L/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	8.61	2	0.23
Aphidius rhopalosiphi	8.61		0.23

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

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of MEPCY in winter wheat

Intended use	**Winter wheat**
Active substance/product	Chlormequat
Application rate (g/ha)	1 x 690 g a.s./ha

MAF	1.0				
vdf	10 (2D) / 1 (3D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 2250	0.0277	1.911	10	0.008
Aphidius rhopalosiphi	> 2200				0.009
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Poecilus cupreus	1395	0.0277	1.911	5	yes
Aleochara bilineata	1395				yes
Chrysoperla carnea	2297.4				yes
Active substance/product	Mepiquat				
Application rate (g/ha)	1 x 230 g a.s./ha				
MAF	1.0				
vdf	10 (2D) / 1 (3D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1530	0.0277	0.637	10	0.004
Aphidius rhopalosiphi	1366				0.005
Active substance/product	**MEPCY**				
Application rate (L/ha)	1 x 2 L f.p./ha				
MAF	1.0				
vdf	10 (2D) / 1 (3D)				
Test species Tier I	**LR_{50} / ER_{50} (lab.) (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	8.61	0.0277	0.006	10	0.01
Aphidius rhopalosiphi	8.61			10	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.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in-field and off-field HQ value calculated for Chlormequat chloride and Mepiquat chloride for the representative species *Typhlodromus pyri* and *Aphidius rhopalosiphi* are lower than the trigger of 2 for Tier I tests, indicating no risk to non-target arthropods in vegetated off-field areas following application

according to the proposed use patterns.

zRMS comment:

Arthropods other than bees:
The study on the effects of MEPCY (SHA 126085 A) on arthropods was not provided by Applicant. In this case, the Applicant used the available data for substance active chlormequat chloride and formulation with mepiquat chloride to indicate acceptable risk for arthropods other than bees. However, in opinion RMS this approach should be not accepted. The data requirements specify that toxicity data for PPP should be provided in the case that a PPP contain more than one active substance. In opinion RMS, the study on the effects of MEPCY (SHA 126085 A) on arthropods other than bees should be provided by RMS. Acceptable risk assessment could not be conclude without the study for PPP and arthropods other than bees.

Data gap:
The study on the effects of MEPCY (SHA 126085 A) on arthropods other than bees (*A.rhopalosiphi* and *T,pyri*) should be provided by Applicant.

zRMS point out that according to commission regulation no 284/2013 standard laboratory studies on *T. pyri* and *A. rhopalosiphi* with formulated product are required for products containing more than 1 active substance.

The risk assessment for arthropods other than bees should be considered at MSs level.

Updated January 2024
To address the current data gap for **arthropods other than bees** were conducted by Applicant according to the OECD Guidelines. The new studies for *T. pyri* and *A. rhopalosiphi* with formulated product MEPCY have been accepted by zRMS. Toxicity data and risk assessments were available for the PPP MEPCY and a low risk was demonstrated for **arthropods other than bees**. The in-field and off-field HQ value calculated for Chlormequat chloride and Mepiquat chloride and **MEPCY** formulation for the representative species *Typhlodromus pyri* and *Aphidius rhopalosiphi* are lower than the trigger of 2 for Tier I tests, indicating no risk to non-target arthropods in vegetated off-field areas following application according to the proposed use patterns. Refinement risk assessment is not needed.

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 Chlormequat and Mepiquat. Full details of these studies are provided in the respective EU DAR and related.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. ~~However, the provision of further data on Chlormequat 34.5% + Mepiquat 11.5% SL is not considered essential, because active substance toxicity data can be used.~~ New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Chlormequat chloride	Mixed into substrate 14 d, acute	LC_{50} = 320 mg a.s./kg dw	EFSA Scientific Report (2008) 179, 1-77
Eisenia fetida	Chlormequat chloride	Mixed into substrate 56 d, chronic	NOEC = 681 mg a.s./kg dw	
Other soil macro-organisms: Not rquired as DT_{90} is 105.1 days and NTA HQ, eartwhorm TER and effect on soil micro-organisms all below triggers.				
Eisenia fetida	Mepiquat chloride	Mixed into substrate 14 d, acute 10 % peat content	LC_{50} = 319.5 mg a.s./kg dw	EFSA Scientific Report (2008) 146, 1-73
Eisenia fetida	Mepiquat chloride	Mixed into substrate 56 d, chronic 10 % peat content	No data submitted or required.	
Other soil macro-organisms: Since the HQ values for the two standard non-target arthropods are below 2 and no effects were observed on the soil dwelling *Aleochara bilineata* no studies are required although DT_{90} in soil is longer than 100 days.				
Eisenia fetida	MEPCY	Mixed into substrate 56 d , chronic 10% peat content	**$NOEC_{reproduction}$ >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mg mepiquat/kg dw)** EC_{10} >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mgmepiquat/ kg dw)	10.4.1.1-01 Parkavi, B., 2023; 13007/2023
Folsomia candida	MEPCY	Mixed into substrate 28 d , chronic 5% peat content	**$NOEC_{reproduction}$ >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mg mepiquat/kg dw)** EC_{10} >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mgmepiquat/ kg dw)	KCP 10.4.2.1-01 Angayarkanni V., 2023, 13009/2023

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	MEPCY	Mixed into substrate 14 d , chronic 5% peat content	**$NOEC_{reproduction}$ >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mg mepiquat/kg dw)** EC_{10} >1000 mg f.p./kg dw (>322 mg chlormequat/kg dw and >107 mgmepiquat/ kg dw)	KCP 10.4.2-02 Angayarkanni V., 2023, 13008/2023
Field studies				
No data, not required.				
Litter bag test				
No data, not required.				

9.8.1.1 Justification for new endpoints

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

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 and 8.7-4. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for Chlormequat and Mepiquat.

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 MEPCY in winter wheat

Intended use	**Winter wheat**		
Acute effects on earthworms			
~~**Product/active substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Chlormequat~~	~~320~~	~~0.736~~	~~434.8~~
~~Mepiquat~~	~~319.5~~	~~0.245~~	~~1304.1~~

Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Chlormequat	681	0.736	925.3
Mepiquat	NR	0.245	NR
MEPCY	> 1000	2.321	< 430.85
Chlormequat*	> 322	0.736	< 437.5
Mepiquat*	> 107	0.245	< 436.73
Chronic effects on other soil macro- and mesofauna (*Folsomia candida*)			
NR			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
MEPCY	> 1000	2.321	< 430.85
Chlormequat*	> 322	0.736	< 437.5
Mepiquat*	> 107	0.245	< 436.73
Chronic effects on other soil macro- and mesofauna (*Hypoaspis aculeifer*)			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
MEPCY	> 1000	2.321	< 430.85
Chlormequat*	> 322	0.736	< 437.5
Mepiquat*	> 107	0.245	< 436.73

TER values shown in bold fall below the relevant trigger.
*Based on the concentration of active substance in MEPCY

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The acute and chronic TER for Chlormequat and Mepiquat are above the Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that Chlormequat and Mepiquat do not poses acute and long-term risk to earthworms and other soil macro- and mesofauna.

zRMS comment:

Earthworms:
The study on the effects of MEPCY (SHA 126085 A) on earthworms was not provided by Applicant. In this case, the Applicant used the available data for substance active chlormequat chloride to indicate acceptable risk for earthworms. However, in opinion RMS this approach should be not accepted. The data requirements specify that toxicity data for PPP should be provided in the case that a PPP contain more than one active substance. It was acknowledged that the active substance chlormequat chloride did not show a high toxicity to earthworms. The RMS noted that the risk assessment for chlormequat chloride indicated a very high margin of safety based on the currently available exposure assessment. However, the toxicity of the plant protection product Mepcy (SHA 126085 A) cannot be predicted on the

basis of the data for the active substance. For mepiquat chloride the toxicity data for long-term toxicity are not available. In opinion RMS, the study on the effects of MEPCY (SHA 126085 A) on earthworms should be provided by RMS. Acceptable risk assessment could not be conclude without the study for PPP and earthworms.

Data gap:
The study on the effects of MEPCY (SHA 126085 A) on earthworms should be provided by Applicant.

Updated January 2024
To address the current data gap for earthworms were conducted by Applicant according to the OECD Guidelines. The new study for MEPCY and *earthworms* have been accepted by zRMS. Toxicity data and risk assessments was available for the PPP MEPCY and a low risk was demonstrated for earthworms. Refinement risk assessment is not needed.

The risk assessment for earthworms should be considered at MSs level.

Other soil macro-organisms
In accordance with the data requirements of the (EU) Regulation 284/2013 data on *Folsomia candida* and *Hypoaspis aculeifer* should be submitted. No toxicity data are available for the PPP. However, the Applicant provided a justification indicating that the data requirements indicate that an assessment is not triggered since it is of low risk to NTAs. However, the RMS noted that the data requirements specify that toxicity data should be provided in the case that a PPP contain more than one active substance.

Data gap:
The study on the effects of MEPCY (SHA 126085 A) on *Folsomia candida* and *Hypoaspis aculeifer* should be provided by Applicant.

The risk assessment for soil macroorganisms should be considered at MSs level.
zRMS point out that according to commission regulation no 284/2013 studies on *F. candida* and *H. aculeifer* with formulated product are required for products containing more than 1 active substance.

Updated January 2024
To address the current data gap for soil macro-organisms (*Folsomia candida* and *Hypoaspis aculeifer*) were conducted by Applicant according to the OECD Guidelines. The new studies for MEPCY and *Folsomia candida* and *Hypoaspis aculeifer* have been accepted by zRMS. Toxicity data and risk assessments were available for the PPP MEPCY and a low risk was demonstrated for soil macro-organisms such as *Folsomia candida* and *Hypoaspis aculeifer*. Refinement risk assessment is not needed.

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

Effects on soil microorganisms of MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. 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	Chlormequat chloride	28 d, aerobic	No effect at day 28 at 18.6 mg a.s./kg dw soil	EFSA Scientific Report (2008) 179, 1-77
C-mineralisation	Chlormequat chloride	28 d, aerobic	No effect at day 28 at 18.6 mg a.s./kg dw soil	
N-mineralisation	BAS 083 34 W (Mepiquat chloride 50 g/L)	28 d, aerobic	< 25% effect at day 28 at 1.352 mg Mepiquat chloride/kg dw soil	EFSA Scientific Report (2008) 146, 1-73
C-mineralisation	BAS 083 34 W (Mepiquat chloride 50 g/L)	84 d, aerobic	< 25% effect at day 84 at 1.352 mg Mepiquat chloride/kg dw soil	
N-mineralisation	MEPCY	28 d, aerobic sandy clay loam soil	No significant effects (< 25% effect compared to untreated control) 4.72% at the applicataion rate of 14.27 mg test item/kg dw (4.61 mg chlormequat/kg dw + 1.51 mg mepiquat/kg dw) 6.92% at the application rate of 71.35 mg test item/kg dw (23.05 mg chlormequat/kg dw + 7.56 mg mepiquat/kg dw)	KCP 10.5.01 Anand, H.S., 2020 G14222
C-mineralisation	MEPCY	28 d, aerobic sandy clay loam soil	No significant effects (< 25% effect compared to untreated control) 6.98% at the applicataion rate of 14.27 mg test item/kg dw (4.61 mg chlormequat/kg dw + 1.51 mg mepiquat/kg dw) 10.30% at the application rate of 71.35 mg test item/kg dw (23.05 mg chlormequat/kg dw + 7.56 mg mepiquat/kg dw)	KCP 10.5.02 Anand, H.S., 2020 G14221

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New endpoints from studies with the MEPCY formulation were included and used in the risk assessment.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 to 8.7-5 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of MEPCY in winter wheat

Intended use	**Winter wheat**		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Chlormequat	18.6 (at 28 d)	0.736	yes
Mepiquat	1.352 (at 28 d)	0.245	yes
MEPCY	71.35 (at 28 d)	2.321	yes
C-mineralisation – not required only as additional source information			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Chlormequat	18.6 (at 28 d)	0.736	yes
Mepiquat	1.352 (at 84 d)	0.245	yes
MEPCY	71.35 (at 28 d)	2.321	yes

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for Chlormequat and Mepiquat in MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

zRMS comment:

The study on the effects of MEPCY (SHA 126085 A) has no significant effect on soil micro-organisms at 14.27 mg formulation/kg dry soil (equivalent to 4.61 mg of chlormequat chloride/ kg dry soil and 1.51 mg of mepiquat chloride/ kg dry soil) and 71.35 mg formulation/kg dry soil (equivalent to 23.05 mg of chlormequat chloride/ kg dry soil and 7.56 mg of mepiquat chloride/ kg dry soil). Based on it, can be concluded that MEPCY (SHA 126085 A) under field conditions, use at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of Chlormequat 34.5% + Mepiquat 11.5% SL were not evaluated as part of the EU assessment of Chlormequat and Mepiquat. ~~However, the provision of further data on MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) is not considered essential, because active substances toxicity data can be used.~~ 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 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Carrot and sunflower (4 other species also tested)	Chlormequat chloride	Vegetative vigour	ER_{50} plant weight > 3750 g a.s./ha	EFSA Scientific Report (2008) 179, 1-77
Oat (*Avena sativa*) Onion (*Allium cepa*) Sugar beet (*Beta vulgaris*) Rape (*Brassica napus*) Carrot (*Daucus carota*) Soy bean (*Glycine max*)	Chlormequat chloride	Vegetative vigour Seedling emergence	ER_{50} plant weight > 2100 g a.s./ha ER_{50} emergence > 2100 g a.s./ha	
Carrot (*Daucus carota*) Linseed (*Linum usitatissimum*) Rape (*Brassica napus*) Pea (*Pisum sativum*) Oat (*Avena sativa*) Onion (*Allium cepa*)	BAS 098 00W (308.2 g mepiquat chloride and 158.8 g ethephon/L)	Vegetative vigour Seedling emergence	ER_{50} > 3000 g f.p./ha (ER_{50} > 924.6 g a.s./ha*) ER_{50} > 3000 g f.p./ha (ER_{50} > 924.6 g a.s./ha*)	EFSA Scientific Report (2008) 146, 1-73
[1] *Zea Mays*$_m$ [2] *Glycine max*$_d$ [3] *Sinapis alba*$_d$ [4] *Avena sativa*$_m$ [5] *Raphanus sativus*$_d$ [6] *Solanum Lycopersico*$_d$	MEPCY	21 d Seedling emergence	**[1)]ER_{50} plant dry weight = 12.23 kg f.p./ha** [2)]ER_{50} shoot lenght = 13.31 kg f.p./ha [3)]ER_{50} shoot lenght = 14.31 kg f.p./ha [4)]ER_{50} plant number = 14.82 kg f.p./ha [5)]ER_{50} plant dry weight = 13.64 kg f.p./ha [6)]ER_{50} plant dry weight = 13.25 kg f.p./ha	KCP 10.6.2-01 Radha, S., 2023; 13010/2023
[1] *Zea Mays*$_m$ [2] *Glycine max*$_d$ [3] *Sinapis alba*$_d$ [4] *Avena sativa*$_m$ [5] *Raphanus sativus*$_d$ [6] *Solanum Lycopersico*$_d$	MEPCY	21 d Vegetative vigour	[1)]ER_{50} shoot lenght = 12.96 kg f.p./ha **[2)]ER_{50} plant number = 12.73 kg f.p./ha** [3)]ER_{50} plant dry weight = 13.01 kg f.p./ha [4)]ER_{50} plant dry weight = 13.28 kg f.p./ha [5)]ER_{50} shoot lenght = 13.01 kg f.p./ha [6)]ER_{50} shoot lenght = 13.49 kg f.p./ha	KCP 10.6.2-02 Radha, S., 2023; 13011/2023

m: monocotyledonous; d: dicotyledonous
* Endpoint calculated from the mepiquat content in the formulation 308.2 g mepiquat chloride + 158.8 g ethephon/L.

9.10.1.1 Justification for new endpoints

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

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 MEPCY in winter wheat

Intended use	**Winter wheat**			
Active substance/product	Chlormequat			
Application rate (g/ha)	1 x 690 g a.s./ha			
MAF	1.0			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Oat, onion, sugar beet, rape, carrot and soybean	> 2100	0.0277	19.11	109.87
Active substance/product	Mepiquat			
Application rate (g/ha)	1 x 230 g a.s./ha			
MAF	1.0			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Carrot, linseed, rape, pea, oat, onion	> 924.6	0.0277	6.37	145.13
Active substance/product	MEPCY			
Application rate (L/ha)	1 x 2 L f.p./ha			
MAF	1.0			
Test species	**ER_{50} (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Zea Mays$_m$ (Seedling emergence)	12.23	0.0277	0.06	220.76
Glycine max$_d$ (Vegetative vigour)	12.73	0.0277	0.06	229.78

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

No risk mitigation needed.

9.10.3 Overall conclusions

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for MEPCY (Chlormequat 34.5% + Mepiquat 11.5% SL) shows that Annex VI trigger of 5 is not exceeded, indicating that MEPCY poses a low risk to non-target plants when applied according to the proposed use rates.

zRMS comment:
The study on the effects of MEPCY (SHA 126085 A) on non-target terrestrial plants for the vegetative vigour test (OECD 227 "Terrestrial Plant Test: Vegetative Vigour Test) and the study on the effects of MEPCY (SHA 126085 A) on non-target terrestrial plants in terms of seedling emergence and seedling growth test (OECD Guideline for the Testing of Chemicals No. 208 “Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test”) were not provided by Applicant. In this case, the Applicant used the available data for substance active to indicate acceptable risk for non-target plants. However, in opinion RMS this approach may be questioned. The data requirements specify that toxicity data for PPP should be provided in the case that a PPP contain more than one active substance. It was acknowledged that both active substance did not show a high toxicity to non-target plants. The RMS noted that the risk assessment for chlormequat chloride indicated a very high margin of safety based on the currently available exposure assessment. Furthermore, mepiquat chloride was indicated not to be of high toxicity to non-target plants. However, according to Regulation 284/2013 to studies of effects on non-target plants shall be required for herbicide and plant growth regulator plant protection products. Mepcy (SHA 126085 A) belongs to plant growth regulators and contains more than 1 substance active and toxicity data for PPP should be provided in opinion RMS (OECD 227 and OECD 208).

Overall, the RMS considered that a low risk from the substance active such as mepiquat chloride and chlormequat chloride can be concluded. Furthermore, it should be noted that additional justification (data and/or risk assessment) for PPP may be needed for risk assessments at product registration. **The risk assessment for non-target plants should be considered at MSs level.**

Data gap:
The study on the effects of MEPCY (SHA 126085 A) on non-target terrestrial plants for the vegetative vigour test (OECD 227) and the study on the effects of MEPCY (SHA 126085 A) on non-target terrestrial plants in terms of seedling emergence and seedling growth test (OECD 208) should be provided by Applicant.

zRMS point out that commission regulation No 284/2013 requires a seedling emergence and vegetative vigour study with formulated product for plant growth regulators.

Updated January 2024
To address the current data gap for non-target terrestrial plants (NTTPs) a seedling emergence and vegetative vigour study for MEPCY were conducted by Applicant according to the OECD Guidelines. The new studies for MEPCY have been accepted by zRMS. Toxicity data and risk assessments were available for the PPP MEPCY and a low risk was demonstrated.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	MEPCY
Common name	Chlormequat 34.5% + Mepiquat 11.5% SL
Classification and proposal labelling	
With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: - Code(s) for hazard pictogram(s): - Signal word: - Hazard statement(s): - EU specific statements: EUH401 Precautionary statement: -

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-01	xxxxxxxxx	2021	Chlormequat 345 g/L + Mepiquat 115 g/L SL: Fish, Acute toxicity test with rainbow trout. Report No. G14214 xxxxxxxxxxxx GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-02	Saiqa Nazhath, M.	2021	Chlormequat 345 g/L + Mepiquat 115 g/L SL: alga, growth inhibition test with *Raphidocelis subcapitata*. Report No. G14215 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-03	Saiqa Nazhath, M.	2021	Chlormequat 345 g/L + Mepiquat 115 g/L SL: *Daphnia magna*, Acute immobilisation test. Report No. G14216 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-04	Kanchana, P.	2023	Study of *Lemna gibba* growth inhibition with Chlormequat 345 g/L + Mepiquat 115g/L SL Report No. 13004/2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.1.1	Likith, N.G.	2020	Chlormequat 345 g/L + Mepiquat 115 g/L SL. Acute oral toxicity test in honey bees Report No. G14217 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.1.2	Likith, N.G.	2020	Chlormequat 345 g/L + Mepiquat 115 g/L SL. Acute contact toxicity test in honey bees Report No. G14218 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.2-01	Lakshmi Prabha, K.	2023	Chronic Oral Toxicity Study of Chlormequat 345 g/L + Mepiquat 115 g/L SL on adult honey bee (*Apis mellifera*) Report No. 11510/2022 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.3-01	Lakshmi Prabha, K.	2023	Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure Report No. 11511/2022	N	SHARDA Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Bioscience Research Foundation. GLP, Unpublished		
KCP 10.3.2.1-01	Parkavi, B	2023	A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115g/l SL on the predatory mite, *Typhlodromus pyri* (Scheuten)". Report No. 13006/2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.2.1-02	Angayarkanni, V	2023	A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115 g/l SL on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani Perez Report No. 13005/2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.1.1-01	Parkavi, B.	2023	Effect of Chlormequat 345g/I + Mepiquat 115g/l SL on reproduction of the earthworm (*Eisenia fetida*) in artificial soil Report No. 13007/2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.2.1-01	Angayarkanni, V.	2023	Effect of Chlormequat 345 g/l + Mepiquat 115 g/1 SL on reproduction of the collembolans (*Folsomia candida*) in artificial soil Report No. 13009/2023. Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.2.1-02	Angayarkanni, V.	2023	Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on the reproductive output of the predatory soil mite *Hypoaspis (Geolaelaps)* aculeifer Canestrini *(*Acari: Laelapidae*)* in artificial soil Report No. 13008 /2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.5.01	Anand, H.S.	2020	Soil microorganisms: nitrogen transformation test of Chlormequat 345 g/L + Mepiquat 115 g/L SL Report No. G14222 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.5.02	Anand, H.S.	2020	Soil microorganisms: carbon transformation test of Chlormequat 345 g/L + Mepiquat 115 g/L SL Report No. G14221 Eurofins Advinus Limited GLP, Unpublished	N	SHARDA Cropchem Limited
KCP 10.6.2-01	Radha, S.	2023	Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on seedling emergence and seedling growth of terrestrial plants. Report No: 13010/2023. Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.6.2-02	Radha, S.	2023.	Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on vegetative vigor on terrestrial plants. 2023. Report No: 13011/2023 Bioscience Research Foundation. GLP, Unpublished	N	SHARDA Cropchem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met: • There was no mortality in the negative control at the end of the test which is less than 10% at the end of the test. • The pH of the test solutions was ranged from 7.67 to 7.74 and the temperature of the test solutions was 13.1 to 13.8°C. • The dissolved oxygen saturation of the test solutions ranged from 90 to 97 % which is more than 60% of the air saturation value throughout the test. • The mean concentration of the test item in the tested concentrations was between 95.768 and 100.293 % for chlormequat and 83.626 and 100.858 for mepiquat of the nominal concentrations which was within ± 20% during the test. **Agreed endpoints:** 96h LC_{50} > 100 mg formulation Mepcy (SHA 126085A), equivalent to 32.3 mg chlormequat /L and 10.6 mg mepiquat/L based on nominal concentrations**.**

Reference:	KCP 10.2.1-01
Report	"Chlormequat 345 g/L + Mepiquat 115 g/L SL: Fish, Acute toxicity test with rainbow trout". xxxxxxxxxxxx., 2021. Ecotoxicology section analytical R & D department xxxxxxxxxxxxx. Study No.: G14214

Guideline(s):	OECD Guideline for the Testing of Chemicals No. 203 (2019).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat 345 g/L +Mepiquat 115 g/L SL Batch number: SCL-99721 Purity: - Chlormequat chloride: 32.3% w/w - Mepiquat chloride: 10.6% w/w Manufacture date: October 2019 Expiry date: October 2021 Density: 1.07 g/cm^3 at 20ºC
Test organism:	Rainbow trout (*Oncorhynchus mykiss*) Length: 4.1 – 4.7 cm Fish were held in the laboratory for 9 days before using it for test. Supplier: - For Range Finding test Neveed Habba Kadal Chawk, Srinagar, Jammu and Kashmir – 190001 - For Definitive Test Kaka Aqua and Agro Associates, Himachal Pradesh
Test design:	Static system (96 h of exposure) One replicate of each test item concentration and control 7 fish in each aquarium
Nominal test item concentration:	100 mg/L plus the control
Test conditions:	Temperature of water: 13.1 – 13.8 °C pH of the control: 7.67 – 7.74 Dissolved oxygen concentration in the test item concentration and the control: 90 – 97% ASV Lighting daily cycle: 16 h light: 8 h dark Light intensity: 543 lux TOC: 0.8 mg/L No feeding
Endpoints:	LC_{50}, LOEC and NOEC

Results

The active ingredient concentration analysis showed that the recovery with the nominal concentration was 100.293 % (RSD was 4.345 %) for Chlormequat and 83.626 % (RSD was 4.633 %) for Mepiquat at the start of the test; 95.768 % (RSD was 0.607 %) for Chlormequat and 100.858 % (RSD was 0.810 %) for Mepiquatat the end of the test (97 hour) indicating that the results were within the acceptable limit (80 to

120 % of the nominal concentration with an RSD of < 20%). There were no mortalities of fish in the negative control and at the tested concentration of 100 mg/L.

There were no mortalities of fish in the negative control and at the tested concentration of 100 mg/L.

Table 1. Intoxication symptoms and mortality of fish in test item concentration 100 mg/L – definitive test.

Group No.	Test concentration (mg test item/L)	Parameter		2h	5h	24h	30h	48h	54h	72h	78h	96h
G1	Negative control	Toxic signs		N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)
		Mortality		-	-	-	-	-	-	-	-	-
		Cumulative mortality	No	-	-	-	-	-	-	-	-	
			%	-	-	-	-	-	-	-	-	
G2	100	Toxic signs		N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)	N (7)
		Mortality		-	-	-	-	-	-	-	-	-
		Cumulative mortality	No	-	-	-	-	-	-	-	-	
			%	-	-	-	-	-	-	-	-	

Total number of fish in each group at the start of test: 7; Numbers in parentheses denote the number of fish
N – Normal

Validity criteria

This test was considered valid, since the test had met the following validity criterions:

- There was no mortality in the negative control at the end of the test which is less than 10% at the end of the test.
- The pH of the test solutions was ranged from 7.67 to 7.74 and the temperature of the test solutions was 13.1 to 13.8°C.
- The dissolved oxygen saturation of the test solutions ranged from 90 to 97 % which is more than 60% of the air saturation value throughout the test.
- The mean concentration of the test item in the tested concentrations was between 95.768 and 100.293 % for Chlormequat and 83.626 and 100.858 for Mepiquat of the nominal concentrations which was within ± 20% during the test.

Conclusion

The LC_{50}, LOEC and NOEC value for Chlormequat 345g/L +Mepiquat 115g/L SLat 96 hours was higher than 100 mg test item/L or 32.3 mg Chlormequat/L and 10.6 mg Mepiquat/L based on nominal concentrations.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met: • There was an increase in cell concentration in the negative control culture by a factor of 80.25, 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 27.49 %, which is within the required limit of 35%. • The coefficient of variation of average growth rate between replicate cultures of negative control was 0.19 %, which is within the required limit of 7%. *Raphidocelis subcapitata* **Agreed endpoints:** 72h E_rC_{50} > 100 mg formulation Mepcy (SHA 126085A), equivalent to 32.3 mg chlormequat /L and 10.6 mg mepiquat/L based on nominal concentrations**.** 72h E_yC_{50} > 100 mg formulation Mepcy (SHA 126085A), equivalent to 32.3 mg chlormequat /L and 10.6 mg mepiquat/L based on nominal concentrations**.**

Reference: KCP 10.2.1-02

Report: "Chlormequat 345 g/L + Mepiquat 115 g/L SL: alga, growth inhibition test with *Raphidocelis subcapitata*". Saiqa Nazhath, M. Sc., 2021. Ecotoxicology section analytical R & D department Eurofins Advinus Limited. Study No.: G14215

Guideline(s): OECD Guideline for the Testing of Chemicals No. 201 (2006).

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

Materials and methods

Test item:	Chlormequat 345 g/L +Mepiquat 115 g/L SL Batch number: SCL-99721 Purity: - Chlormequat chloride:32.3%w/w - Mepiquat chloride:10.6%w/w Manufacture date: October 2019 Expiry date: October 2021 Density:1.07 g/cm^3 at 20ºC
Reference substance:	Potassium dichromate.
Test organism:	The unicellular freshwater green algae, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*) SAG 61.81 (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz).
Test design:	72 hours of exposure Six replicates for the test item concentration and six for the control Initial algal cell density: 1 x 10^4 cells/mL.
Nominal test item concentration:	100 mg/L plus the control.
Test conditions:	Temperature: 22.2 – 23.1ºC pH of the control: 7.88 – 7.99 Mean light intensity: 6800 – 7200 lux Constant illumination and shaking Medium: OECD
Chemical determinations:	Validate an Ion Chromatograph method.
Endpoints:	E_rC_{50}, E_yC_{50}, LOEC and NOEC

Results

The active ingredient concentration analysis in all test concentrations showed that the recovery with nominal concentration was 95.904 % (RSD was 0.181 %) at the start and 94.965 % (RSD was 0.907 %) at the end of the test (72 hour) for Chlormequat. Recovery of nominal concentrations was 101.220 % (RSD was 0.708) at the start, and 101.179 % (RSD was 5.770 %) at the end of the test (72 hour) for Mepiquat indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

At test concentration of 100 mg test item /L, the observed growth rate and yield of algal biomass was comparable with the control during the test period.

Observed cells were found morphologically normal in the control and the tested concentration of 100 mg test item /L.

Table 1. Definitive Test-Average Specific Growth Rate - Control

Replicate	Specific Growth rate			Replicate-mean specific growth rate	S.D.	% CV
	0 h- 24 h	24 h - 48 h	48 h - 72 h			
R1	1.83	1.03	1.52	1.46	0.40	27.63
R2	1.83	1.05	1.50	1.46	0.39	26.82
R3	1.83	1.03	1.52	1.46	0.40	27.63
R4	1.83	1.03	1.53	1.46	0.40	27.62
R5	1.83	1.03	1.54	1.47	0.41	27.61
R6	1.83	1.03	1.52	1.46	0.40	27.63
Mean ± SD (% CV)	1.83 ± 0 (0)	1.03 ± 0.01	1.52 ± 0.01	1.46 ± 0(0.19)	-	27.49
Mean coefficient of variation (% CV) for section by section specific growth rates						27.49
The coefficient of variation (% CV) of average specific growth rates during the whole test period in replicate control cultures						0.19

Table 2. Definitive Test- Specific Growth Rate: Treatment Group

Replicate	Specific Growth rate			Replicate-mean specific growth rate	S.D.	% CV
	0 h- 24 h	24 h - 48 h	48 h - 72 h			
R1	1.83	1.03	1.53	1.46	0.40	27.62
R2	1.87	1.01	1.51	1.46	0.43	29.51
R3	1.75	1.14	1.50	1.46	0.31	20.96
R4	1.79	1.09	1.51	1.46	0.35	24.08
R5	1.91	0.97	1.51	1.46	0.47	32.24
R6	1.83	1.06	1.49	1.46	0.39	26.43
Mean ± SD (% CV)	1.83 ± 0.06 (3.09)	1.05 ± 0.06 (5.75)	1.51 ± 0.01 (0.88)	1.46 ± 0 (0.09)	-	26.81

Table 3. Definitive Test- Yield: Control

Group	Test Concen-tration (mg test item/L)	Replicate	Specific Growth rate			Replicate-mean specific growth rate	S.D.	% CV
			0 h- 24 h	24 h - 48 h	48 h - 72 h			
G1	0	R1	5.30	16.50	79.00	33.60	39.71	118.20
		R2	5.30	16.80	79.00	33.70	39.65	117.66
		R3	5.30	16.50	79.00	33.60	39.71	118.20
		R4	5.30	16.50	79.25	33.68	39.86	118.33
		R5	5.30	16.50	80.25	34.02	40.43	118.85
		R6	5.30	16.50	79.00	33.60	39.71	118.20
		Mean ± SD (% CV)	5.3 ± 0 (0)	16.55 ± 0.12 (0.74)	79.25 ± 0.5 (0.63)	33.7 ± 0.16 (0.48)	-	118.24

Table 4. Definitive Test- Yield: Treatment group

Group	Test Concen-tration (mg test item/L)	Replicate	Specific Growth rate			Replicate-mean specific growth rate	S.D.	% CV
			0 h- 24 h	24 h - 48 h	48 h - 72 h			
G2	100	R1	5.25	16.5	79.75	33.83	40.16	118.70
		R2	5.50	16.75	79.25	33.83	39.73	117.44

		R3	4.75	17.00	79.75	33.83	40.23	118.92
		R4	5.00	16.75	79.50	33.75	40.05	118.68
		R5	5.75	16.75	79.25	33.92	39.64	116.88
		R6	5.25	17.00	78.75	33.67	39.48	117.28
		Mean ± SD (% CV)	5.25 ± 0.35 (6.73)	16.79 ± 0.19 (1.12)	79.38 ± 0.38 (0.48)	33.81 ± 0.09 (0.25)	-	117.98

Validity criteria
There was an increase in cell concentration in the negative control culture by a factor of 80.25, 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 27.49 %, which is within the required limit of 35%.

The coefficient of variation of average growth rate between replicate cultures of negative control was 0.19 %, which is within the required limit of 7 %.

Conclusion

The EC_{50}, LOEC and NOEC values for growth rate and yield are higher than 100 mg test item/L or 32.30 mg Chlormequat /L and 10.60 mg Mepiquat /L on the basis of the nominal concentration of the test item.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met: • There was no immobilization of daphnia in the negative control during the test period, which is within the allowed 10 per cent immobilization of daphnids. • The dissolved oxygen concentration at the end of the test was more than ≥ 3 mg/L in negative control and treatment test vessels. *Daphnia magna* **Agreed endpoints:** 48-h EC_{50} > 100 mg formulation Mepcy (SHA 126085A), equivalent to 32.3 mg chlormequat /L and 10.6 mg mepiquat/L based on nominal concentrations**.**

Reference:	KCP 10.2.1-03
Report	"Chlormequat 345 g/L + Mepiquat 115 g/L SL: *Daphnia magna*, Acute immobilisation test". Saiqa Nazhath, M. Sc., 2021. Ecotoxicology section analytical R & D department Eurofins Advinus Limited. Study No.: G14216
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 202 (2004).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Chlormequat 345 g/L +Mepiquat 115 g/L SL
Batch number: SCL-99721
Purity:
- Chlormequat chloride: 32.3% w/w

- Mepiquat chloride: 10.6% w/w

Manufacture date: October 2019
Expiry date: October 2021
Density: 1.07 g/cm^3 at 20ºC

Reference substance: Potassium dichromate.

Test organism: *Daphnia magna* Straus (< 24 h old at exposure initiation)

Test design: Static test (exposure: 48 h)
Four replicates per each test item concentration and the control Five Daphnia magna in each replicate.

Nominal test item concentration: 100 mg/L plus the control.

Test conditions: Temperature: 19.8 – 20.6ºC
pH of the control: 7.81 – 7.90
Dissolved oxygen concentration in the control: 6.7 – 7.0 mg/L Daily cycle: 16 h light: 8 h dark
No feeding
No aeration

Chemical determinations: Validate Chromatograph method.

Endpoints: EC_{50}, LOEC and NOEC

Results

The active ingredient concentration analysis in all test concentrations showed that the recovery with the nominal concentration was 96.945 % (RSD was 0.480%) at the start of the test and 96.315 % at the end of the test (48 hour) (RSD was 0.860 %) for Chlormequat and 85.459 % (RSD was 3.865 %) at the start of the test and 100.723 % at the end of the test (48 hour) (RSD was 0.887 %) for Mepiquat indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

There was no immobilization of daphnia in the negative control and at the limit test concentration of 100 mg/L at 24 and 48 hours of exposure.

Table 1. Immobilisation of *Daphnia magna.*

Group	Treatment (mg test item/L)	No. of Daphnia introduced	Number of immobilised Daphnia magna								Inmobilisation [%]	
			24h				48h				24h	48h
			R1	R2	R3	R4	R1	R2	R3	R4		
G1	Negative control	20	0	0	0	0	0	0	0	0	0	0
G2	100	20	0	0	0	0	0	0	0	0	0	0

Validity criteria
This test was considered valid, because:

- There was no immobilization of daphnia in the negative control during the test period, which is within the allowed 10 per cent immobilization of daphnids.
- The dissolved oxygen concentration at the end of the test was more than ≥ 3 mg/L in negative control and treatment test vessels.

Conclusion

The EC_{50}, LOEC and NOEC value for immobilization of daphnia at 24 and 48 hours are higher than 100 mg test item/L or 32.30 mg Chlormequat /L and 10.60 mg Mepiquat /L on the basis of the nominal concentration of the test item.

Comments of zRMS:	The study was accepted by zRMS. The results are considered valid because the following criteria were satisfied: • The doubling time of frond number in the control was 2.0 days, criterion: less than 2.5 days (the factor of frond number in the control between O and 7 day was 10.9). • The average specific growth rate in the control between day O and day 7 was 0.341 d^{-1} (minimum requirement: higher than 0.275 d^{-1}). **Agreed toxicity endpoints:**

The endpoint values showing the impact of the test item on the growth rate of *Lemna gibba* after 7th days of the exposure period are presented in Table given below.

Endpoint	Test item mg/L (based on nominal concentrations)	Chlormequat[a] mg/L (based on nominal concentrations)	Mepiquat[b] mg/L (based on nominal concentrations)
Growth rate – based on frond number			
E_rC_{10}	**49.56** (41.77-57.00)	**15.96** (13.45 – 18.35)	**5.30** (4.47 – 6.10)
E_rC_{20}	**86.78** (79.27 – 96.52)	**27.94** (25.52 – 31.08)	**9.29** (8.48 – 10.33)
E_rC_{50}	**>100**	**>32.20**	**>10.7**
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68
Growth rate – based on dry weight			
E_rC_{10}	**58.81** (44.66 – 71.56)	**18.94** (14.38 – 23.04)	**6.29** (4.78 – 7.66)
E_rC_{20}	**>100**	**>32.20**	**>10.7**
E_rC_{50}	**>100**	**>32.20**	**>10.7**
NOEC	25	8.05	2.68
LOEC	50	16.10	5.35

*a: based on the content of Chlormequat in the test item, i.e. 32.2(%) w/w

*b: based on the content of Mepiquat in the test item, i.e. 10.7(%) w/w
Density: 1.07 g/cm^3 provided by the sponsor.

The endpoint values showing the impact of the test item on yield of *Lemna gibba* after 7 days of the exposure period are presented in Table given below.

Endpoint	Test item mg/L (based on nominal concentrations)	Chlormequat[a] mg/L (based on nominal concentrations)	Mepiquat[b] mg/L (based on nominal concentrations)
Yield – based on frond number			
E_yC_{10}	**26.06** (19.91 – 33.30)	**8.39** (6.41 – 10.72)	**2.79** (2.13 – 3.56)
E_yC_{20}	**43.76** (36.66 – 50.73)	**14.09** (11.80 – 16.34)	**4.68** (3.92 – 5.43)
E_yC_{50}	**>100**	**>32.20**	**>10.7**
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68
Yield – based on dry weight			
E_yC_{10}	**29.87** (18.81 – 44.14)	**9.62** (6.06 – 14.21)	**3.20** (2.01 – 4.72)
E_yC_{20}	**48.31** (34.74 – 61.48)	**15.56** (11.19 – 19.80)	**5.17** (3.72 – 6.58)
E_yC_{50}	**>100**	**>32.20**	**>10.7**
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68

*a: based on the content of Chlormequat in the test item, i.e. 32.2(%) w/w
*b: based on the content of Mepiquat in the test item, i.e. 10.7(%) w/w
Density: 1.07 g/cm^3 provided by the sponsor.

Reference:	KCP 10.2.1.-04
Report	"Study of *Lemna gibba* growth inhibition with Chlormequat 345 g/L + Mepiquat 115g/L SL". Kanchana, P. 2023. Bioscience Research Foundation. Report number: 13004/2023.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 221 (2006).
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The test was conducted in a static design for 7th days based on the stability analysis. Six concentrations of the test item plus the control were maintained. Each concentration was divided into three replicates with nine initial frond numbers each, whereas the control group was divided into six replicates with nine initial frond numbers each.

The total number of fronds in each test vessel was counted twice during exposure (days 2 and 4) and at exposure termination (day 7). The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time.

No distinctive changes from the normal development of plants in the test item concentrations of 6.25, 12.5, 25 and in the control group, whereas in the test item concentrations of 50 and 100 mg/L spots of chlorosis were observed during the 7th day of experiment.

The results of stability test showed that the concentrations of Chlormequat 345 g/L + Mepiquat 115 g/L SL were stable during 7th days under test conditions. The concentrations of Chlormequat 345 g/L + Mepiquat 115 g/L SL were determined using a validated chromatographic method. Samples of all the test item concentrations and the control collected at exposure initiation (day 0) and at exposure termination (day 7) were chemically determined.

In fresh samples of the test item concentrations collected at exposure initiation, the determined concentrations of Chlormequat 345 g/L were between 98.9 and 101.3% of the nominal concentrations, respectively. The results confirmed that the test item concentrations were prepared correctly. In spent samples of the test item concentrations collected at exposure termination, the determined concentrations of Chlormequat 345 g/L were between 99.0 and 102.1% of the nominal concentrations, respectively.

In fresh samples of the test item concentrations collected at exposure initiation, the determined concentrations of Mepiquat 115 g/L were between 99.1 and 101.6% of the nominal concentrations, respectively. The results confirmed that the test item concentrations were prepared correctly. In spent samples of the test item concentrations collected at exposure termination, the determined concentrations of Mepiquat 115 g/L were between 98.5 and 101. 7% of the nominal concentrations, respectively. Therefore, the concentrations of Chlormequat 345 g/L + Mepiquat 115 g/L SL were stable during 7th days under test conditions.

The endpoint values were determined based on the nominal test item concentrations, and nominal concentrations of Chlormequat 345 g/L + Mepiquat 115 g/L SL in the test item.

Materials and methods

Test item:	Chlormequat 345 g/L + Mepiqüat 115 g/L SL batch no.: SCL- 120820
Active substance concentration:	Chlormequat Chloride: 32.2 (%) w/w Mepiquat Chloride: 10.7 (%) w/w
Test organism:	The freshwater aquatic plant, *Lemna gibba*, culture maintained at BRF test facility. Colonies were cultured for 7-10 days before exposure initiation.
Test design:	Static; 7 days of exposure; three replicates of each test item concentration; six replicates of control.
Normal test item concentrations:	6.25, 12.5, 25, 50 and 100 mg/L plus the control
Test conditions:	Medium: 20X AAP pH of the control: 7.6 – 8.1 average light intensity: 7411 – 8756 lux temperature: 22.1 – 24.7°C
Statistical analysis:	The endpoint values were determined after 7th days of the exposure period by using a Probit analysis in the NCSS (Number Cruncher Statistical System), non-linear regression and LOEC and NOEC was determined by using Tukey's multiple comparison test using Graphpad Prism version 9.5.1
Endpoints:	E_rC_x, E_yC_x, LOEC and NOEC

Results

Table 1. Growth of *Lemna gibba* after 7 days

Endpoint	Test item mg/L (based on nominal concentrations)	Chlormequat[a] mg/L (based on nominal concentrations)	Mepiquat[b] mg/L (based on nominal concentrations)
Growthrate – based on frond number			

E_rC_{10}	49.56	15.96	5.30
E_rC_{20}	86.78	27.94	9.29
E_rC_{50}	>100	>32.20	>10.7
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68
Growthrate – based on dry weight			
E_rC_{10}	58.81	18.94	6.29
E_rC_{20}	>100	>32.20	>10.7
E_rC_{50}	>100	>32.20	>10.7
NOEC	25	8.05	2.68
LOEC	50	16.10	5.35

*a: based on the content of Chlormequat in the test item, i.e. 32.2(%) w/w
*b: based on the content of Mepiquat in the test item, i.e. 10.7(%) w/w

Table 2. Yield of *Lemna gibba* after 7 days

Endpoint	Test item mg/L (based on nominal concentrations)	Chlormequat[a] mg/L (based on nominal concentrations)	Mepiquat[b] mg/L (based on nominal concentrations)
Yield – based on frond number			
E_yC_{10}	26.06	8.39	2.79
E_yC_{20}	43.76	14.09	4.68
E_yC_{50}	>100	>32.20	>10.7
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68
Yield – based on dry weight			
E_yC_{10}	29.87	9.62	3.20
E_yC_{20}	48.31	15.56	5.17
E_yC_{50}	>100	>32.20	>10.7
NOEC	12.5	4.03	1.34
LOEC	25	8.05	2.68

*a: based on the content of Chlormequat in the test item, i.e. 32.2(%) w/w
*b: based on the content of Mepiquat in the test item, i.e. 10.7(%) w/w

Validity criteria
The results are considered valid because the following criteria were met:

- The doubling time of frond number in the control was 2.0 d, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 10.9).
- The average specific growth rate in the control between day 0 and day 7 was 0.341 d^{-1} (minimum requirement: higher than 0.275 d^{-1}).

Conclusions
The results obtained for the growth rate based on the frond number led to the following conclusions:

The concentration of Chlormequat 345 g/L + Mepiquat 115 g/L SL causing a 50% inhibition of Lemna gibba growth rate based on the frond number within the exposure period (ErC50) is equal to > 100 mg/L, i.e. >32.20 mg Chlormequat/L (based on the content in the test item) and > 10.7 mg Mepiquat/L (based on the content in the test item).

The highest concentration at which Chlormequat 345 g/L + Mepiquat 115 g/L SL is observed to have no statistically significant effects on the growth rate of Lemna gibba based on the frond number (NOEC) is 12.5 mg/L, i.e. 4.03 mg Chlormequat/L (based on the content in the test item) and 1.34 mg Mepiquat/L (based on the content in the test

item).

The results obtained for the growth rate based on the dry weight led to the following conclusions:

The concentration of Chlormequat 345 g/L + Mepiquat 115 g/L SL causing a 50% inhibition of Lemna gibba growth rate based on the dry weight within the exposure period (ErC50) is equal to >100 mg/L, ie. >32.20 mg Chlormequat/L (based on the content in the test item) and >10.7 mg Mepiquat/L (based on the content in the test item).

The highest concentration at which Chlormequat 345 g/L + Mepiquat 115 g/L SL is observed to have no statistically significant effects on the yield of Lemna gibba based on the frond number (NOEC) is 12.5 mg/L, i.e. 4.03 mg Chlormequat/L (based on the content in the test ítem) and 1.34 mg Mepiquat/L(based on the content in the test item).

The results obtained for the yield based on the frond number led to the following conclusions:

The concentration of Chlormequat 345 g/L + Mepiquat 115 g/L SL causing a 50% inhibition of *Lemna gibba* yield based on the frond number within the exposure period (EyC50) is >100 mg/L, i.e. >32.20 mg Chlormequat/L (based on the content inthe test item) and >10.7 mg Mepiquat/L (based on the content in the test item).

The highest concentration at which Chlormequat 345 g/L + Mepiquat 115 g/L SL is observed to have no statistically significant effects on the yield of *Lemna gibba* based on the frond number (NOEC) is 12.5 mglL, i.e. 4.03 mg Chlormequat/L(based on the content in the test ítem) and 1.34 mg Mepiquat/L(based on the content in the test item).

The results obtained for the yield based on the dry weight led to the following conclusions:

The concentration of Chlormequat 345 g/L + Mepiquat 115 g/L SL causing a 50% inhibition of *Lemna gibba* yield based on the dry weight within the exposure period (EyC50) is equal to >100 mglL, i.e. >32.20 mg Chlormequat/L (based on the content in the test item) and >10.7 mg Mepiquat/L (based on the content in the test item).

The highest concentration at which Chlormequat 345 g/L + Mepiquat 115 g/L SL is observed to have no statistically significant effects on the yield of *Lemna gibba* based on the dry weight (NOEC) is 12.5 mg/L, i.e. 4.03 mg Chlormequat/L (based on the content in the test item) and 1.34 mg Mepiquat/L (based on the content in the test item).

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met: • No mortality was observed in control and is within the specified 10 percent limit at the end of the test. • The LD_{50} of the toxic standard, dimethoate at 24-h and 48h was 0.17 µg a.i./bee and 0.15 µg a.i./bee which met the reported range of 0.10 to 0.30 µg a.i./bee. *Apis mellifera* **Agreed endpoints:** 48-h LD_{50} > 100 µg formulation Mepcy (SHA 126085A)/bee

Reference: KCP 10.3.1.1.1

Report "Chlormequat 345 g/L + Mepiquat 115 g/L SL: Acute Oral Toxicity Test in Honey Bees". Likith Nidasale Girish, 2020, G14217. Eurofins Advinus Limited

Guideline(s): Yes, OECD Guideline No. 213 (1998)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) Yes

Materials and methods

Test item:

Description:	Chlormequat 345 g/L + Mepiquat 115 g/L SL
Production batch:	SCL - 99721
A.i. content:	Chlormequat chloride 32.3% w/w Mepiquat chloride 10.6% w/w

Test system:

Species:	*Apis mellifera*
Strain:	-
Age:	Active adult foraging workers
Average weight:	-
Average length:	-
Source:	A colony maintained at Eurofins Advinus Limited, India
Acclimation period:	Bees were acclimatized for a period of 18 h 20 minutes
Diet:	50% (w/v) aqueous sucrose solution

Experimental conditions:

Temperature:	23.2 – 24.7°C
Humidity:	65 – 68%
Hardness:	-
pH:	-
Light and photoperiod:	24h darkness (except during observations).
Loading:	-
Test procedure:	For the oral toxicity test, the test substance was added to a 50% w/v sucrose solution reaching the concentration of 100 µg a.i./bee. Feeders were filled with the di-

lution. Bees were kept unfed for approximately 2 hours. The bees were then fed for 4 hours and observed on a period of 48 hours.

Experimental period: 48h

Test design and treatment

Stainless steel cages with an opening on each side to allow the feeding with glass tubes. The bees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
A preliminary test was conducted with doses of 0.1, 1, 10, 50 and 100 µg a.i./bee with three replicates each. According to the results, the following nominal test item concentrations were used: 100 µg a.i./bee in the oral test. Each treatment group had 3 replicates with 10 bees per replicate.
The mortality data was analysed with the statistical method of Probit analysis using an in-house developed and validated computer program. The dose response curves were plotted and the LD_{50} calculation was calculated using a validated computer program.

Results

There was no mortality and behavioral changes of the bees observed in the control group during 4, 24 and 48 h post-treatment.
There was no mortality and behavioral changes of the bees at the tested concentration of 100 µg a.i./bee at 4, 24 and 48 h post-treatment.
The percent mortalities for the toxic standard, Dimethoate at 48h post-treatment were 16.67, 66.67 and 90.0% at the tested concentrations of 0.075, 0.15 and 0.30 µg a.i./bee respectively.
The condition of the test system was observed at 4, 24 and 48 hours after treatment. The following table shows the endpoints.

Oral toxicity test results

Endpoint	Nominal concentration of test item (µg/bee)	~~Endpoints based on active ingredients (µg a.i./bee)~~	
LD_{50}	> 100	~~> 100~~	~~Chlormequat chloride 32.3%~~
		~~> 100~~	~~Mepiquat chloride 10.6%~~

Conclusion

The LD_{50} value of the test item, Chlormequat 345 g/L + Mepiquat 115 g/L SL at 48 h is higher than 100 µg ~~a.i.~~ formulation/bee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met: • No mortality was observed in control and is within the specified 10 percent limit at the end of the test. • The LD_{50} of the toxic standard, dimethoate at 24-h and 48h was 0.14 µg a.i./bee and 0.13 µg a.i./bee which met the reported range of 0.10 to 0.35 µg a.i./bee. *Apis mellifera* **Agreed endpoints:** 48-h LD_{50} > 100 µg formulation Mepcy (SHA 126085A)/bee

Reference: KCP 10.3.1.1.2

Report "Chlormequat 345 g/L + Mepiquat 115 g/L SL: Acute Contact Toxicity Test in Honey Bees". Likith Nidasae Girish, 2020, G14218. Eurofins Advinus

Limited

Guideline(s): Yes, OECD Guideline No. 214 (1998)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item:

Description:	Chlormequat 345 g/L + Mepiquat 115 g/L SL
Production batch:	SCL - 99721
A.i. content:	Chlormequat chloride 32.3% w/w Mepiquat 10.6% w/w

Test system:

Species:	*Apis mellifera*
Strain:	
Age:	Active adult foraging workers
Average weight:	-
Average length:	-
Source:	A colony maintained at Eurofins Advinus Limited, India
Acclimation period:	20 hours
Diet:	50% (v/v) honey and sugar solution

Experimental conditions:

Temperature:	23.1 – 24.8°C
Humidity:	64 – 67%
Hardness:	-
pH:	-
Light and photoperiod:	24h darkness (except during observations).
Loading:	-
Test procedure:	The honeybees were anaesthetized with carbon dioxide, transferred to plastic trays and dosed on the dorsal side of the thorax with 1 µl of test solution containing the test substance or reference substance.

Experimental period: 48h

Test design and treatment

Stainless steel cages with an opening on each side to allow the feeding with glass tubes.
A preliminary test was done at the dose of 0.1, 1.0, 10.0, 50.0 and 100 µg a.i./bee. Each treatment group had 3 replicates with 10 bees per replicate. According to the results, the following nominal test item concentrations were used: 100.0 µg a.i./bee. Each treatment group had 3 replicates with 10 bees per replicate. The honeybees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
The mortality data was analysed with the statistical method of Probit analysis using an in-house developed and validated computer program. The dose response curves were plotted at observation time (24 and 48 h) and the sopes of the curves and the LD_{50} was calculated using a validated computer pro-

gram.

Results

There was no mortality and behavioral changes of bees observed in the control and at the tested concentrations of 100 µg a.i./bee at 4, 24 and 28 hours post-treatment.
The percent mortalities for the toxic standard, Dimethoate at 48h post-treatment were 23.33, 60.00 and 83.33 at the tested concentrations of 0.075, 0.15, 0.30 µg a.i./bee respectively.
The condition of the test system was observed at 4, 24 and 48 hours after treatment. The following table shows the endpoints.

Contact toxicity test results

Endpoint	Nominal concentration of test item (µg/bee)	~~Endpoints based on active ingredients (µg a.i./bee)~~	
LD_{50}	> 100	~~> 100~~	~~Chlormequat 32.3%~~
		~~> 100~~	~~Mepiquat chloride 10.6%~~

Conclusion

The LD_{50} value of the test item, Chlormequat 345 g/L + Mepiquat 115 g/L SL at 48 h is higher than 100 µg ~~a.i.~~ formulation/bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS: The study was accepted by zRMS.

Validity criteria

- There was no mortality in control group.
- The average mortality in the reference substance treated group is 76.67 % at the end of the test [(Test Guideline criteria: should be ≥50% at the end of the test (10 days following start of exposure)]

Agreed toxicity endpoints:

Initial		Consumed		No. of tested bees	Mortality total		LC_{50}	LDD_{50}
Concentration [mg/kg of food]	Dose [µg/20mg/ bee/day]	Concentration [mg/kg of food]	Dose [µg/bee/ day]		No. of bees[%]		[mg/kg]	[µg/bee/ day]
Chlormequat 345 g/l + Mepiquat 115 g/l SL								
Control				30	0	0.00	3033.77 (2941.31 – 3126.23)	63.12 (61.35 – 64.89)
1301.5	26.0	1301.50	29.81	30	1	3.33		
1822.2	36.4	1822.20	39.62	30	4	13.33		
2551.0	51.0	2551.00	52.85	30	11	36.67		
3571.4	71.4	3571.40	73.58	30	16	53.33		
5000	100	5000.00	100.87	30	28	93.33		
Dimethoate								
0.8 mg a.i./kg	0.016 µg a.i./bee	0.8 mg a.i/kg	0.01 µg a.i./bee	30	23	76.67	Not determined	
Chlormequat 345 g/l + Mepiquat 115 g/l SL	NOEC [mg/kg]			1822.2				
	NOEDD [µg/bee/ day]			39.62				

Reference: KCP 10.3.1.2-01

Report: "Chronic Oral Toxicity Study of Chlormequat 345 g/L + Mepiquat 115 g/L SL on adult honey bee (*Apis mellifera*)". Lakshmi Prabha, K. 2023. Biosci-

	ence Research Foundation. Report No. 11510/2022.
Guideline(s):	OECD test No. 245 Guideline for the Testing of Chemicals: Honey bee (*Apis mellifera* L.), Chronic Oral Toxicity Test – 10 Day Feeding (9 October 2017).
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Initially, a range finding study was conducted for Chlormequat 345 g/L + Mepiquat 115 g/L SL with 1, 10, 50, 100 and 200 µg/bee in sucrose solution (50% w/v) to determine the concentrations for the Dose Response Test. Honey bees were acclimatized in the test unit for 24 hours before treating with test substance at different concentrations. A concurrent control group (50% w/v sucrose solution) was also maintained. After 24 h of treatment, the treated diet was withdrawn from the respective treatment groups, feeder tube was weighed and replaced with freshly prepared treated diet.

The Cumulative mortality observed at the end of the 10 th day of exposure was 0.00, 0.00, 3.33, 33.33, 93.33 and 100.00% in bees exposed to control, 1, 10, 50, 100 and 200 µg/bee respectively.

Based on the results of range finding study, the Dose Response Test was conducted and no deviation was observed in range finding study. Honeybees were acclimatized for 24 hours.
The bees were orally treated with control, 26.0, 36.4, 51.0, 71.4 and 100 µg/bee concentrations in 50% w/v sucrose solution. A concurrent control group with 50% w/v sucrose solution was also maintained.

Materials and methods

Name:	Chlormequat 345 g/1 + Mepiquat 115 g/1 SL
Batch number:	SCL-120820
Species:	*Apis mellifera* (L) strain carnica
Source:	Bee hive maintained at BRF test facility
Stage:	2 days old young adult bee
Dose Response Test:	Untreated control The test item: 5 concentrations: 1301.5, 1822.2, 2551, 3571.4 and 5000 food corresponding to daily doses of 26.0, 36.4, 51.0, 71.4 and 100 µg/bee/day (nominal, assuming daily uptake of lg feeding solution/ bee/day). Consumed dose: 29.81, 39.62, 52.85, 73.58 and 100.87 µg/bee/day.
Number of adult bees:	100 bees/replicate 3 replicates/concentration
Duration:	10 days

Endpoints: LC_{50}, LDD_{50}, NOEC. And NOEDD

Statistical analysis: NCSS 2000, statistical software (Number Cruncher Statistical System).

Results and discussions

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

Honey bees were acclimatized for 24 hours. The bees were orally treated with control, 26.0, 36.4, 51.0, 71.4 and 100 µg/bee concentrations in 50% w/v sucrose solution. A concurrent control group with 50% w/v sucrose solution was also maintained. The average feed consumption recorded for 10 days of treatment with test substance was 29.81, 39.62, 52.85, 73.58 and 100.87 µg/bee corresponding to the nominal dose 26.0, 36.4, 51.0, 71.4 and 100 µg/bee respectively.

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

- On day 1 to 4, toxicity signs (affected) were observed in bees exposed to 71.4 and 100 µg /bee whereas bees exposed to control, 26.0, 36.4 and 51.0 µg/bee appeared normal.
- On day 5 to 7 toxicity signs (affected) was observed in bees exposed to 36.4, 51.0, 71.4 and 100 µg /bee whereas bees exposed to control and 26.0 µg/bee appeared normal.
- On day 8 to 10, toxicity signs (affected) were observed in bees exposed to 26.0, 36.4, 51.0, 71.4 and 100 µg/bee whereas bees exposed to control appeared normal.

Table 1: The chronic oral toxicity study of the test item on honeybees (*Apis mellifera* L.) in the laboratory test is summarized below.

Initial		Consumed		No. of tested bees	Mortality		LC_{50}	LDD_{50}
Concentration [mg/kg of food]	Dose [µg/20 mg/bee / day]	Concentration [mg/kg of food]	Dose [µg/20 mg/bee/ day]		Total No. of bees	[%]	[mg/ kg]	[µg/ /bee/ day]
Chlormequat 345 g/L + Mepiquat 115 g/L SL								
Control				30	0	0.00	3033.77 (2941.31 - 3126.23)	63.12 (61.35-64.89)
1301.5	26	1301.50	29.81	30	1	3.33		
1822.2	36.4	1822.20	39.62	30	4	13.33		
2551	51	2551.00	52.85	30	11	36.67		
3571.4	71.4	3571.40	73.58	30	16	53.33		
5000	100	5000.00	100.87	30	28	93.33		
Dimethoate								
0.8 mg a.i./kg	0.016 µg a.i./bee	0.8 mg a.i./kg	0.01 µg a.i./bee	30	23	76.67	Not determined	
Chlormequat 345 g/L + Mepiquat 115 g/L SL	NOEC [mg/kg]				1822.2			
	NOEDD [µg/bee/ day]				39.62			

Validity criteria

- There was no mortality in control group.
- The average mortality in the reference substance treated group is 76.67 % at the end of the test [(Test Guideline criteria: should be ≥50% at the end of the test (10 days following start of exposure)]

-

Conclusion

Based on feed consumption (test item intake) LDD_{50} was calculated to be 63.12 µg/bee i.e., 19.01 µg Chlormequat /bee and 6.31 µg Mepiquat /bee,

LC_{50} was determined as 3033.77 mg/kg food i.e., 913.53 mg Chlormequat/kg food and 303.38 mg Mepiquat /kg food,

The NOEC was determined as 1822.2 mg /kg i.e., 548.70 mg Chlormequat/kg food and 182.22 mg Mepiquat /kg food,

NOEDD was detennined as 39.62 µg/bee i.e., 11.93 µg Chlormequat /bee and 3.96 µg Mepiquat/bee when exposed to bees (Apis mellifera) for 10 days under experimental conditions.

Reference substance study: The mortality of reference substance 0.8 mg a.i/kg was found to be 76.67% between the stipulated range of 0.5 - 1.0 mg a.i./kg for 10 days exposure on *Apis mellifera*.

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

Comments of zRMS:	The study was accepted by zRMS. **Validity criteria:** - Larval mortality in the control: In control (A1), the cumulative larval mortality from D3 to D8 was 8.33% (Criterion: should be ≤15% across all control replicates). - Adult emergence rate: In control (A1), the adult emergence rate on D22 was 86.11% respectively. (Criterion: should be ≥ 70% across all control replicates). - Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 69.44% (Criterion: should be > 50% across all reference replicates). **Agreed toxicity endpoints:**

Conclusion

		Up to D22		
Test item doses	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on content (µg Chlormequat[a] /larva)**	**Value based on content (µg Mepiquat[b] /larva)**
	ED_{10}	71.73 (68.34 – 75.13)	21.59 (20.66 – 22.51)	7.17 (6.86 – 7.48)
	ED_{20}	85.47 (82.34 – 88.59)	25.72 (24.79 – 1.86)	8.55 (8.24 – 0.62)
	ED_{50}	119.48 (116.40 – 122.56)	35.96 (35.03 – 36.88)	11.95 (11.64 – 12.26)
	NOED	70.0	21.07	7.0
Test item Concentrations	**Endpoint with 95% CL**	**Value based on Nominal concentrations (mg/kg food)**	**Value based on content (mg Chlormequat[a] /larva)**	**Value based on content (mg Mepiquat[b] /larva)**
	EC_{10}	466.47 (444.37 – 488.57)	140.38 (846.58 – 146.41)	46.65 (93.48 – 48.65)
	EC_{20}	555.73 (535.39 – 576.07)	167.24 (161.21 – 173.27)	55.57 (53.57 – 57.58)
	EC_{50}	776.85 (756.81 – 796.89)	233.78 (227.75 – 239.81)	77.69 (75.68 – 79.69)
	NOEC	455.2	136.99	45.52

a: based on the content of Chlormequat, i.e., 32.2% w/w
b:based on the content of Mepiquat, i.e.10.7% w/w and 1.07 g/cm^3 density given by the sponsor

Reference:	KCP 10.3.1.3-01
Report	"Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure". Lakshmi Prabha, K. 2023. Bioscience Research Foundation. Report No. 11511/2022
Guideline(s):	OECD Guideline for the testing of chemicals, No. 239 (2016): Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat 345g/L + Mepiquat l 15g/L SL Batch No.: SCL-120820
Test system:	Species: Honey bee - *Apis mellifera* (L.); Hymenoptera, (*Apoidea*) (strain: carnica) Source: Bee hive maintained at BRF test facility Stage: First instalar larvae (one day old) during grafting of queen-right colonies in good health conditions.
Dose response test:	Control, 70.0, 91.0, 118.3, 153.8 and 200 µg/larvae
Number of adult bees:	12 bees/ replicate 3 replicates 5 concentrations
Duration:	22 days
Endpoints:	ED_{10}, ED_{20} and ED_{50}, NOED and NOEC
Statistical analysis:	The NOED and NOEC was determined by one-way ANOVA test using Graph Pad Prism 8.2. The ED_{10}, ED_{20} and ED_{50} of adult emergence (D22) was determined by probit analysis using NCSS-2000.

Results and discussions

On D8, larval mortalities of 5.56% was observed in control group. Pupal mortality (between D8 and D15) was 8.33% in the control group. The control group showed a total mortality of 11.11% (A1) on D22.

In the test item group 104.1, 145.8, 204.1, 285.7 and 400 µg/larvae, larval mortalities on D8 was 8.82, 8.82, 14.71, 14.71 and 47.06%. Pupal mortalities 2.86, 5.71, 8.57, 8.57 and 11.43% in the test item treatment groups. Total mortalities at D22 was 18.75, 21.88, 25.00, 37.50 and 75.00%.

Mortality in the reference (R1) was 55.88% across all replicates on D8, Pupal mortality was D(15) 14.29%. Total mortalities at D22 was 87.50% respectively.

Table 1: Toxicity of Chlormequat 345 g/L + Mepiquat 115 g/L SL to larvae of *Apis mellifera L.*

Treatment group	Test solution (ID)	Dose (µg/larva)	Conc. (mg/kg food)	On D8			On D15		On D 22		
				Larval mortality D3 to D8		Mean (OO)	Pupal stage D8 to D15		Total mortality D3-D22		Adult emergence rate %
				mor (%)	corr (%)	(%)	mor (%)	corr (%)	mor (%)	corr (%)	(%)
Control	A1	-	-	8.33	-	0	2.78	0	13.89	0	86.11
Test item	T1	70.0	455.2	8.33	0.00	0	5.56	2.86	22.22	9.68	77.78
	T2	91.0	591.7	22.22	15.15	0	8.33	5.71	38.89	29.03	61.11
	T3	118.3	769.2	36.11	30.30	0	8.33	5.71	52.78	45.16	47.22
	T4	153.8	1000	52.78	48.48	0	8.33	5.71	69.44	64.52	30.56
	T5	200	1300	72.22	69.70	0	13.89	11.43	97.22	96.77	2.78
Ref. item	R1	7.39 µg a.i./larva	48 mg a.i./kg	63.89	60.61	0	8.33	5.71	88.89	87.10	11.11

Note:D-Day, Mor- Mortality, corT.-Corrected Mortality, OO-Other observationResults are averages based on 3 replicates, containing 12 larvae each;
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test item and reference item were corrected by A1;
negative values are set to "0"; calculations are performed with non-rounded values; CL. confidence limit
OO: Other observations (e. g. remaining food)
1 Average% of pupal mortality was calculated according to the following formula:
Sum of dead between D8 and D22 / Sum of living larvae on D8 x 100%
2 Adult emergence [%] = 100 [%] – Mortality of D22 [%]

		Up to D22		
Test item doses	Endpoint with 95% CL	Value based on Nominal Dose (µg/larva)	Value based on Chlormequat[a] content (µg a.s./larva)	Value based on Mepiquat[b] content (µg a.s./larva)
	ED_{10}	71.73	21.59	7.17
	ED_{20}	85.47	25.72	8.55
	ED_{50}	119.48	35.96	11.95
	NOED	70.0	21.07	7.0
Test item concentrations	Endpoint with 95% CL	Value based on Nominal Dose (µg/larva)	Value based on Chlormequat[a] content (µg a.s./larva)	Value based on Mepiquat[b] content (µg a.s./larva)
	EC_{10}	466.47	140.38	46.65
	EC_{20}	55.73	167.24	55.57
	EC_{50}	776.85	233.78	77.69
	NOEC	455.2	136.99	45.52

a: based on the content of Chlormequat, i.e:, 32.2% w/w
b: based on the content of Mepiquat, i.e.10.7% w/w and 1.07 glcm3 density given by the sponsor

Validity criteria

- Larval mortality in the control: In control (A1), the cumulative larval mortality from D3 to D8 was 8.33% (Criterion: should be ≤15% across all control replicates).

- Adult emergence rate: In control (A1), the adult emergence rate on D22 was 86.11% respectively. (Criterion: should be ≥ 70% across all control replicates).

- Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 69.44% (Criterion: should be > 50% across all reference replicates).

Conclusion

Under the laboratory test conditions, honeybee larvae (Apis mellifera L.) were repeatedly exposed to Chlormequat 345 g/1 + Mepiquat 115 g/1 SL for 22 days.

All the validity criteria were rnet (curnulative rnortality in control and reference treatrnent groups and adult ernergence in the control).

In a repeated exposure larval toxicity study with Chlormequat 345 g/L + Mepiquat 115 g/L SL, the ED_{50} was calculated to be 119.48 µg/larva i.e., 35.96 µg Chlormequat /larva and 11.95 µg Mepiquat/larva which is equivalent to an EC 50 of 776.85 mg/kg diet i.e., 233.78 mg Chlormequat/kg diet and 77.69 mg Mepiquat/kg diet.

The ED_{10} was calcuiated to be 71.73 µ,g/larva i.e., 21.59 µ,g Chlormequat/larva and 7.17 µg Mepiquat/larva which is equivalent to an EC 10 of 466.47 mg/kg diet i.e., 140.38 mg Chlormequat/kg diet and 46.65 mg Mepiquat/kg of diet.

The ED_{20} was calculated to be 85.47 µg/larva i.e., 25.72 µg Chlormequat/larva and 8.55 µg Mepiquat/larva) which is equivaient to an EC20 of 555.73 mg/kg, 167.24 mg Chlormequat/kg diet and 55.57 mg Mepiquat/kg diet.

The NOED was calcuiated to be 70.0 µg/larva i.e., 21.07 µg Chlormequat/larva and 7.00 µg Mepiquat/larva the corresponding NOEC was 455.2 mg/kg diet i.e., 136.99 mg Chlormequat/kg diet and 45.52 mg Mepiquat/kg diet.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.4.1.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study was accepted by zRMS. **Validity criteria:** The following validity criteria were met during the study: - mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%) - mortality of the mites exposed to the reference item at the rate of 16.0 L/ha was 87% on day 7 of exposure (criterion: from 50 to 100%) - the mean number of eggs per female in the control group was 5.61 (required: ≥ 4 eggs per female). **Agreed toxicity endpoints:**

Study group (application rate) (kg test item/ha)	Parameter (endpoint)				
	Mortality after 7 days		Reproduction		
	Total (%)	LR_{50}	Mean no. of eggs/ female (Rr)	Reproduction reduction Pr [%]	ER_{50}
Control	0	-	5.61	-	-
Chlormequat 345g/l + Mepiquat 115g/l SL					
1.00	5	**8.93 kg/ha** (2875^a + 956^b g/ha)	5.19	7.63	**8.61 kg/ha** (2772^a + 921^b g/ha)
2.00	7		4.82^+	14.20^+	
4.00	25^+		3.58^+	36.24^+	
8.00	46^+		2.85^+	45.09^+	
16.00	70^+		-	-	
$NOER_{mortality}$		**2.00 kg/ha** (644^a + 214^b g/ha)	$NOER_{reproduction}$		**1.00 kg/ha** (322^a + 107^b g/ha)
ROGOR	Mortality after 7 days				
16.0 mL/ha	87%				

+: statistically significant differences at $p < 0.0001$ compared to control
-: the reproduction was not determined due to the mortality was higher than 50% in comparison with the control group
a: based on the **Chlormequat** content in the test item, i.e. 32.2 % w/w
b: based on the **Mepiquat** content in the test item, i.e. 10.7% w/w , provided by the Sponsor

Reference:	KCP 10.3.2.1-01
Report	"A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115g/l SL on the predatory mite, *Typhlodromus pyri* (Scheuten)". Parkavi, B. 2023. Bioscience Research Foundation. Report No.: 13006/2023
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115g/I SL mortality and reproduction of the predatory mite, *T pyri* (Sch.) was conducted for Sharda Cropchem Ltd., India at Bio-science Research Foundation according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, and the study plan.

The study was carried out based on the sponsor recommended rates for the test item as the definitive test. There were 1.00, 2.00, 4.00, 8.00 and 16.00 kg/ha of the test item/ha. A 24 hours old (protonymphal stage) of predatory mi tes *T pyri* were exposed to the test item applied to discs and fed with pine pollen (*Pinus* sp.) during the experimental period.

Mortality was observed after 7 days of post treatment of the test item. Observations of reproduction in the control and other groups treated with the test item were made after 8, 11 and 14 days post treatment of the test item.

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

To verify the sensitivity of the mites and the precision of the test procedure, the insecticide, ROGOR (30% dimethoate) was used as a reference item. The rate of the reference item was 16.0 mL/ha (4.8 g a.i./ha). The control group was treated with distilled water.

Materials and methods

Test item:	Chlormequat 345g/l + Mepiquat 115 g/l SL batch number: SCL-120820 production date: 06.07.2022 expiry date: 05.06.2024
Biological test:	The predatory mite, *Typhlodromus pyri* (Scheuten) (Acari: *Phytoseiidae*) Age: 24-hour-old protonymphs Source: BRF Insectary
Experimental design:	7 study groups: - 1 control - 5 treatments - 1 reference item Number of protonymphs per treatment/replicate: 100/20 Number of replicates: 5
Test conditions:	Temperature: 23.5 – 25.9ºC Relative air humidity: 66 – 85% Photoperiod: 16 hours light: 8 hours dark Light intensity: 915-1110 lux
Statistical analysis:	The endpoint values for mortality and reproduction were determined by using Probit analysis in the NCSS (Number Cruncher Statistical System), one-way ANOVA and Non linear regression using Graphpad Prism. The means and standard deviations were calculated using Excel sheets.
Endpoints:	– LR_{50} and $NOER_{mortality}$ – ER_{50} and $NOER_{reproduction}$

Results

The effects of Chlormequat 345g/l + Mepiquat 115g/l SL on mortality and reproduction of *Typhlodromus pyri* in a laboratory test are summarized in Table below.

Study group (application rate) (kg test item/ha)	Parameter (endpoint)				
	Mortality after 7 days		Reproduction		
	Total (%)	LR_{50}	Mean no. of eggs/female (Rr)	Reproduction reduction Pr [%]	ER_{50}
Control	0	-	5.61	-	-
Chlormequat 345 g/l + Mepiquat 115 g/l SL					
1.00	5	**8.93 kg/ha (2875[a] + 959[b] g/ha)**	5.19	7.63	**8.61 kg/ha (2772[a] + 921[b] g/ha)**
2.00	7		4.82+	14.20+	
4.00	25+		3.58+	36.24+	
8.00	46+		2.85+	45.09+	
16.00	70+		-	-	
$NOER_{mortality}$		**2 kg/ha (644[a] + 214[b] g/ha)**	**$NOER_{reproduction}$**		**1 kg/ha (322[a] + 107[b] g/ha)**
ROGOR	**Mortality after 7 days**				
16.0 mL/ha	87%				

a: based on the Chlormequat content in the test item, i.e. 32.2% w/w

b: based on the Mepiquat content in the test item, i.e. 10.7% w/w, provided by the Sponsor

+: statistically significant differences at $p<0.0001$ compared to control

-: the reproduction was not determined due to the mortality was higher than 50% in comparison with the control group

Mortality of the control group after 7 days of exposure was 0%. After 7 days of exposure to Chlormequat 345g/I + Mepiquat 115g/I SL at rates of 1.00, 2.00, 4.00, 8.00 and 16 kg/ha, the percentages of *T. pyri* mortalities were 5, 7, 25, 46 and 50%, respectively. There were no statistically significant differences in mortality between group treated with the Chlormequat 345g/l + Mepiquat 115g/I SL rates of 1.00, 2.00 kg/ha and the control group.

On the basis of the obtained mortality results, the LR50 value was 8.93 kg Chlormequat 345g/I + Mepiquat 115g/l SL/ha i.e., 2875 g Chlormequat/ha + 956 g Mepiquat /ha. The NOERmortality value is equal to 2.00 kg Chlormequat 345g/l + Mepiquat 115g/l SL /ha i.e., 644 g Chlormequat/ha + 214 g Mepiquat /ha.

For the reference item ROGOR (Dimethoate 30% EC), the mortality of mites after 7 days of exposure at the rate of 16.0 mL/ha, was 87%, hence the criterion specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.

The mean reproduction rate (Rr) in the control group was 5.61 eggs/female after 14 days, whereas in the group treated with Chlormequat 345g/l + Mepiquat 115g/l SL at rates of 1.00, 2.00, 4.00 and 8.00 kg/ha was 5.19, 4.82, 3.58 and 2.85, respectively. Reduction in reproduction (Pr) in the group treated with Chlormequat 345g/l + Mepiquat 115g/l SL at rates of 1.00, 2.00, 4.00 and 8.00 kg/ha was 7.63, 14.20, 36.24 and 45.09 % respectively.

There was no statistically significant differences in reproduction between group treated with the test item at the rates of 1.00 kg /ha and the control group.

On the basis of the obtained reproduction results, the ER50value is 8.61 kg Chlormequat 345g/l + Mepi-

quat 115g/l SL /ha i.e., 2772 g Chlormequat/ha + 921 g Mepiquat /ha .The NOERreproduction value is equal to 1.00 kg Chlormequat 345g/l + Mepiquat 115 g/l SL ha i.e., 322 g Chlormequat/ha + 107 g Mepiquat /ha.

Validity criteria

The following validity criteria were met during the study:

- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%)
- mortality of the mites exposed to the reference item at the rate of 16.0 L/ha was 87% on day 7 of exposure (criterion: from 50 to 100%)
- the mean number of eggs per female in the control group was 5.61 (required: ≥ 4 eggs per female).

Conclusion

On the basis of the obtained results, it can be concluded that Chlormequat 345g/l + Mepiquat 115 g/1 SL had no adverse effects on mortality and reproduction of the predatory mite, *T pyri* at the rates of 1.00 and 2.00 kg/ha for mortality and 1.00 kg/ha far reproduction.

Comments of zRMS:

The study was accepted by zRMS.

Validity criteria:

The following validity criteria were met during the study:

- after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- After 48 hours, mortality of the group treated with the reference item at the rate of 0.4 mL/ha was 82.50% (criterion: a minimum of 50%),
- All wasps in control survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- The mean number of mummies per female in the control group was 13 .3 (criterion: a minimum of 5.0 mummies/female),
- All wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring)

Agreed toxicity endpoints:

Study group (application rate) (kg test item/ha)	Parameter (endpoint)				
	Mortality after 48 h		Reproduction		
	Total (%)	LR_{50}	Mean no. of mummies/ female	Fecundity reduction Pr [%]	ER_{50}
Control	-	-	13.3	-	-
Chlormequat 345g/l + Mepiquat 115g/l SL					
1	2.50	8.79 kg/ha (2830g[a] + 940g[b] a.i/ha)	12.9	3.01	8.61 kg/ha (2772g[a] + 921g[b] a.i/ha)
2	5.00		11.9	10.53+	
4	17.50+		9.9	25.56+	
8	32.50+		7.1	46.62+	
16	85.00+		-*	-*	
$NOER_{mortality}$		2 kg/ha (644g[a] + 214g[b] a.i/ha)	$NOER_{fecundity}$		1 kg/ha (322g[a] + 107g[b] a.i/ha)

ROGOR	Mortality after 48 h
0.4 mL/ha	82.50%

+: statistically significant differences at $p < 0.05$
a: based on the **Chlormequat** content in the test item, i.e. 32.2 % w/w
b: based on the **Mepiquat** content in the test item, i.e. 10.7 % w/w, provided by the Sponsor
*: Due to more than 50% mortality in mortality phase; reproduction phase for these concentrations was not applicable

Reference:	KCP 10.3.2.1-02
Report	"A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115 g/l SL on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani Perez)". Angayarkanni, V. 2023. Bioscience Research Foundation. Study No.: 13005/2023
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

A laboratory test for evaluating the effects of Chlormequat 345g/l + Mepiquat 115g/l SL on mortality and fecundity of *Aphidius rhopalosiphi* was conducted for Sharda Cropchem Ltd, India at Bioscience Research Foundation, India according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, and the study plan.

The study was carried out based on the sponsor recommended rates for the test item as the definitive test. They were 1, 2, 4, 8 and 16kg of the test item/ha. Adult wasps were exposed to the test item applied to glass plates. Mortality was determined at 2, 24 and 48 hours after the release of wasps to the test arenas. Females, which survived 48-hour exposure to the test item and the ones from the control group were subjected to fecundity assessments. To allow the oviposition, fifteen female wasps from the groups treated with the test item and the control group were individually introduced into fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After 24 hour oviposition, the wasps were removed from the test arenas and the number of mummies (parasitized aphids in which wasps in pupae were developing) was recorded after 12 days. Mortality of the wasps after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.

To verify the sensitivity of the biological test system and the precision of the test procedure, the insecticide, ROGOR (30% dimethoate) was used as a reference item. The rate of the reference item was 0.4 mL/ha (0.12 g dimethoate/ha). The control group was treated with distilled water.

Materials and methods

Test item:	Chlormequat 345g/l + Mepiquat 115g/l SL; batch number: SCL-120820
Test system:	the parasitic wasp, *Aphidius rhopalosiphi* (De Stephani-Perez); Hymenoptera: Braconidae Age: adult wasps (less than 48 hours old) Source: BRF Insectary
Test design:	7 test groups: 1 control group, 5 test item group and 1 reference group number of replicates: 4 replicates in mortality unit, 15 replicates in reproduction unit

Test organism per treatment Group: 40, divided in 4 parallel replicates, each containing 10 test organisms (for mortality).15 individually confined female survivors from the control and treatment which have lower than 50% mortality on mortality phase were used for reproduction phase.

Test conditions: Temperature: 19.1 – 21.3°C
Relative air humidity: 69 – 81%
Photoperiod: 16 hours light (mortality assessment and oviposition: 1796-1941 lux; fecundity assessment: 5275-5518 lux): 8 hours dark

Statistical analysis: The endpoint values for mortality and reproduction were determined by using a Probit analysis in the NCSS (Number Cruncher Statistical System), one-way ANOVA using Graphpad Prism and Non-Linear regression Calculation Result using Graphpad Prism. The means, reduction and standard deviations were calculated using Excel sheets.

Endpoints:

Mortality
– LR_{50} the rate of the test item causing a 50% mortality of the wasps within the 48-hour period.
– NOER the rate of the test item at which no statistically significant effects on the mortality of the wasps within the 48-hour period are observed

Fecundity
– ER_{50} the rate of the test item causing a 50% reduction in fecundity within a given exposure period.
– NOER the rate of the test item at which no statistically significant effects on the fecundity within a given exposure period are observed.

Results

The effects of Chlormequat 345g/l + Mepiquat 115g/l SL on mortality and fecundity of *Aphidius rhopalosiphi* in a laboratory test are summarized in Table below.

Table 1: Endpoint values

Study group (application rate) (kg test item/ha)	Parameter (endpoint)				
	Mortality after 48 h		Reproduction		
	Total (%)	LR_{50}	Mean no. of mummies/ female	Fecundity reduction Pr [%]	ER_{50}
Control	-	-	13.3	-	-
Chlormequat 345 g/l + Mepiquat 115 g/l SL					
1	2.50	8.79 kg/ha (2830g[a] + 940g[b] a.i./ha)	12.9	3.01	8.61 kg/ha (2772g[a] + 921g[b] a.i./ha)
2	5.00		11.9	10.53+	
4	17.50+		9.9	25.56+	
8	32.50+		7.1	46.62+	
16	85.00+		-*	-*	
$NOER_{mortality}$		2 kg/ha (644g[a] + 214g[b] a.i./ha)	$NOER_{fecundity}$		1 kg/ha (322g[a] + 107g[b] a.i./ha)
ROGOR	Mortality after 48 h				
0.4 mL/ha	82.50%				

a: based on the Chlormequat content in the test item, i.e. 32.2% w/w
b: based on the Mepiquat content in the test item, i.e. 10.7% w/w, provided by the Sponsor
*: Due to more than 50% mortality in mortality phase; reproduction phase for these concentrations was not applicable
+: statistically significant differences at $p<0.05$

The validity criterion for mortality was met, because mortality of the control group after 48 hours of exposure was 0.0%, whereas mortality of wasps after 48 hours of the exposure to Chlormequat 345g/l + Mepiquat 115g/l SL at rates of 1, 2, 4, 8 and 16 kg/ha was 2.50, 5.00, 17.50, 32.50 and 85.00%, respectively.

There were statistically significant differences in mortality between groups treated with the test item at rates of 4, 8 and 16kg/ha and the control group.

On the basis of the obtained mortality results, the LRso value is 8. 79 kg of Chlormequat 345g/I + Mepiquat 115g/l SL/ha, i.e., 2830g Chlormequat + 940g Mepiquat/ha. The NOERmortality value is 2 kg/ha (i.e., 644g Chlormequat/ha + 214g Mepiquat/ha).

For the reference ítem ROGOR (Dimethoate 30% EC), the mortality of wasps after 48 hours of exposure at the rate of 0.4 mL/ha was 82.50%, hence the criterion specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.

The fecundity assessment showed that the mean number of mummies per female in the control group was 13.3, whereas in the group treated with Chlormequat 345g/I + Mepiquat 115g/l SL at rates of 1, 2, 4 and 8 kg/ha was 12.9, 11.9, 9.9 and 7.1 respectively. Fecundity reduction (Pr) in the group treated with Chlormequat 345g/l + Mepiquat 115g/l SL at rates of 1, 2, 4 and 8 kg/ha was 3.01, 10.53, 25.56 and 46.62%, respectively.

There were statistically significant differences in fecundity between the groups treated with the test item at rates of 2, 4 and 8 kg/ha and the control group.

Fecundity reduction results, the ER50 value is 8.61 kg of Chlormequat 345g/I + Mepiquat 115g/I SL ha, i.e., 2772g Chlormequat + 921g Mepiquat/ha. The NOERrecundity value is 1 kg/ha, (i.e., 322g Chlormequat + 107g Mepiquat/ha).

Validity criteria

The following validity criteria were met during the study:

- after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- After 48 hours, mortality of the group treated with the reference item at the rate of 0.4 mL/ha was 82.50% (criterion: a minimum of 50%),
- All wasps in control survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- The mean number of mummies per female in the control group was 13 .3 (criterion: a minimum of 5.0 mummies/female),
- All wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring)

Conclusion
On the basis of the obtained results, it can be concluded that Chlormequat 345g/l + Mepiquat 115g/l SL had no adverse effects on mortality and fecundity of *Aphidius rhopalosiphi* at rates of 1 and 2 kg/ha and at rate of 1 kg/ha, respectively.

A 2.4.1.2 KCP 10.3.2.2 Extended laboratory testing, aged residue with non-

target arthropods

A 2.5 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.5.1 KCP 10.4.1 Earthworms

Comments of zRMS:

The study was accepted by zRMS.

Validity criteria:

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

- Mean adult mortality: 0.0% (criterion: ≤ 10%),
- The mean number of juveniles per vessel at the end of the test: 164.50 (criterion: ≥ 30 juveniles at the end of the test),
- The coefficient of variation calculated for the number of juveniles: 4.0 % (criterion: ≤ 30%).

Agreed toxicity endpoints:

Survival parameter:

Endpoint	Value [mg of the test item /kg d.w. soil]	Value Chlormequat [a] (mg/kg d.w. soil)	Value Mepiquat [b] (mg/kg d.w. soil)
LC_{10}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LC_{20}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LC_{50}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
NOEC	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LOEC	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)

a: Chlormequat
b: Mepiquat
n.d. - not determined

Reproduction parameter:

Endpoint	Value [mg test item/kg d.w. soil]	Value Chlormequat [a] (mg/kg d.w. soil)	Value Mepiquat [b] (mg/kg d.w. soil)
EC_{10}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
EC_{20}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
EC_{50}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
NOEC	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LOEC	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)

a: Chlormequat
b: Mepiquat
n.d. - not determined

Reference: KCP 10.4.1.1-01

Report	"Effect of Chlormequat 345g/I + Mepiquat 115g/l SL on reproduction of the earthworm (*Eisenia fetida*) in artificial soil" Parkavi, B., 2023. Bioscience Research Foundation. Report No: 13007/2023.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 222 (2016).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the study was to assess the impact of Chlormequat 345g/I + Mepiquat 115g/I SL on mortality of the earthworms to determine the LC10, LC20, LC5o, LOEC and NOEC values and on their reproduction to determine the EC10, EC20, EC50, LOEC and NOEC values. The study was conducted for Sharda Cropchem Ltd, India at Bioscience Research Foundation, India according to the OECD guideline No. 222, Adopted 29th July, 2016 and the study plan.

Based on the sponsor recommendation ten concentrations of the test item were used. They are 5.40, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also untreated control group (with deionized water and without the test item) divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all the adult earthworms were removed from the test containers and observed. All changes in their behaviour and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after an additional 4-week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Materials and methods

Product name:	Chlormequat 345g/l + Mepiquat 115g/l SL
Batch number:	SCL – 120820
Production date:	6th July 2022
Appearance:	Slightly Yellow liquid
Formulation:	Soluble concentrate
Storage conditions:	Ambient temperature (+15 to +25 ºC)

Results

Mortality of earthworms at concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil was 0.00 - 2.5% after 4 weeks of the experiment. In the control group it was 0.0%.

The concentration of the test item causing 50% mortality of adults within the exposure period (LC50) is >1000 mg/kg dry weight of the artificial soil, i.e. (>322.00mg Chlormequat /kg dry+ > 107.00 mg Mepiquat/kg dry weight of the artificial soil).

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

Endpoints	Value [mg of the test item/kg d.w. soil]	Value Chlormequat[a] (mg/kg d.w. soil)	Value Mepiquat[b] (mg/kg d.w. soil)
LC_{10}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LC_{20}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LC_{50}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
NOEC	>1000	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LOEC	(n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)

a: chlormequat
b: mepiquat
n.d.: not determined

After the exposure of earthworms to the test item at concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 148.25- 164.00 per replicate. As for the control group, the mean number of juveniles was equal to 164.50 per replicate.

The endpoint values showing the impact of the test ítem on reproduction of the earthworms are presented in given Table.

Endpoints	Value [mg of the test item/kg d.w. soil]	Value Chlormequat[a] (mg/kg d.w. soil)	Value Mepiquat[b] (mg/kg d.w. soil)
EC_{10}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
EC_{20}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
EC_{50}	>1000 (n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)
NOEC	>1000	>322.00[a] (n.d.)	>107.00[b] (n.d.)
LOEC	(n.d.)	>322.00[a] (n.d.)	>107.00[b] (n.d.)

a: chlormequat
b: mepiquat
n.d.: not determined

Validity criteria

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

- Mean adult mortality: 0.0% (criterion: ≤ 10%),
- The mean number of juveniles per vessel at the end of the test: 164.50 (criterion: ≥ 30 juveniles at the end of the test),
- The coefficient of variation calculated for the number of juveniles: 4.0 % (criterion: ≤ 30%).

Conclusions

The concentration of **Chlormequat 345g/l + Mepiquat 115g/l SL** causing a 10% reduction in the number of juveniles produced within the exposure period **(EC10)** is equal to **>1000 mg/kg dry weight of the artificial soil (>322.00 mg Chlormequat +> 107.00 mg Mepiquat kg dry weight of the artificial soil).**

The concentration of **Chlormequat 345 g/l + Mepiquat 115 g/l SL** causing a 20% reduction in the number of juveniles produced within the exposure period **(EC20)** is equal to **>1000 mg/kg dry weight of the artificial soil (>322.00 mg Chlormequat + >107.00 mg Mepiquat kg dry weight of the artificial soil).**

The concentration of **Chlormequat 345g/l + Mepiquat 115g/l SL** causing a 50% reduction in the number of juveniles produced within the exposure period **(EC50)** is equal to **> 1000 mg/kg dry weight of the artificial soil (>322.00 mg Chlormequat + >107.00 mg Mepiquat kg dry weight of the artificial soil).**

The lowest concentration at which **Chlormequat 345g/l + Mepiquat 115g/l SL** observed to have statistically significant effects on earthworm reproduction **(LOEC**) is **> 1000 mg/kg dry weight of the artificial soil (>322.00 mg Chlormequat + >107.00 mg Mepiquat kg dry weight of the artificial soil).**

The highest concentration at which **Chlormequat 345g/l + Mepiquat 115g/l SL** is observed to have no statistically significant effects on earthworm reproduction (**NOEC**) is **> 1000 mg/kg dry weight of the artificial soil (>322.00 mg Chlormequat + >107.00 mg Mepiquat kg dry weight of the artificial soil).**

A 2.5.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.5.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.5.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.5.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was accepted by zRMS. **Validity criteria:** The results are considered valid because the following criteria were satisfied in the control: - Mean adult mortality: 0.0% (criterion: ≤ 20%), - The mean number of juveniles per vessel at the end of the test: 716.75 (criterion: ≥ 100 juveniles at the end of the test), - The coefficient of variation calculated for the number of juveniles: 1.95 (criterion: ≤ 30%). **Agreed toxicity endpoints:** Survival parameters:

Endpoint	Value [mg of the test item/kg d.w. soil]	Value [mg of Chlormequat /kg d.w. soil]	Value [mg of Mepiquat /kg d.w. soil]
LC_{10}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
LC_{20}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
LC_{50}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
NOEC	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
LOEC	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)

Reproduction parameter:

Endpoint	Value [mg of the test item/kg d.w. soil]	Value [mg of Chlormequat /kg d.w. soil]	Value [mg of Mepiquat /kg d.w. soil]
EC_{10}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
EC_{20}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
EC_{50}	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
NOEC	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)
LOEC	>1000 (n.d)	> 322.00 (n.d)	> 107.00 (n.d)

Reference: KCP 10.4.2.1-01

Report: "Effect of Chlormequat 345 g/l + Mepiquat 115 g/1 SL on reproduction of the collembolans (*Folsomia candida*) in artificial soil". Angayarkanni, V., 2023. Bioscience Research foundation India. Report No: 13009/2023.

Guideline(s): OECD 232 (2016): OECD Guidelines for the testing of chemicals, No. 232 Adopted 29th July 2016; Collembolan Reproduction Test in soil".

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

The aim of the study was to assess the impact of Chlormequat 345g/L + Mepiquat 115 g/L SL on mortality of the collembolans, Folsomia candida to determine the LC_{10}, LC_{20}, LC_{50} LOEC and NOEC values and on their reproduction to determine the EC10, EC20. EC50. LOEC and NOEC values.

Ten concentrations of the test item were used. They were 5.04, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

Materials and methods

Test item: Chlormequat 345 g/L + Mepiquat 115 g/L SL

	batch number: SCL-120820
Active substance content:	Chlormequat Chloride: 32.2 % w/w Mepiquat Chloride: 10.7 % w/w
Artificial soil:	5% sphagnum peat(a particle size of 2+1mm), 20% kaolin clay, and 75% air-dried industrial sand with more than 50% of the particles between 50 and 200 µm.
Test system:	Specie: *Folsomia candida* Source: The culture maintained at BRF,India. Age: 9-12 days old.
Concentrations of the test item:	A control, 5.04, 9.07,16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg test item/kg dry weight of the artificial soil.
Test conditions:	- Temperature: 19.0 – 21.3°C - Glass containers with a capacity of 100mL with covers were used. - pH at the beginning of the test: 5.82 – 5.93 - pH at the end of the test:6.22 – 6.23 - soil moisture content at the beginning of the test: 32.65-33.42 % (50.62 – 51.81% of the maximum water holding capacity) - soil moisture content at the end of the test: 33.48– 33.56% (51.91 – 52.03% of the maximum water holding capacity) - light-dark cycle: 16 h light and 8 h dark - light intensity: 590-653 lux
Statistical analysis:	EC_{10}, EC_{20}, EC_{50} , LC_{10}, LC_{20}, LC_{50}, NOEC, LOEC – probit analysis in the NCSS(Number cruncher statistical system),one-way ANOVA and Nonlinear regression using Graphpad prism 8.0
Endpoints:	EC_{10}, EC_{20}, EC_{50} , NOEC LC_{10}, LC_{20}, LC_{50}, NOEC

Results and discussions

Mortality at the concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil ranged from 0.0 to 2.5%. As for the control group, it was 0.0%.

The concentration of Chlormequat 345 g/1 + Mepiquat 115g/1 SL causing 50% mortality of adults within the exposure period (LC50) is >1000mg/kg dry weight of the artificial soil. (i.e., > (322.00 mg Chlormequat + 107.00 mg Mepiquat /kg dry weight of the artificial soil). After the exposure of collembolans to the test item at the concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil the mean number of juveniles ranged from 726.25 and 711.75 per replicate. As for the control group, the mean number of juveniles was equal to 726.38 per replicate.

Table 1. Mortality of adult collembolans (*Folsomia candida*) after 28 days of the experiment

Sample/concentration (mg/kgd.w.soil)		Total mortality	
		NO	%
Control	-	0	0
T1	5.04	0	0

T2	9.07	0	0
T3	16.33	0	0
T4	29.40	0	0
T5	52.92	0	0
T6	95.26	0	0
T7	171.47	0	0
T8	308.64	0	0
T9	555.56	0	0
T10	1000.00	1	2.5

Table 2. Number of juvenile collembolans (*Folsomia candida*) after 28days of the experiment

Sample/concentration (mg/kgd.w.soil)		**Reduction in comparison to the control (%)**	**CV*(%)**
Control	-	NA	0.42
T1	5.04	0.02	0.48
T2	9.07	0.09	0.56
T3	16.33	0.50	0.50
T4	29.40	0.88	1.88
T5	52.92	1.29	1.26
T6	95.26	1.81	0.60
T7	171.47	1.84	0.89
T8	308.64	1.88	1.46
T9	555.56	1.94	0.96
T10	1000.00	2.01	1.14

Table 3. Endpoint values – the impact of the test item on mortality of adult collembolans (*Folsomia candida*)

Endpoint	**Value [mg of the test item/kg dry weight of the artificial soil]**	**Value [mg of Chlormequat/kg d.w.soil**	**Value [mg of Mepiquat/kg d.w.soil]**
LC10	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LC20	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LC50	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

NOEC (survival)	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

Table 4. Endpoint values – the impact of the test item on reproduction of collembolans (*Folsomia candida*)

Endpoint	**Value [mg of the test item/kg dry weight of the artificial soil]**	**Value [mg of Chlormequat/kg d.w.soil**	**Value [mg of Mepiquat/kg d.w.soil]**
EC10	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
EC20	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
EC50	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
NOEC (repro-duction)	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

Validity criteria

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

- Mean adult mortality: 0.0% (criterion: ≤ 20%),
- The mean number of juveniles per vessel at the end of the test: 716.75 (criterion: ≥ 100 juveniles at the end of the test),
- The coefficient of variation calculated for the number of juveniles: 1.95 (criterion: ≤ 30%).

Conclusions

The obtained results led to the following conclusions:

- The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is >1000 mg/kg dry weight of the artificial soil. (i.e. 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).
- The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is >1000 mg/kg dry weight of the artificial soil. (i.e. 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).
- The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is >1000 mg/kg dry weight of the artificial soil. (i.e. 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).
- The lowest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (LOEC) is >1000 mg/kg dry weight of the artificial soil. (i.e. 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is >1000 mg/kg dry weight of the artificial soil. (i.e. 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

Comments of zRMS:

The study was accepted by zRMS.

Validity criteria:

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

- Mean adult mortality: 0.0% (criterion: ≤ 20%),
- The mean number of juveniles per replicate at the end of the test: 128.25 (criterion: ≥ 50 juveniles at the end of the test),
- The coefficient of variation for the number of juveniles: 2.49 (criterion: ≤ 30%).

Agreed toxicity endpoints:

Survival parameter:

Endpoint	Value [mg test item/kg d.w. soil]	Value [mg Chlormequat /kg d.w. soil]	Value [mg Mepiquat /kg d.w. soil]
LC_{10}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
LC_{20}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
LC_{50}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
NOEC	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
LOEC	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)

Reproduction parameter:

Endpoint	Value [mg test item/kg d.w. soil]	Value [mg Chlormequat /kg d.w. soil]	Value [mg Mepiquat /kg d.w. soil]
EC_{10}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
EC_{20}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
EC_{50}	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
NOEC	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)
LOEC	>1000 (n.d.)	> 322.00 (n.d.)	> 107.00 (n.d.)

a – Chlormequat
b - Mepiquat
n.d. - not determined

Reference: KCP 10.4.2.1-02

Report: "Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on the reproductive output of the predatory soil mite *Hypoaspis (Geolaelaps)* aculeifer Canestrini *(*Acari: Laelapidae*)* in artificial soil". Angayarkanni V. Bioscience Research foundation, India. Report No.: 13008 /2023.

Guideline(s): OECD 226 (2016): OECD Guidelines for the testing of chemicals, No. 226 Adopted:29th July 2016)

Deviations: No

GLP: Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the study was to assess the impact of Chlormequat 345 g/1 + Mepiquat 115 g/1 SL on mortality of the predatory soil mite, *Hypoaspis aculeifer* to determine the LC10, LC20, LC50, LOEC and NOEC values and on its reproductive output to determine the EC10, EC20, EC50, LOEC and NOEC values. The study was conducted for Sharda Cropchem Ltd., India at Bioscience Research Foundation, India according to the OECD guideline No. 226, Adopted: 29th July 2016 and the study plan.

Ten concentrations of the test item were used. They were 5.04, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted for 14 days. After that, the mites were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

Materials and methods

Test item:	Chlormequat 345 g/1 + Mepiquat 115 g/1 SL batch number: SCL-120820
Active substance:	Chlormequat Chloride: 32.2 % w/w Mepiquat Chloride: 10.7 % w/w
Artificial soil:	5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand with more than 50% of the particles between 50 and 200 µm.
Test system:	*Hypoaspis (Geolaelaps)* aculeifer *canestrini (*Acari: Laelapidae) Source: BRF Insectary.The developed females were introduced into the test units 33 days after the start of the egg-laying period for synchronisation. *H.aculeifer* is considered to be a relevant representative of soil fauna and predatory mites in particular.
Test design:	test duration: 14 days number of treatment groups:1 control group/10 test item group. test organisms per treatment:40,(80 for the control group) replicates per treatment group:4
Concentrations of the test item:	5.04, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.0 mg test item/kg dry weight of the artificial soil.
Test conditions:	temperature: 20.0-21.4°C pH at the beginning of the test: 6.03– 6.02 pH at the end of the test: 6.07– 6.08 The water content at the initiation of the test soil was 32.48 and 33.15%(corresponding to 50.33-51.40 %of the WHC)

The water content at the end of the test soil was 32.52 and 33.97%(corresponding to 50.42-52.67%of the WHC)

light-dark cycle: 16 h light and 8 h dark

light intensity:560 to 635lux

Statistical analysis: EC_{10}, EC_{20}, EC_{50} , LC_{10}, LC_{20}, LC_{50},NOEC,LOEC- determined by using a Probit analysis in the NCSS(Number Cruncher Statistical System),One-way ANOVA and non-linear regression using Graphpad Prism.

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

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

Results and discussions

Based on the observed results, the mortality at the concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil ranged from 0.0 to 2.5 %. As for the control group, it was 0.0% when exposed to hypoaspis under experimental condition. The concentration of the test item causing 50% mortality of adults within the exposure period (LCSO) is >1000 mg/kg dry weight of the artificial soil. (i.e., > (322.00 mg Chlormequat+107.00 mg Mepiquat)/kg dry weight of the artificial soil). After the exposure of Hypoaspis aculeifer to the test item at the concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 128.25 and 124.25per replicate. As for the control group, the mean number of juveniles was equal to 128.25 per replicate.

The results are summarized in the table given below.

Table 1. Mortality of adult females of after 16 of exposure to the test soil.

Sample/concentration (mg/kgd.w.soil)		Total number of adult females introduced	Mean Mortality (%)
Control	-	80	0.0
T1	5.04	40	0.0
T2	9.07	40	0.0
T3	16.33	40	0.0
T4	29.40	40	0.0
T5	52.92	40	0.0
T6	95.26	40	0.0
T7	171.47	40	0.0
T8	308.64	40	0.0
T9	555.56	40	0.0
T10	1000.00	40	2.50

Table 2. Results of reproductive output of *Hypoaspis aculeifer* after 16 days of exposure to the test soil.

Sample/concentration (mg/kgd.w.soil)		Mean no of juveniles	Reduction in reproduction output compared to control (%)
Control	-	128.25	-
T1	5.04	128.25	0.00
T2	9.07	127.75	0.39
T3	16.33	127.50	0.58
T4	29.40	127.25	0.78
T5	52.92	127.00	0.97
T6	95.26	126.75	1.17
T7	171.47	125.50	1.36
T8	308.64	125.75	1.95
T9	555.56	124.50	2.92
T10	1000.00	124.25	3.12

Table 3. Endpoint values – the impact of the test item on survival of *Hypoaspis aculeifer* are presented in Table given below.

Endpoint	Value [mg of the test item/kg dry weight soil]	Value (mg of chlormequat/kg d.w.soil)	Value (mg of Mepiquat/kg d.w.soil)
LC10	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LC20	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LC50	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
NOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

Table 4.Endpoint values – the impact of the test item on reproductive output of *Hypoaspis aculeifer* are presented in Table given below.

Endpoint	Value [mg of the test item/kg dry weight soil]	Value (mg of chlormequat/kg d.w.soil)	Value (mg of Mepiquat/kg d.w.soil)
EC10	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
EC20	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

EC50	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
NOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)
LOEC	>1000 (n.d)	>322.00 (n.d)	>107.00 (n.d)

Validity criteria

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

- Mean adult mortality: 0.0% (criterion: ≤ 20%),
- The mean number of juveniles per replicate at the end of the test: 128.25 (criterion: ≥ 50 juveniles at the end of the test),
- The coefficient of variation for the number of juveniles: 2.49 (criterion: ≤ 30%).

Conclusions

The obtained results led to the following conclusions:

- The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is >1000 mg/kg dry weight of the artificial soil. (i.e. >322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

- The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is >1000 mg/kg dry weight of the artificial soil. (i.e. >322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

- The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is >1000 mg/kg dry weight of the artificial soil. (i.e. >322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

- The lowest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (LOEC) is >1000 mg/kg dry weight of the artificial soil. (i.e. > 322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is >1000 mg/kg dry weight of the artificial soil. (i.e. >322.00 mg Chlormequat + 107.00 Mepiquat /Kg dry weight of the artificial soil).

A 2.5.2.2 KCP 10.4.2.2 Higher tier testing

A 2.6 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. <u>Nitrogen transformation test:</u> The Mepcy (SHA 126085 A), at 28 days after treatment, did not affect the microbial nitrogen transformation in soil since the treated samples deviated less than 25% from the control after 28 days from treatment: - 4.72% at the concentration of 14.27 mg of product/kg dry soil equivalent to 4.61 mg of chlormequat chloride/ kg dry soil and 1.51 mg of mepiquat chloride/ kg dry soil;

	- 6.92% at the concentration of 71.35 mg of product/kg dry soil equivalent to 23.05 mg of chlormequat chloride/ kg dry soil and 7.56 mg of mepiquat chloride/ kg dry soil.

Reference	KCP 10.5.01
Report	"Soil Microorganisms: Nitrogen Transformation Test of Chlormequat 345 g/L + Mepiquat 115 g/L SL". H. S. Anand, M. Sc, 2020. Study code: G14222. Analytical R & D Department Eurofins Advinus Limited, India
Guideline(s)	OECD Guidelines for Testing of Chemicals. Test No. 216 (OECD, 2000)
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Material and methods

Test material	Chlormequat 345 g/L + Mepiquat 115 g/L SL, batch number SCL-99721
Soil	Agricultural soil collected from a site where no crop protection products have been applied for a minimum of one year before sampling and no organic fertilizer have been applied for at least six months before.
Test design	Three portions of soil (3 x 75 g. dry weight), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 25 g). The soil was amended with a suitable organic substrate, i.e. powdered Lucerne-grass meal with a C/N ratio 13/1 at dose of 5 g/kg dry weight of soil. Test duration: 28 days.
Concentrations of the test material	Control; 14.27 mg of test item/kg dry weight soil (as Chlormequat chloride 4.61 mg a.s./kg of dry weight of soil, as Mepiquat 1.51 mg a.s./kg of dry weight of soil) and 71.35 mg of test item/kg of dry weight soil (as Chlormequat chloride 23.05 mg a.s./kg of dry weight of soil, as Mepiquat 7.56 mg a.s./kg of dry weight of soil)
Test conditions	Temperature: 19.7 – 20.3°C, soil moisture: 50 ± 5 % MWHC, pH in Milli-Q water: 6.62 ± 0.02
Endpoints	The concentration of nitrate ions [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation and percent deviation from the control in nitrate formation.
Statistical analysis	- SYSTAT Statistical Package Ver.12.0., - ANOVA

Study design

The aim of the study was to detect long-term adverse effects of Chlormequat 345 g/L + Mepiquat 115 g/L SL on the processes of nitrogen transformation in aerobic surface soils.

Sandy clay loam soil was used. It was manually cleared of large objects and sieved to a particle size of 2 mm. The concentrations of the test item were 14.27 (PEC) and 71.35 (5 x PEC) mg of test item/kg of dry weight soil. The treated and the control soils were divided into three replicates. On days 0, 7, 14 and 28 of incubation, soil samples were collected to determine the quantities of nitrates.

The method involves a measurement of the nitrate ion concentration in a soil extract obtained by using 0.1 M KCl, then adding 6 N Hydrochloric acid and Chromotrophic acid (0.0125%) reagent. The absorbance of the solution was measured at 362 nm.

The nitrate formation rate in each treated group was compared with that in the control and the percent deviation of the treated from the control was calculated.

Results

After 28 days of incubation, the lowest treatment group deviated by 4.72% and the highest treatment group deviated by 6.92% from control with respect to the nitrate content.

All analyses and comparisons were evaluated at the 5% ($p<0.05$) level and it was found that p-value = 0.72, 0.07, 0.02 and 0.05 on day 0, 7, 14 and 28 days respectively, at 5% level of significance (i.e. α = 0.05). The analysis data indicated that there was a significant difference between control vs high and low dose only on the day 14. The results of day 0, after 7 and 28 days, $p \geq 0.05$ and hence, there was no significant difference between control vs high dose and low dose.
The calculated % variations among the replications and control samples were less than 15% indicating the validity of the test on all the intervals.
The difference between nitrate content of the treated and the control samples after 28 day interval were less than 25% indicating that the test item does not have a long term influence on Nitrogen transformation in soil microorganisms.

Table 1: Nitrate content in soil - deviations from the control [%]:
Determination of nitrate formation rate on day 0, 7, 14, 28.

Day	Sample details	Nitrate content in soil (mg/kg/day)	% of Deviation from control
0	Control	94.38 ± 1.77	NA
	Lower Concentration	95.45 ± 1.02	1.13
	Higher Concentration	94.91 ± 1.81	0.56
7	Control	12.48 ± 0.50	NA
	Lower Concentration	12.53 ± 0.21	0.40
	Higher Concentration	13.17 ± 0.14	5.53
14	Control	5.89 ± 0.10	NA
	Lower Concentration	6.37 ± 0.29	8.15
	Higher Concentration	6.45 ± 0.05	9.51
28	Control	3.18 ± 0.06	NA
	Lower Concentration	3.33 ± 0.12	4.72
	Higher Concentration	3.40 ± 0.07	6.92

Conclusions
The effect of the test item on nitrogen transformation activity of soil microorganisms was investigated in a sandy clay loam soil. The test was performed at 20 ± 2ºC for 28 days and the average recorded maximum and minimum temperatures were 20.3ºC and 19.7ºC, respectively.
The results of the measurement of nitrate content for control (soil treated with Mili-Q water), low concentration level of 14.27 mg test item/kg dry weight of soil (as Chlormequat Chloride 4.61 mg a.s./kg of dry weight of soil, as Mepiquat chloride 1.51 mg a.s./kg of dry weight of soil) and high concentration level of 71.35 mg of test item/kg of soil (as Chlormequat Chloride 23.05 mg a.s./kg of dry weight of soil, as Mepiquat Chloride 7.56 mg a.s./kg of dry weight of soil) on each of 0, 7, 14 and 28 day intervals.
After 28 days of incubation, the lowest treatment group deviated by 4.72% and highest treatment group deviated 6.92% from the control with respect to the nitrate content.
Based on the experiment results, it can be concluded that the test item, of Chlormequat 345 g/L + Mepiquat 115 g/L SL does not have long-term influence on nitrogen transformation in soil microorganisms.

Comments of zRMS:	The study is considered as additional source of information (According to Regulation 284/2013 the carbon transformation study this is not required). All validity criteria were met. <u>Carbon transformation test:</u> The Mepcy (SHA 126085 A)**,** at 28 days after treatment, did not affect the microbial carbon transformation in soil since the treated samples deviated less than 25% from the control after 28 days from treatment: - 6.98% at the concentration of 14.27 mg of product/kg dry soil equivalent to 4.61 mg of chlormequat chloride/ kg dry soil and 1.51 mg of mepiquat chloride/ kg dry soil; - 10.30% at the concentration of 71.35 mg of product/kg dry soil equivalent to 23.05 mg of chlormequat chloride/ kg dry soil and 7.56 mg of mepiquat chloride/ kg dry soil.

Reference: KCP 10.5.02

Report	"Soil Microorganisms: Carbon Transformation Test of Chlormequat 345 g/l + Mepiquat 115 g/L SL", H. S. Anand, M. Sc., 2020, G14221. Analytical R & D Department Eurofins Advinus Limited, India.
Guideline(s):	OECD Guidelines for Testing of Chemicals. Test No. 217 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Materials

Test item:	
Description:	Chlormequat 345 g/l + Mepiquat 115 g/L SL
Production batch:	SCL – 99721
Active ingredients content:	Chlormequat 345 g/L Mepiquat 115 g/L
Vehicle and control:	Milli-Q water
Test system:	
Species:	Microorganisms
Source:	From a site where no crop protection products have been applied for a minimum of one year before sampling and no organic fertilizer have been applied for at least six months before
Experimental conditions:	
Temperature:	20 ± 2°C
Humidity:	50 ± 5% MWHC
Air changes:	-
Light and photoperiod:	Dark (24/24h)

Study design and methods

Experimental period:	24/01/2020 – 04/03/2020
Test design and treatment:	3 portions of soil, each containing 25 g of dry weight, for each of the untreated and treated groups. Test duration: 28 days. Concentrations of the test material: Control; 14.27 mg of the test item/kg soil (PEC) and 71.35 mg of the test item/kg soil (5 x PEC). The glucose induced respiration rate in the treated soil samples was compared with that in the control, and the percent deviation of the treated from the control was calculated after 0, 7, 14, and 28 days of incubation.
Statistics:	The statistical analysis of the experimental data was carried out using licensed copies of SYSTAT Statistical Package Ver.12.0. The quantitative variable (CO_2, mg/kg dry weight of soil/h) was tested using ANOVA. Comparison of means between treatment groups and control group was done. All analyses and comparisons were evaluated at the 5% ($p < 0.05$) level. Statistically non-significant differences ($p < 0.05$) indicated as non-significant (NS)

Results

The calculated % deviations in the glucose induced respiration rates (i.e., carbon dioxide released rates) between treated and control are < 25%.

After 28 days of incubation, the lowest treatment group deviated by 6.98% and the highest treatment group deviated by 10.30% from control with respect to the glucose induced respiration rates (carbon dioxide released rates). The variations between results of replicate control samples were within ±15% on every occasion tested.

All analyses and comparisons were evaluated at the 5% ($p < 0.05$) level. The day 0, 7, 14 and 28 analysis data evaluated at the 5% ($p<0.05$) level and the statistical data showed that p-values= 0.00, 0.00, 0.00 and 0.09 respectively, at 5% level of significance (i.e. $\alpha = 0.05$). The results after 28 days, $p \geq 0.05$ and hence, there was no significant difference between control vs high dose and low dose.

The difference in respiration rates between the treated and the control was <25% on day 28 and hence, the experiment was concluded after 28 days interval.

Table 3: Mean Glucose induced respiration rate - deviations from the control [%]

Day	Sample details	Mean Glucose induced respiration rate (mg/kg/hr)	% of Deviation from control
0	Control	81.51	NA
	Lower Concentration	93.32	14.49
	Higher Concentration	95.72	17.43
7	Control	86.63	NA
	Lower Concentration	92.60	6.89
	Higher Concentration	98.98	14.26
14	Control	82.00	NA
	Lower Concentration	93.10	13.54
	Higher Concentration	95.94	17.00
28	Control	88.41	NA
	Lower Concentration	94.58	6.98
	Higher Concentration	97.52	10.30

NA: Not Applicable
Control: Milli-Q water
Lower Concentration: 14.27 mg of test item/kg of dry weight soil
Higher Concentration: 71.35 mg of test item/kg of dry weight soil

Conclusion

The effect of the test item on carbon transformation activity of soil microorganisms was investigated in a sandy clay loam soil. The test was performed at 20 ± 2ºC for 28 days and the recorded maximum and minimum temperatures were 20.3ºC and 19.7ºC, respectively. The application rates of test item were control (only Milli-Q water), low concentration level of 14.27 mg test item/kg dry weight of soil and as Chlormequat Chloride: 4.61 mg a.s./kg dry weight of soil and as Mepiquat Chloride: 1.51 mg a.s./kg dry weight of soil and high concentration level of 71.35 mg test item/kg dry weight of soil and as Chlormequat Chloride: 23.05 mg a.s./kg dry weight of soil and as Mepiquat Chloride: 7.56 mg a.s./kg dry weight of soil.

The variations between results of replicate control samples were within ±15%. After 28 days of incubation, the lowest treatment group deviated by 6.98% and the highest treatment group deviated by 10.30% from control with respect to the glucose induced respiration rates which was below the threshold value of < 25%. Hence the experiment was concluded after 28 days of incubation.

Based on the experiment results, it can be concluded that the test item, Chlormequat 345 g/L + Mepiquat 115 g/L SL does not have long-term influence on carbon transformation in soil microorganisms.

A 2.7 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.7.1 KCP 10.6.1 Summary of screening data

A 2.7.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:

The study was accepted by zRMS.

Validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Chlormequat 345 g/L + Mepiquat 115 g/L SL on seedling emergence and seedling growth of terrestrial plants were met:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
  95% – Corn
  100% – Soybean
  95% – White mustard
  100% – Oats
  100% – Radish
  95% – Tomato
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%)
- the control seedlings did not exhibit any visible phytotoxic symptoms.
- environmental conditions for all plants of the same species were identical.

Agreed toxicity endpoints:

The endpoint values showing the impact of the Chlormequat 345 g/l on seedling emergence and seedling growth of the plant species tested are presented in Table given below.

Endpoint value		Corn (*Zea mays*)	Soybean (*Glycine max*)	White Mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum lycopersicon*)
Plant number							
ER_{50}	kg/ha[a]	11.77	12.56	12.73	14.82	12.17	12.47
	g/ha[b]	3789.9	4044.3	4099.1	4772	3918.7	4015.3
NOER	kg/ha[a]	3	3	3	3	3	3
	g/ha[b]	966	966	966	966	966	966
Shoot length (plants without roots)							
ER_{50}	kg/ha[a]	12.04	13.31	14.31	12.39	11.94	12.64
	g/ha[b]	3876.9	4285.8	4607.8	3989.6	3844.7	4070.1
NOER	kg/ha[a]	1.5	1.5	3	1.5	1.5	1.5
	g/ha[b]	483	483	966	483	483	483
Plant dry weight (plants without roots)							
ER_{50}	kg/ha[a]	12.23	12.82	11.95	13.40	13.64	13.25
	g/ha[b]	3938.1	4128	3847.9	4314.8	4392.1	4266.5
NOER	kg/ha[a]	1.5	1.5	1.5	1.5	1.5	3
	g/ha[b]	483	483	483	483	483	966

[a]: value for the test item, i.e Chlormequat i.e. 32.2 (% w/w) expressed as kg/ha
[b]: value for active substance, i.e Chlormequat expressed as g/ha

The endpoint values showing the impact of the Mepiquat 115 g/l on seedling emergence and seedling growth of the plant species tested are presented in Table given below.

Endpoint value		Corn (*Zea mays*)	Soybean (*Glycine max*)	White Mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum lycopersicon*)
Plant number							
ER_{50}	kg/ha[a]	11.77	12.56	12.73	14.82	12.17	12.47
	g/ha[b]	1259.4	1343.9	1362.1	1585.7	1302.2	1334.3
NOER	kg/ha[a]	3	3	3	3	3	3
	g/ha[b]	321	321	321	321	321	321
Shoot length (plants without roots)							
ER_{50}	kg/ha[a]	12.04	13.31	14.31	12.39	11.94	12.64
	g/ha[b]	1288.3	1424.2	1531.2	1325.7	1277.6	1352.5
NOER	kg/ha[a]	1.5	1.5	3	1.5	1.5	1.5
	g/ha[b]	160.5	160.5	321	160.5	160.5	160.5
Plant dry weight (plants without roots)							
ER_{50}	kg/ha[a]	12.23	12.82	11.95	13.40	13.64	13.25
	g/ha[b]	1308.6	1371.7	1278.7	1433.8	1459.5	1417.8
NOER	kg/ha[a]	1.5	1.5	1.5	1.5	1.5	3
	g/ha[b]	160.5	160.5	160.5	160.5	160.5	321

[a]: value for the test item, i.e. Mepiquat i.e 10.7 (% w/w) expressed as kg/ha
[b]: value for active substance, i.e Mepiquat expressed as g/ha

Phytotoxicity parameter:
The phytotoxicity parameter was assessment by RMS based on data in the study report without statistical analysis :
Corn ER_{50} > 12 kg formulation **MEPCY**/ha (6.5% effect)
Soybean ER_{50} > 12 kg formulation **MEPCY**/ha (9.0% effect)
White mustard ER_{50} > 12 kg formulation **MEPCY**/ha (13.75% effect)
Oats ER_{50} > 12 kg formulation **MEPCY**/ha (15.0% effect)
White mustard ER_{50} > 12 kg formulation **MEPCY**/ha (13.75% effect)
Radish ER_{50} > 12 kg formulation **MEPCY**/ha (10.0% effect)
Tomato ER_{50} > 12 kg formulation **MEPCY**/ha (11.5% effect)

Reference:	KCP 10.6.2-01
Report	"Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on seedling emergence and seedling growth of terrestrial plants". Radha, S. 2023. Bioscience Research Foundation. Report number: 13010/2023.
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The study, aimed at evaluating the effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species to determine the ER10 , ER25 , ER50 , and NOER values for plant number, shoot length and shoot weight.

Seeds of the test plant species were sown in plastic pots. The number of seeds per pot as well as the total number of seeds per application rate were 3 seeds/pot, i.e. 21 seeds/application rate (7 pots/application

for radish, except large size seeds of corn, soybean and tomato per application rate were 2 seeds/pot i.e. 20 seeds/application rate (10 ports/application rate) and small size seeds of white mustard and oats per application rate were 5 seeds/pot i.e. 20 seeds/application rate (4 ports/application rate). The number of seeds per pot selection provided the adequate growth conditions and avoided over crowing plants during the experiment.

The test item as sprayed onto the soil surface. The experiment was conducted in a plant growth chamber with suitable environmental conditions for each test species were provided. The experiment was finished 14 days after the emergence of 50% of the control seedlings. During the experiment, the plants were observed for emergence on every day and visual phytotoxicity (7 and 14 days after the emergence of 50% of the control seedlings). At the end of the experiment, the number of plants was counted. Next, the plants were cut down and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed.

The results concerning the shoot length, the dry weight and the number of plants at the end of the experiment were statistically analyzed to determine the ER_{10}, ER_{25}, and ER_{50} and NOER values.

Materials and methods

Test item:	Chlormequat 345 g/L + Mepiquat 115 g/L SL batch number: SCL-120820
Test species:	Corn (*Zea Mays*), soybean (*Glycine max*), radish (*Raphanus sativus*), white mustard (*Sinapis alba*), tomato (*Solanum Lycopersicon*) and oats (*Avena sativa*).
Soil:	Sandy loam containing 1.3% organic carbon.
Study design:	number of rates: • Corn - 2 seeds/pot - 20 seeds/application rate (10 pots/application rate) • Tomato - 2 seeds/pot- 20 seeds/application rate (10 pots/application rate). • Soybean - 2 seeds/pot - 20 seeds/application rate (10 pots/application rate)), • Radish - 3 seeds/pot - 21 seeds/application rate (7 pots/application rate). • White Mustard - 5 seeds/pot - 20 seeds/application rate (4 pots/application rate). • Oats - 5 seeds/pot - 20 seeds/application rate (4 pots/application rate).
Exposure termination:	14 days after the emergence of 50% of the control seedlings
Application rates:	- a control, - 1.5 of the test item /ha - 3 of the test item /ha - 6 of the test item /ha - 12 of the test item /ha - 24 of the test item /ha
Test conditions:	Temperature: 21.2 – 24.1°C humidity: 69.2 – 87.1% lighting: 16 h light : 8 h dark light intensity: 323 – 376 µE/m2/s carbon dioxide concentration: 341 – 368 ppm

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

Endpoints: ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

The results are summarized in the table below.

Table 1. The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

Endpoint value		Corn (*Zea Mays*)	Soybean (*Glycine max)*	White mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum Lycopersicon*)
Plant number							
ER_{50}	Kg/ha	11.77	12.56	12.73	14.82	12.17	12.47
NOER		3	3	3	3	3	3
Shoot length (plants without roots)							
ER_{50}	Kg/ha	12.04	13.31	14.31	12.39	11.94	12.64
NOER		1.5	1.5	3	1.5	1.5	1.5
Plant dry weight (plants without roots)							
ER_{50}	Kg/ha	12.23	12.82	11.95	13.40	13.64	13.25
NOER		1.5	1.5	1.5	1.5	1.5	1.5

The test item, i.e. Chlormequat 345 g/L + Mepiquat 115 g/L SL applied at rates ranging from 1.5 to 24 kg/ha had a varied impact on seedling emergence and seedling growth of all the plant species tested. The impact depended on the rate of the test item and species used. After the application of the test item 12 and 24 kg/ha, seedling emergence was delayed for all the species including corn, white mustard, soybean, oats, radish and tomato in comparison with the control. However, all the plant species tested emerged after the application of the test item at rates ranging from 1.5 to 24 kg/ha. The phytotoxic symptoms for all the plant species tested were observed at all the rates of the test item used on day 14 after the emergence of 50% of the control seedlings. There were phytotoxic symptoms observed for all the six plant species. The following phytotoxic symptoms were observed:

- Corn, Soybean, Radish, White Mustard and Tomato: chlorosis, necrosis, wilting, leaf deformation or stem deformation.
- Oats: chlorosis, necrosis, leaf deformation and stem deformation.

Validity criteria

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Chlormequat 345 g/L + Mepiquat 115 g/L SL on seedling emergence and seedling growth of terrestrial plants were met:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
  95% – Corn
  100% – Soybean
  95% – White mustard
  100% – Oats
  100% – Radish
  95% – Tomato
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%)
- the control seedlings did not exhibit any visible phytotoxic symptoms.
- environmental conditions for all plants of the same species were identical.

Comments of zRMS:

The study was accepted by zRMS.

Validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Chlormequat 345 g/L + Mepiquat 115 g/L SL on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:

100% – Corn
95% – Soybean
90% – White mustard
95% – Oats
100% – Radish
100% – Tomato

- the mean survival of the emerged control seedlings was 100% for corn, radish and tomato, 95% for Oat and soybean, 90% white mustard in case of all experimental species (validity criterion: at least 90%)
- the control plants did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Agreed toxicity endpoints:

The endpoint values showing the impact of the Chlormequat on vegetative vigour of the plant species tested are presented in Table given below.

Endpoint value		Corn (*Zea mays*)	Soybean (*Glycine max*)	White Mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum lycopersicon*)
Plant number							
ER_{50}	kg /ha[a]	12.66	12.73	11.76	12.31	12.24	11.98
	g/ha[b]	4076.5	4099.1	3786.7	3963.8	3941.3	3857.6
NOER	kg /ha[a]	3	3	3	3	3	3
	g/ha[b]	966	966	966	966	966	966
Shoot length (plants without roots)							
ER_{50}	kg /ha[a]	12.96	11.94	12.05	12.27	13.01	13.49
	g/ha[b]	4173.1	3844.7	3880.1	3950.9	4189.2	4343.8
NOER	kg ha[a]	1.5	1.5	1.5	1.5	1.5	1.5
	g/ha[b]	483	483	483	483	483	483
Plant dry weight (plants without roots)							
ER_{50}	kg /ha[a]	12.71	12.34	13.01	13.28	11.87	12.18
	g/ha[b]	4092.6	3973.5	4189.2	4276.2	3822.1	3922
NOER	kg /ha[a]	1.5	1.5	1.5	1.5	1.5	1.5
	g/ha[b]	483	483	483	483	483	483

[a]: value for the test item i.e. **Chlormequat** expressed as Kg/ha
[b]: value for active substance, i.e. **Chlormequat** expressed as g/ha.

The endpoint values showing the impact of the Mepiquat on vegetative vigour of the plant species tested are presented in Table given below.

Endpoint value		Corn (*Zea mays*)	Soybean (*Glycine max*)	White Mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum lycopersicon*)
Plant number							
ER_{50}	Kg/ha[a]	12.66	12.73	11.76	12.31	12.24	11.98
	g/ha[b]	1354.6	1362.1	1258.3	1317.2	1309.7	1281.9
NOER	kg /ha[a]	3	3	3	3	3	3
	g/ha[b]	321	321	321	321	321	321
Shoot length (plants without roots)							
ER_{50}	kg /ha[a]	12.96	11.94	12.05	12.27	13.01	13.49
	g/ha[b]	1386.7	1277.6	1289.4	1312.9	1392.1	1443.4
NOER	kg /ha[a]	1.5	1.5	1.5	1.5	1.5	1.5
	g/ha[b]	160.5	160.5	160.5	160.5	160.5	160.5
Plant dry weight (plants without roots)							
ER_{50}	kg /ha[a]	12.71	12.34	13.01	13.28	11.87	12.18
	g/ha[b]	1360	1320.4	1392.1	1421	1270.1	1303.3
NOER	kg ha[a]	1.5	1.5	1.5	1.5	1.5	1.5
	g/ha[b]	160.5	160.5	160.5	160.5	160.5	160.5

[a]: value for the test item i.e. **Mepiquat** expressed as Kg/ha

[b]: value for active substance, i.e. **Mepiquat** expressed as g/ha.

Phytotoxicity parameter:

The phytotoxicity parameter was assessment by RMS based on data in the study report without statistical analysis :

Corn ER_{50} > 12 kg formulation **MEPCY**/ha (11% effect)
Soybean ER_{50} > 12 kg formulation **MEPCY**/ha (23.5% effect)
White mustard ER_{50} > 12 kg formulation **MEPCY**/ha (20% effect)
Oats ER_{50} > 12 kg formulation **MEPCY**/ha (26.3% effect)
White mustard ER_{50} > 12 kg formulation **MEPCY**/ha (13.75% effect)
Radish ER_{50} > 12 kg formulation **MEPCY**/ha (12.14% effect)
Tomato ER_{50} > 12 kg formulation **MEPCY**/ha (15.0% effect)

Reference:	KCP 10.6.2-02
Report	"Effect of Chlormequat 345 g/L + Mepiquat 115 g/L SL on vegetative vigor on terrestrial plants". Radha, S. 2023. 2023. Bioscience Research Foundation. Report number: 13011/2023.
Guideline(s):	according to the OECD Guideline No. 227 (2006)
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Seeds of the test plant species were sown in plastic pots. The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. The number of seeds per pot as well as the total number of seeds per application rate were 3 seeds/pot, i.e. 21 seeds/application rate (7 pots/application rate) for radish, except large size seeds of corn, soybean and tomato per application rate were 2 seeds/pot i.e. 20 seeds/application rate (10 pots/application rate) and small size seeds of white mustard and oats per application rate were 5 seeds/pot i.e. 20 seeds/application rate (4 pots/application rate).

The test item was sprayed onto the plant leaf surface. The experiment was conducted in a plant growth chamber with suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application) and mortality. The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60°C to a constant weight and weighed.

The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER10, ER25, ER50 and NOER values.

Materials and methods

Test item:	Chlormequat 345 g/L + Mepiquat 115 g/L SL batch number: SCL-120820
Test species:	Corn (*Zea Mays*), soybean (*Glycine max*), radish (*Raphanus sativus*), white mustard (*Sinapis alba*), tomato (*Solanum Lycopersicon*) and oats (*Avena sativa*).
Soil:	Sandy loam containing 1.3% organic carbon.
Study design:	number of rates: • Corn - 2 seeds/pot - 20 seeds/application rate (10 pots/application rate) • Tomato - 2 seeds/pot- 20 seeds/application rate (10 pots/application rate). • Soybean - 2 seeds/pot - 20 seeds/application rate (10 pots/application rate)), • Radish - 3 seeds/pot - 21 seeds/application rate (7 pots/application rate). • White Mustard - 5 seeds/pot - 20 seeds/application rate (4 pots/application rate). • Oats - 5 seeds/pot - 20 seeds/application rate (4 pots/application rate).
Exposure termination:	14 days after the emergence of 50% of the control seedlings
Application rates:	- a control, - 1.5 of the test item /ha - 3 of the test item /ha - 6 of the test item /ha - 12 of the test item /ha - 24 of the test item /ha
Test conditions:	Temperature: 21.2 – 24.5°C humidity: 69.5 – 82.5% lighting: 16 h light : 8 h dark light intensity: 322 – 373 µE/m2/s carbon dioxide concentration: 342 – 365 ppm

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

Endpoints: ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

Table 1. The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

Endpoint value		Corn (*Zea Mays*)	Soybean (*Glycine max*)	White mustard (*Sinapis alba*)	Oats (*Avena sativa*)	Radish (*Raphanus sativus*)	Tomato (*Solanum Lycopersicon*)
Plant number							
ER_{50}	Kg/ha	12.66	12.73	11.76	12.31	12.24	11.98
NOER		3	3	3	3	3	3
Shoot length (plants without roots)							
ER_{50}	Kg/ha	12.96	11.94	12.05	12.27	13.01	13.49
NOER		1.5	1.5	3	1.5	1.5	1.5
Plant dry weight (plants without roots)							
ER_{50}	Kg/ha	12.71	12.34	13.01	13.28	11.87	12.18
NOER		1.5	1.5	1.5	1.5	1.5	1.5

The test item, i.e. Chlormequat 345 g/I + Mepiquat 115 g/I SL applied at rates ranging from 1.5 to 24 kg/ha of the test item/ha, had a varied impact on vegetative vigour of all the plant species tested. The impact depended on the rate of test item and species used.

There was mortality observed for corn, white mustard, radish and tomato tested at rates ranging from 3 to 24 L of the test item/ha and for corn, soybean and oats mortality was observed at 1.5 to 24 kg/ha of the test item/ha. The phytotoxic symptoms were observed at rates of 6 to 24 kg/ha of the test item used for soybean, white mustard, oats and tomato. The phytotoxic symptoms were observed at rates of 12 to 24 kg/ha of the test item used for corn and radish The following phytotoxic symptoms were observed on 21 days after the test item application: chlorosis, necrosis, wilting, leaf deformation and stem deformation.

Validity criteria

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Chlormequat 345 g/L + Mepiquat 115 g/L SL on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:

  100% – Corn
  95% – Soybean
  90% – White mustard
  95% – Oats
  100% – Radish
  100% – Tomato
- the mean survival of the emerged control seedlings was 100% for corn, radish and tomato, 95% for Oat and soybean, 90% white mustard in case of all experimental species (validity criterion: at least 90%)
- the control plants did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

A 2.7.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.8 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.9 KCP 10.8 Monitoring data