

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 8500 A

Product name(s): MEPISHA

Chemical active substances:

Mepiquat chloride, 50 g/L

(Mepiquat 38 g/L)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: February 2021

MS Finalisation date: September 2021, February 2022

Version history

When	What
September 2021	Finalisation of the assessment by zRMS.
February 2021	Final Version of RR after Commenting Period

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel- opmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. num- ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	Winter wheat, winter barley, spring barley	F	Reduction of crop height	Foliar Spray	BBCH 31-39	a) 1 b) 1	-	a) 0.75 b) 0.75	a) 0.0285 b) 0.0285	200-400		0.0375 mepiquat chloride/ha							
2	CEU	Winter Oilseed rape	F	Reduction of crop height	Foliar Spray	BBCH 31-39	a) 1 b) 1	-	a) 0.75 b) 0.75	a) 0.0285 b) 0.0285	200-400		0.0375 mepiquat chloride/ha							

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:	<ul style="list-style-type: none">(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 (<i>e.g.</i> 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 <u>and</u> 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	<ul style="list-style-type: none">(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
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9.1.1 Overall conclusions

zRMS comments:

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Not agreed or not relevant information is struck through. In blue corrected values or information were added by zRMS, if relevant.

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

- Birds

According to the screening assessment, all the TER_a and TER_{lt} values for the active substance mepiquat **chloride** are greater than the annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to birds according to the intended uses.

Moreover, the risk for birds due to uptake of contaminated drinking water was considered as low and mepiquat **chloride** shows low potential for bioaccumulation, hence, there is no risk to earthworm-eating and fish-eating birds according to the intended uses of MEPISHA.

- Mammals

According to the screening and first tier assessment, all the TER_a and TER_{lt} values for the active substance mepiquat **chloride** are greater than the annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to mammals according to the intended uses.

Moreover, the risk for mammals due to uptake of contaminated drinking water was considered as low and mepiquat **chloride** shows low potential for bioaccumulation, hence, there is no risk to earthworm-eating and fish-eating mammals according to the intended uses of MEPISHA.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

For all intended uses, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for *Daphnia* acute as characterised by an EC_{50} of 68500 µg/L in connection with an assessment factor of 100) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

9.1.1.3 Effects on bees (KCP 10.3.1)

Studies on the toxicity to honeybees show that hazard quotients (oral and contact) for mepiquat **chloride** and the formulation MEPISHA are clearly under the cut-off value. An application of MEPISHA to cereals and oilseed rape in respect of the GAP does not present an unacceptable risk for honeybees.

According to EU Reg. 284/2009 the chronic studies for bees (adult and larvae) should be submitted by the applicant.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field HQ values calculated for the representative species *T. pyri* and *A. rhopalosiphi*, are lower than the trigger of 2 for first-tier tests, indicating no risk to non-target arthropods in vegetated in-field and off-field areas following application of MEPISHA according to the proposed use patterns.

9.1.1.5

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

The acute and chronic TER for Mepiquat **chloride** are above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, according to the risk assessment and the effects on other soil arthropods, it is concluded that Mepiquat **chloride** and MEPISHA formulation do not pose acute and long-term risk to earthworms and other soil macro- and mesofauna.

Risk assessments conducted with relevant PEC_{soil} for the active substance mepiquat **chlorided** indicate a low risk to soil microorganisms when applied according to the proposed use rates. The use of MEPISHA at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

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

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for mepiquat **chloride** shows that the Annex VI trigger value of 5 is reached, indicating that MEPISHA poses a low risk to non-target plants when applied according to the proposed use rates.

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

Mepiquat:

Effects on biological methods for sewage treatment:

Activated sludge respiration > 1000 g mepiquat chloride/L

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of MEPISHA grouped according to criterion

Grouping according to criterion			
Group	Intended uses	Relevant use parameters for grouping	Relevant parameter or value for sorting
Cereals	Winter wheat Winter barley Spring barley	Application rate	Application rate (1 x 0.0285 –0.0375- kg mepiquat chloride/ha correspond to 0.0285 kg mepiquat/ha) for: - Birds and mammals risk assessment - Aquatic organisms
Oilseed rape	Winter oilseed rape	Application rate	Application rate (1 x 0.0285 –0.0375- kg mepiquat chloride /ha correspond to 0.0285k g mepiquat/ha) for: - Birds and mammals risk assessment - Aquatic organisms
All crops	All crops	Application rate	- Bees risk assessment - Arthropods other than bees risk assessment - Earthworms risk assessment - Soil microorganisms

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

Effects on birds of MEPISHA (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of mepiquat [chloride](#). However, the provision of further data on the MEPISHA is not considered essential, because endpoints obtained with the active substance 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
Bobwhite quail <i>Colinus virginianus</i>	Mepiquat chloride	Acute	LD ₅₀ > 2000 mg a.s./kg bw/day	EFSA Scientific Report (2008) 146, 1-73
Bobwhite quail <i>Colinus virginianus</i>	Mepiquat chloride	Dietary 8 d, Short-term	LD ₅₀ > 1326 mg a.s./kg bw/day	
Japanese quail <i>Coturnix japonica</i>	Mepiquat chloride	Reproductive toxicity (long-term)	NOED = 100.7 mg a.s./kg bw/day	

9.2.1.1 Justification for new endpoints

The EU agreed endpoints are used for the risk assessment.

Selection of the endpoint used for acute risk assessment:

According to the Guidance EFSA/2009/1438, where dietary LC₅₀ is lower than the acute LD₅₀, the dietary value should be used in the acute risk assessment. Therefore, LC₅₀ > 1326 mg a.s./kg bw/d was used in the acute risk assessment as worst-case.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use groups “cereals” and “oilseed rape” were assessed separately (see 9.1.2).

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

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

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

Intended use		Cereals				
Active substance/product		Mepiquat chloride				
Application rate (g/ha)		1 x 28.5 / 1 x 37.5 g mepiquat chloride /ha equivalent to 1 x 28.5g mepiquat/ha				
Acute toxicity (mg/kg bw)		> 1326				
TER criterion		10				
Crop scenario	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	4.53 5.95	293.0 222.85	
Reprod. toxicity (mg/kg bw/d)		100.7				
TER criterion		5				
Crop scenario	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 x 0.53	0.98 1.29	102.9 78.06	

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

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of MEPISHA in oilseed rape

Intended use		Oilseed rape				
Active substance/product		Mepiquat chloride				
Application rate (g/ha)		1 x 28.5 1 x 37.5 g mepiquat chloride/ha equivalent to 1 x 28.5 g mepiquat/ha				
Acute toxicity (mg/kg bw)		> 1326				
TER criterion						
Crop scenario		Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage	Oilseed rape	Indicator species for screening	158.8	1.0	4.53 5.95	293.0 222.85
Reprod. toxicity (mg/kg bw/d)		100.7				
TER criterion						
Crop scenario		Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Growth stage	Oilseed rape	Indicator species for screening	64.8	1 x 0.53	0.98 1.29	102.9 78.06

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.

Conclusion:

According to the screening, all the TER_a and TER_{lt} values for the active substance mepiquat are greater than the annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to birds according to the intended uses.

zRMS comment:

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 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.

9.2.2.2 Higher-tier risk assessment

Not required.

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 MEPISHA 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of ~~702.02–890~~ (geometric mean, $n = 12$ (EFSA Scientific Report (2005) 45, 1–61 EFSA Scientific Report (2008) 146, 1–73)), Mepiquat **chloride** belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for birds from all other intended uses (see 9.1.2).

Effective application rate (g/ha) =	28.5–37.5		
Acute toxicity (mg/kg bw) =	> 1326	quotient =	0.02 0.023
Reprod. Toxicity (mg/kg bw/d) =	100.7	quotient =	0.28 0.37

As the ratios do not exceed the value of 3000 for mepiquat, it is not necessary to conduct a drinking water risk assessment for birds.

zRMS comments:

We agree that hazard quotient for Puddle scenario for Mepiquat chloride below trigger value 50, so no specific calculations of exposure and TER are necessary

9.2.2.4 Effects of secondary poisoning

The log Pow of Mepiquat chloride amounts to -3.45 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

zRMS comment:

As active substance has log Pow of less than 3 it is therefore considered that secondary poisoning is not expected to occur from the proposed use of Mepisha.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

According to the screening assessment, all the TER_a and TER_{lt} values for the active substance mepiquat chloride are greater than the annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to birds according to the intended uses.

Moreover, the risk for birds due to uptake of contaminated drinking water was considered as low and mepiquat shows low potential for bioaccumulation, hence, there is no risk to earthworm-eating and fish-eating birds according to the intended uses of MEPISHA.

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

Effects on mammals of MEPISHA (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of mepiquat [chloride](#). However, the provision of further data on the formulation is not considered essential, because endpoints obtained with the active substance 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 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Mepiquat chloride	Oral Acute	LD ₅₀ = 200 mg/kg bw	EFSA Scientific Report (2008) 146, 1-73
Rat	Mepiquat chloride	Oral Long-term	NOAED = 155 mg/kg bw	

9.3.1.1 Justification for new endpoints

The used endpoints were the EU agreed ones.

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 Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use groups “cereals” and “oilseed rape” were assessed separately (see 9.1.2).

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

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

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

Intended use	Cereals
Active substance/product	Mepiquat chloride
Application rate (g/ha)	1 x 28.5 / 1 x 37.5 g mepiquat chloride /ha equivalent to 1 x 28.5 g mepiquat/ha
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	Indicator species for screening		118.4	1.0	3.37 4.44	59.3 45.04
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 x 0.53	0.73 0.96	212.5 161.45

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

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of MEPISHA in oilseed rape

Intended use		Oilseed rape				
Active substance/product		Mepiquat chloride				
Application rate (g/ha)		1 x 28.5 / 1 x 37.5 g mepiquat chloride/ha equivalent to 1 x 28.5g mepiquat/ha				
Acute toxicity (mg/kg bw)		200				
TER criterion		10				
Crop scenario	Indicator/generic focal species		SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage						
Oilseed rape	Indicator species for screening		118.4	1.0	3.37 4.44	59.3 45.04
Reprod. toxicity (mg/kg bw/d)		155				
TER criterion		5				
Crop scenario	Indicator/generic focal species		SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Growth stage						
Oilseed rape	Indicator species for screening		48.3	1 x 0.53	0.73 0.96	212.5 161.45

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.

Conclusions:

According to the screening and first tier assessment, all the TER_a and TER_{lt} values for the active substance mepiquat **chloride** are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to mammals according to the intended uses.

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 mammals 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.

9.3.2.2 Higher-tier risk assessment

Not required.

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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of ~~702.02~~ **890** (geometric mean, $n = 12$ (EFSA Scientific Report (2005) 45, 1-61)), Mepiquat **chloride** belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for mammals from all other intended uses (see 9.1.2).

Effective application rate (g/ha) =	28.5 37.5		
Acute toxicity (mg/kg bw) =	200	quotient =	0.14 0.19
Reprod. toxicity (mg/kg bw/d) =	155	quotient =	0.18 0.24

As the ratios do not exceed the value of 3000 for mepiquat **chloride**, it is not necessary to conduct a drinking water risk assessment for mammals.

zRMS comments:

We agree that hazard quotient for Puddle scenario for Mepiquat chloride are below trigger value 50, so no specific calculations of exposure and TER are necessary.

9.3.2.4 Effects of secondary poisoning

The log Pow of Mepiquat amounts to -3.45 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

According to the screening and first tier assessment, all the TER_a and TER_{lt} values for the active substance mepiquat are greater than the annex VI trigger of 10 and 5, respectively, indicating that MEPISHA presents no unacceptable acute and long-term risk to mammals according to the intended uses.

Moreover, the risk for mammals due to uptake of contaminated drinking water was considered as low and mepiquat shows low potential for bioaccumulation, hence, there is no risk to earthworm-eating and fish-eating mammals according to the intended uses of MEPISHA.

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

Effects on aquatic organisms of MEPISHA (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of mepiquat [chloride](#). 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 – mepiquat [chloride](#)

Species	Substance	Exposure System	Results	Reference
Fish				EFSA Scientific report (2008) 146, 1-73
<i>Oncorhynchus mykiss</i>	Mepiquat chloride	96 h, s	$LC_{50} > 100 \text{ mg a.s./L}$	
<i>Oncorhynchus mykiss</i>	Mepiquat chloride	28 d, f	$NOEC = 100 \text{ mg a.s./L}$	
<i>Oncorhynchus mykiss</i>	Mepiquat chloride	95 d, f	$NOEC = 100 \text{ mg a.s./L}$	

Species	Substance	Exposure System	Results	Reference
Aquatic invertebrate				
Daphnia magna	Mepiquat chloride	48 h, s	EC ₅₀ = 68.5 mg a.s./L	
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 _b C ₅₀ = 14.4 mg a.s./L E _r C ₅₀ = 44.8 mg a.s./L	
Higher plant				
Lemna gibba	Mepiquat chloride	14 d, s	E _b C ₅₀ = 2.6 mg a.s./L E _r C ₅₀ = 15.41 mg a.s./L	
Higher-tier studies (micro- or mesocosm studies)				
No study submitted, not required.				

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

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

Species	Substance	Exposure System	Results	Reference
<i>O. mykiss</i>	MEPISHA	96 h, s	LC ₅₀ = 333.5 mg/L _{nom} (LC ₅₀ = 16.8 mg a.i./L)*	KCP 10.2.1-01 Nazhath, S., 2021 G14244
<i>R. subcapitata</i>	MEPISHA	72 h, s	E _r C ₅₀ = 75.83 mg/L _{nom} (E _r C ₅₀ = 3.81 mg a.i./L)* E _y C ₅₀ = 21.31 mg/L _{nom} (E _y C ₅₀ = 1.07 mg a.i./L)*	KCP 10.2.1-02 Nazhath, S., 2021 G14245
<i>D. magna</i>	MEPISHA	48 h, s	EC ₅₀ = 203.6 mg/L _{nom} (EC ₅₀ = 10.42 mg/L)*	KCP 10.2.1-03 Nazhath, S., 2021 G14246
<i>L. gibba</i>	MEPISHA	7 d, s	Fronde: E _r C ₅₀ = 16.8312 mg/L _{nom} (E _r C ₅₀ = 0.8466 mg a.i./L)* E _y C ₅₀ = 7.9474 mg/L _{nom} (E _y C ₅₀ = 0.7964 mg a.i./L)* Dry weight: E _r C ₅₀ = 20.3396 mg/L _{nom} (E _r C ₅₀ = 1.0231 mg a.i./L)* E _y C ₅₀ = 7.9596 mg/L _{nom} (E _y C ₅₀ = 0.8950 mg a.i./L)*	KCP 10.2.1-04 Radha, S., 2021 6090/2019
Higher-tier studies (micro- or mesocosm studies)				
No study submitted, not required.				

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

*mepiquat chloride

9.5.1.1 Justification for new endpoints

The LoEP from EFSA Scientific report (2008) 146, 1-73 for mepiquat was used. **Studies were con-ducted with MEPISHA and were also considered for 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, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

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

Group		Fish acute	Fish pro- longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. capricornu- tum</i>	<i>L. gibba</i>
Endpoint (µg/L)		LC ₅₀ 100000	NOEC 100000	EC ₅₀ 68500	NOEC 12500	ErC ₅₀ 44800	ErC ₅₀ 15410
AF		100	10	100	10	10	10
RAC (µg/L)		1000	10000	685	1250	4480	1541
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	5.17 6.81	0.005 0.00681	0.001 0.000681	0.008 0.0099	0.004 0.0054	0.001 0.0015	0.003 0.044
Step 2							
N-Europe	0.40	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
S-Europe	0.63	0.001	<0.001	0.001	0.001	<0.001	<0.001

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Mepiquat chloride for each organism group based on FOCUS Steps 1 and 2 calculations for the use of MEPISHA in winter oilseed rape

Group		Fish acute	Fish pro- longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. capricornu- tum</i>	<i>L. gibba</i>
Endpoint (µg/L)		LC ₅₀ 100000	NOEC 100000	EC ₅₀ 68500	NOEC 12500	E _r C ₅₀ 44800	E _r C ₅₀ 15410
AF		100	10	100	10	10	10
RAC (µg/L)		1000	10000	685	1250	4480	1541
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
-	5.17 6.80	0.005 0.0068	0.001 0.00068	0.008 0.099	0.004 0.0054	0.001 0.0015	0.003 0.0044
Step 2							
N-Europe	0.36	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
S-Europe	0.55	0.001	<0.001	0.001	<0.001	<0.001	<0.001

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

zRMS comment:

The zRMS calculated the risk assessment with the above endpoints according to the EFSA conclusions. Regarding the algal and macrophytes toxicity endpoints, the following should be noted:

The endpoint E_rC₅₀ is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae and shall be performed at EU level.

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 ppp Mepisha.

9.5.3 Overall conclusions

For all intended uses, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for *Daphnia* acute as characterised by an EC₅₀ of 68500 µg/L in connection with an assessment factor of 100) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

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

Effects on bees of MEPISHA (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of mepiquat. New data submitted with this application are listed in Table 9.6-1 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
<i>Apis mellifera</i>	Mepiquat chloride	Oral	LD₅₀ > 107.4 µg a.s./bee	EFSA Scientific report (2008) 146, 1-73
<i>Apis mellifera</i>	Mepiquat chloride	Contact	LD₅₀ > 100 µg a.s./bee	
<i>Apis mellifera</i>	MEPISHA	Oral	LD₅₀ > 100 µg/bee	KCP 10.3.1.1.1 Nazhath, S., 2019 G14247
<i>Apis mellifera</i>	MEPISHA	Contact	LD₅₀ > 100 µg/bee	KCP 10.3.1.1.2 Nazhath, S., 2019 G14248
Higher-tier studies (tunnel test, field studies)				
Not required.				

9.6.1.1 Justification for new endpoints

The endpoints used did not differ from the EU agreed one (EFSA Scientific report (2008) 146, 1-73). Additionally new studies were conducted with the formulation MEPISHA and the risk assessment was also done considering these new endpoints.

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for bees from all other intended uses (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of MEPISHA in all crops

Intended use	All crops		
Active substance	Mepiquat chloride		
Application rate (g a.s./ha)	1 x 28.5 / 1 x 37.5 g mepiquat chloride/ha equivalent to 1 x 28.5 g mepiquat/ha		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 107.4	28.5	0.27 0.35
Contact toxicity	> 100	37.5	0.29 0.375
Product	MEPISHA		
Application rate (g/ha)	1 x 764.7*		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 100	764.7	7.65
Contact toxicity	> 100		7.65

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

* 0.5 L/ha taking into account a density value of 1.0196 g/mL.

zRMS comments:

The Q_{HO} and Q_{HC} values for the a.s. – mepiquat chloride and the formulation Mepisha are all below the trigger of 50 indicating an acceptable acute risk to adult bees based on the maximum intended use of Mepisha.

According to Reg. 284/2009 the chronic adult and chronic larvae tests for bees should be submitted by the applicant.

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

Studies on the toxicity to honeybees show that hazard quotients (oral and contact) for mepiquat and the formulation MEPISHA are clearly under the cut-off value. An application of MEPISHA to cereals and oilseed rape in respect of the GAP does not present an unacceptable risk for honeybees.

[According to Reg. 284/2009 the chronic adult and chronic larvae tests for bees should be submitted by the applicant.](#)

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

Effects on non-target arthropods of MEPISHA (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of 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 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	BAS 083 52 W (Mepiquat chloride 617.6 g/L)	Laboratory test glass plates (2D)	LR ₅₀ = 1366 g a.s./ha	ESFSA Scientific Report (2008) 146, 1-73
<i>Typhlodromus pyri</i>	BAS 083 52 W (Mepiquat chloride 617.6 g/L)	Laboratory test glass plates (2D)	LR ₅₀ = 1530 g a.s./ha	
<i>Chrysoperla carnea</i>	BAS 098 00W (308.2 g mepiquat chloride/L and 158.9 g ethephon/L)	Laboratory test glass plates (2D) Larvae 2-3 days old	LR ₅₀ > 5.00 L/ha (> 1525 g mepiquat-chloride/ha)	
<i>Aleochara billineata</i>	BAS 098 00W (308.2 g mepiquat chloride/L and 158.9 g ethephon/L)	Laboratory test sand (2D). Adults 2-6 days old	ER ₅₀ > 3.00 L/ha (> 915 g mepiquat-chloride/ha)	
<i>Aphidius rhopalosiphi</i>	MEPISHA	Laboratory test glass plates (2D)	LR ₅₀ > 3.8 L/ha (> 147.8 g a.s./ha)	KCP 10.3.2.1-01 Sonali, G., 2020 6050/2019
<i>Typhlodromus pyri</i>	MEPISHA	Laboratory test glass plates (2D)	LR ₅₀ > 3.8 L/ha (> 147.8 g a.s./ha)	KCP 10.3.2.1-02 Bala, P., 2020 6051/2019
Field or semi-field tests				
Not required.				

9.7.1.1 Justification for new endpoints

The used endpoints were the EU agreed ones. Studies were conducted with MEPISHA and were also considered for the risk assessment.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for non-target arthropods from all other intended uses (see 9.1.2).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of MEPISHA in all crops

Intended use		All crops	
Active substance/product		Mepiquat chloride	
Application rate (g/ha)		1 x 28.5 / 1 x 37.5 g mepiquat chloride equivalent to 1 x 28.5 mepiquat/ha	
MAF		1	
Test species Tier I	LR₅₀ (lab.)/ER₅₀ (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Aphidius rhopalosiphi</i>	1366	28.5 37.5	0.02
<i>Typhlodromus pyri</i>	1530		0.02
<i>Aphidius rhopalosiphi</i>	147.8 >194*		0.19 0.25
<i>Typhlodromus pyri</i>	147.8 >194* 122.5**		0.19 0.25* 0.306**

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.

*LR₅₀ for mepiquat chloride

*ER₅₀ for mepiquat chloride

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for non-target arthropods from all other intended uses (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of MEPISHA in all crops

Intended use		All crops			
Active substance/product		Mepiquat chloride			
Application rate (g/ha)		1 x 28.5 / 1 x 37.5 g mepiquat chloride /ha equivalent to 1 x 28.5 mepiquat/ha			
MAF		1			
vdf		10 (Tier 1)			
Test species	LR₅₀ (lab.) (g/ha)	Drift rate	PER_{off-field} (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
Tier I					
<i>Aphidius rhopalosiphi</i>	1366	0.0277	0.079 1.03	10	0.001
<i>Typhlodromus pyri</i>	1530				0.001
<i>Aphidius rhopalosiphi</i>	147.8 >194 *				0.005 0.0053*
<i>Typhlodromus pyri</i>	147.8 >194 122.5**				0.005 0.0053* 0.0084**

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.

*LR₅₀ for **mepiquat chloride**

ER₅₀ for **mepiquat chloride

zRMS comment:

The risk from the formulation Mepisha based on the results from laboratory studies for two indicator species T.pyri and Aphidius rhopalosiphi.

The HQ_{in-field} and HQ_{off-field} values were below trigger of 2, indicating an acceptable risk to NTA from exposure of Mepisha (expressed in mepiquat chloride).

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 values calculated for the representative species *T. pyri* and *A. rhopalosiphi*, are lower than the trigger of 2 for first-tier tests, indicating no risk to non-target arthropods in vegetated in-field and off-field areas following application of MEPISHA according to the proposed use patterns.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mepiquat chloride and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of MEPIQUAT (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of 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 9.8-1: 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
<i>Eisenia fetida</i>	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
<i>Eisenia fetida</i>	Mepiquat chloride	Mixed into substrate 56 d, chronic 10 % peat content	No data submitted or required.	EFSA Scientific Report (2008) 146, 1- 73
<i>Eisenia fetida</i>	MEPISHA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 52.92 mg f.p./kg dw soil NOEC = 2.03 mg mepiquat/kg dw soil (NOEC _{corr} = 1.02 mg-mepiquat/kg dw*) NOEC=2.66 mg mepiquat chloride/kg dws	KCP 10.4.1.1 Murali, K., 2021 9549/2021
<i>Folsomia candida</i>	MEPISHA	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 171.47 mg f.p./kg dw soil NOEC = 6.57 mg mepiquat /kg dw soil (NOEC _{corr} = 3.29 mg mepiquat/kg dw*) NOEC = 8.62 mg mepiquat chloride/kg dw soil	KCP 10.4.2.1-01 Murali, K., 2020 6091/2019

Species	Substance	Exposure System	Results	Reference
<i>Hypoaspis aculeifer</i>	MEPISHA	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 308.64 mg f.p./kg dw soil NOEC = 11.83 mg mepiquat/kg dw soil (NOEC_{corr} = 5.92 mg /kg dw*) NOEC = 15.52 mg mepiquat chloride/kg dw soil	KCP 10.4.2.1-02 Rajeshwari, S., 2020 6092/2019
Since the HQ values for the two standard non-target arthropods are below 2 and no effects were observed on the soil dwelling <i>Aleochara bilineata</i> no studies are required although DT ₉₀ in soil is longer than 100 days.				
Field studies				
Not required.				
Litter bag test				
Not required.				

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

9.8.1.1 Justification for new endpoints

The endpoints used were the EU agreed ones, except for formulation, corresponding to data proper to MEPISHA formulation.

Chronic studies with MEPISHA on **earthworms**, collembolan and predatory soil mite were submitted by the Applicant and no unacceptable risk was obtained after the risk assessment. Moreover, the long-term risk to earthworms exposed to mepiquat **chloride** is regarded as low according to approach as per EFSA conclusions (EFSA Scientific Report (2008) 146, 1-73) and the risk assessment for NTA was acceptable with endpoints for tested indicator species. In addition, *Chrysoperla carnea* and *Aleochara bilineata* extended laboratory studies with representative formulation present in the EFSA conclusions also concluded low risk on mortality and reproduction. Therefore, the Applicant considers that an acceptable risk to **earthworm non-target soil organisms** for MEPISHA formulation can be concluded.

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 is considered for mepiquat **chloride**.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses (see 9.1-2).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of MEPISHA in all crops

Intended use		Cotton-All crops	
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Mepiquat chloride	319.5	0.008	39937.5
Chronic effects on earthworms			
Product/active substance	NOEC (mg a.s /kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER > 5)
MEPISHA (Mepiquat)	NR	0.008	NR
MEPISHA (expressed in mepiquat)	1.02	0.008	127.5
MEPISHA (expressed in mepiquat chloride)	2.03*	0.008	253
	2.66**	0.010	266
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER > 5)
MEPISHA (Folsomia candida) (expressed in mepiquat)	3.29	0.008	411.3
MEPISHA (expressed in mepiquat chloride)	6.57*	0.008	821.25
	8.62**	0.010	862
MEPISHA (Hypoaspis aculeifer) (expressed in mepiquat)	5.92	0.008	740
MEPISHA (expressed in mepiquat chloride)	11.83*	0.008	1478.8
	15.52**	0.010	1552

TER values shown in bold fall below the relevant trigger.

*the endpoint for mepiquat

** the endpoint for mepiquat chloride

zRMS comment:

The risk assessment was verified by zRMS in the Table above.

The PEC soil calculations evaluated by e-fate experts in Section 8 was taken into account.

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).

The TER_{LT} values were above trigger of 5 for ppp Mepisha for earthworm and other soil macro-organism indicating an acceptable risk.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The acute and chronic TER for Mepiquat are above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, according to the risk assessment and the effects on other soil arthropods, it is concluded that Mepiquat and MEPISHA formulation do not pose acute and long-term risk to earthworms and other soil macro- and mesofauna.

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

Effects on soil microorganisms of MEPIQUAT (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of 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	Mepiquat chloride	28 days	< 25% effect at day 28 at 1.352 mg a.s./kg dw soil	EFSA Scientific report (2008) 146, 1-73
C-mineralisation	Mepiquat chloride	84 days	< 25% effect at day 84 at 1.352 mg a.s./kg dw soil	
N-mineralisation	MEPISHA	28 d, aerobic sandy clay loam soil	Effects <25% at 10.14 mg f.p./kg soil (0.388 mg mepiquat/kg d.w. soil) Effects <25% at 33.80 mg f.p./kg soil (1.29 mg mepiquat/kg d.w. soil)	KCP 10.5.1 Anand, H. S. 2020 G14252
C-mineralisation	MEPISHA	28 d, aerobic sandy clay loam soil	Effects <25% at 10.14 mg f.p./kg soil (0.388 mg mepiquat/kg d.w. soil) Effects <25% at 33.80 mg f.p./kg soil (1.29 mg mepiquat/kg d.w. soil)	KCP 10.5.2 Anand, H. S. 2020 G14251

9.9.1.1 Justification for new endpoints

The used endpoints were the EU agreed ones, except for formulation, corresponding to data proper to MEPISHA formulation.

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 and 8.7-4 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for the soil microorganisms from all other intended uses (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of MEPISHA in all crops

Intended use	All crops		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Mepiquat chloride	1.352 (at 28 d)	0.008	yes
MEPISHA (product) Mepisha (expresses in mepiquat)	33.80 (at 28 d) 0.388-0.129	0.2039 0.008	Yes yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Mepiquat	1.352 (at 28 d)	0.008	yes
MEPISHA	33.80 (at 28 d)	0.2039	yes

ZRMS comments:

The risk assessment for soil micro-organism after exposure of active substance was accepted by zRMS with consideration $PECs$ values agreed by e-fate experts in Section 8. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant $PECs$ soil for the maximum application rate of active substances mepiquat chloride (expressed in mepiquat) and the product Mepisha.

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for the active substance mepiquat indicate a low risk to soil microorganisms when applied according to the proposed use rates. The use of MEPISHA 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 mepiquat. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of MEPIQUAT (Mepiquat 3.8% SL) were not evaluated as part of the EU assessment of mepiquat.

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
<i>Daucus carota</i> <i>Linum usitatissimum</i> <i>Brassica napus</i> <i>Pisum sativum</i> <i>Avena sativa</i> <i>Allium cepa</i>	BAS 098 00W (308.2 g mepiquat chloride and 158.8 g ethephon/L)	Seedling emergence	ER ₅₀ > 3000 mL formulation/ha ER ₅₀ > 924.6 g a.s./ha	EFSA Scientific Report (2008) 146, 1-73
<i>Daucus carota</i> <i>Linum usitatissimum</i> <i>Brassica napus</i> <i>Pisum sativum</i> <i>Avena sativa</i> <i>Allium cepa</i>	BAS 098 00W (308.2 g mepiquat chloride and 158.8 g ethephon/L)	Vegetative vigour	ER ₅₀ > 3000 mL *formulation/ha ER ₅₀ > 924.6 g a.s./ha	
<i>Glycine max</i> <i>Solanum lycopersicon</i> <i>Raphanus sativus</i> <i>Pisum sativum</i> <i>Sinapis alba</i> <i>Zea mays</i>	MEPISHA	Seedling emergence	ER ₅₀ > 3000 mL formulation/ha (ER ₅₀ > 116.7 g mepiquat/ha) ER ₅₀ >153.2 g mepiquat chloride/ha	KCP 10.6.2-01 Radha, S., 2020 6093/2019
<i>Glycine max</i> <i>Solanum lycopersicon</i> <i>Raphanus sativus</i> <i>Pisum sativum</i> <i>Sinapis alba</i> <i>Zea mays</i>	MEPISHA	Vegetative vigour	ER ₅₀ > 3000 mL formulation/ha (ER ₅₀ > 116.7 g mepiquat/ha) ER ₅₀ >153.2 g mepiquat chloride/ha	KCP 10.6.2-02 Radha, S., 2020 6094/2019

9.10.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones.

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”, (SAN-CO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “all crops” covers the risk for non-target terrestrial plants from all other intended uses (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of MEPISHA in all crops

Intended use		All crops		
Active substance/product		Mepiquat chloride		
Application rate (g a.s./ha)		1 x 28.5 / 1 x 37.5 g mepiquat chloride/ha equivalent to 1 x 28.5g mepiquat/ha		
MAF		1		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
All tested plants	924.6 (Seedling emergence)	0.0277	0.79	1171.2
All tested plants	924.6 (Vegetative vigour test)	0.0277	0.79	1171.2
All tested plants	> 116.7 (Seedling emergence)* >153.2 (Seedling emergence)**	0.0277	0.79 1.03	147.8* 148.73
All tested plants	> 116.7 (Vegetative vigour test)* >153.2 (Seedling emergence)**	0.0277	0.79 1.03	147.8* 148.73

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

*the endpoint for mepiquat

** the endpoint for mepiquat chloride

The calculated TER values are higher than the trigger of 5 both for seedling emergence and vegetative vigour test, indicating no unacceptable risk to non-target plants. Therefore, appropriate risk mitigation measures are not necessary.

zRMS comment:

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SAN-CO/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. The deterministic risk based on the ER₅₀ >116.7 g mepiquat /ha and PER_{off-field} values 0.79 g mepiquat /ha indicated an acceptable risk for non-target plants. No mitigation measures are required.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not necessary.

9.10.3 Overall conclusions

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for mepiquat shows that the Annex VI trigger value of 5 is reached, indicating that MEPISHA poses a low risk to non-target plants when applied according to the proposed use rates.

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

Mepiquat:

Effects on biological methods for sewage treatment:

Activated sludge respiration > 1000 g mepiquat chloride/L

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	MEPISHA
Common Name	MEPISHA, Mepiquat 3.8% SL
Classification and proposed 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	xxxxxxxxxxxxxxxx	2021	Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: Fish, acute toxicity test with rainbow trout. xx GLP Unpublished	Y	Sharda Cropchem Limited
KCP 10.2.1-02	Nazhath, S.	2021	Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: Alga, Growth Inhibition Test with <i>Raphidocelis subcapita</i> . Eurofins Advinus Limited. Report No. G14245 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-03	Nazhath, S.	2021	Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: <i>Daphnia magna</i> , Acute Immobilization Test. Eurofins Advinus Limited. Report No. G14246 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-04	Radha, S.	2021	Study of <i>Lemna gibba</i> growth inhibition with Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL. Bioscience Research Foundation. Report No. 6090/2019 GLP	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
			Unpublished		
KCP 10.3.1.1.1	Nazhath, S.	2019	Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL: acute oral toxicity test in honey bees Eurofins Advinus Limited. Report No. G14247 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.1.2	Nazhath, S.	2019	Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL: acute contact toxicity test in honey bees Eurofins Advinus Limited. Report No. G14248 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1- 01	Sonali, G.	2020	A laboratory test for evaluating the effects of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani - Perez) Bioscience research foundation. 6050/2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1- 02	Bala, P.	2020	A laboratory test for evaluating the effects of Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v on the predatory mite, <i>Typhlodromus pyri</i> (Scheuten) Bioscience research foundation. 6051/2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.4.1.1	Murali, K.	2021	Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on reproduction of the earthworms (<i>Eisenia fetida</i>) in artificial soil. Bioscience research foundation. 9549/2021 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.4.2.1- 01	Murali, K.	2020	Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the collembolans (<i>Folsomia candida</i>) in artificial soil. Bioscience research foundation. 6091/2019	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
			GLP Unpublished		
KCP 10.4.2.1-02	Rajeshwari, S.	2020	Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the Reproductive Output of the Predatory Soil Mite <i>Hypoaspis (Geolaelaps) aculeifer</i> Canestrini (Acari: Laelapidae) in Artificial Soil Bioscience research foundation. 6092/2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.5.1	Anand, H. S.	2020	Soil microorganisms: nitrogen transformation test of mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL. Eurofins Advinus Limited. Report No. G14252 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.5.2	Anand, H. S.	2020	Soil microorganisms: carbon transformation test of mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL Eurofins Advinus Limited. Report No. G14251 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-01	Radha, S.	2020	Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL on seedling emergence and seedling growth of terrestrial plants. Bioscience Research Foundation. Report No. 6093/2019 GLP Unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-02	Radha, S.	2020	Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on vegetative vigour of terrestrial plants Bioscience Research Foundation. Report No. 6094/2019 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 valid. All validity criteria were met.		
	<ul style="list-style-type: none"> 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 7.73 to 7.79 and the temperature of the test solutions was 13.0 to 13.9 °C. The dissolved oxygen saturation of the test solutions ranged from 85 to 98 % which is more than 60% of the air saturation value throughout the test. The concentration of the test item in all tested concentrations was between 81.452 and 102.700 % of the nominal concentrations which was within \pm 20% during the test. 		
	Agreed endpoints:		
	Endpoint at 96 h	Test item (mg/L)	Mepiquat chloride (mg/L)
	LC ₅₀	333.5 (253.2 – 435.4)*	16.8
	NOEC	88.0	4.43
	LOEC	194	9.76
*: fiducial limits at 95 per cent			

Reference:	KCP 10.2.1 - 01
Report	“Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: Fish, acute toxicity test with rainbow trout”. XX
Guideline(s):	Yes, From study plan. The criteria for acceptance of analysis results of test concentration was 80 to 120 % of nominal concentration instead of claimed concentration. This deviation did not have any impact on the outcome of the study
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Test item:	Description:	Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL
	Production batch:	SCL - 18246
	A.i. content:	Chloride, 51.05 g/L Ion, 38.9 g/L

Test system:	Species: Rainbow trout (<i>Oncorhynchus mykiss</i>)
	Strain: -
	Average weight: 12.2468 g
	Average length: 4.1 – 4.6 cm
	Source: Neveed Habba Kadal Chowk, Srinagar, Jammu and Kashmir – 190001.
	Acclimation period: 7 days

Experimental conditions:	
Temperature:	13.0 – 13.9°C
Dissolved O ₂ :	85 – 98%
Hardness:	250 as mg CaCO ₃ /L
pH:	7.73 – 7.79
Light and photoperiod:	569 (lux), 16h light and 8h dark
Test procedure:	Static
Experimental period:	96h ± 1 h

Static system (96 hours, one replicate of ten fish for each test item concentration and the control). The definitive test was carried out using nominal test concentrations 40.0, 88.0, 194, 427 and 939 mg/L plus a control in a static mode. Seven fish were used for each test solution with single replicate per test concentration. The fish were observed for mortalities and visible abnormalities after 2 ± 0.5 h, 5 ± 1 h, 24 ± 2 , 30 ± 2 , 48 ± 2 , 54 ± 2 , 72 ± 2 , 78 ± 2 and 96 ± 2 h. The concentrations of the active substances were chemically determined using a validated analytical method.

The active ingredient concentration analysis in all test concentrations showed that the recovery with the nominal concentration was 81.452 to 97.821% (RSD was 1.213 to 2.687 %) at the start and 89.756 to 102.700 % (RSD was 1.601 to 4.320 %) at the end of the test (96 hour) indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

Results

There were no mortalities of fish in the negative control and at the tested concentrations of 40.0 and 88.0 mg/L. There was 28.6, 57.1 and 100% mortality at the tested concentrations of 194, 427 and 939 mg/L at 96 hour exposure respectively.

Conclusion

Endpoint at 96 h	Test item (mg/L)	Mepiquat chloride (mg/L)
LC ₅₀	333.5 (253.2 – 435.4)*	16.8
NOEC	88.0	4.43
LOEC	194	9.76

*: fiducial limits at 95 per cent

Comments of zRMS:	The study is considered valid. All validity criteria were met.		
	<ul style="list-style-type: none"> There was an increase in cell concentration of the negative control culture by a factor of 77.29, 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 22.35 %, which is within the required limit of 35%. The coefficient of variation of average growth rate between replicate cultures of negative control was 0.12 %, which is within the required limit of 7 %. 		
	Agreed endpoints:		
	Observations	EC values (µg test item/L)	EC values (µg Mepiquat chloride/L)
	72 hours	ErC ₅₀	75.83 (47.56 – 124.00)
		ErC ₂₀	22.30 (9.54 – 42.92)
		ErC ₁₀	10.90 (3.14 – 26.42)
		EyC ₅₀	21.31 (0.00 – 0.00)
		EyC ₂₀	18.04 (13.63 – 0.00)
		EyC ₁₀	16.37 (8.83 – 0.00)
		LOEC	6.25
		NOEC	0.00 (<6.25)
	Note: 1) ErC refers to growth rate, EyC refers to yield. 2) Range mentioned after EC value refers to 95% fiducial limits.		

Reference:

KCP 10.2.1-02

Report

“Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: Alga, Growth Inhibition Test with *Raphidocelis subcapita*”, Saiqa Nazhath, (2021), Report No. G14245. Eurofins Advinus Limited.

Guideline(s):

OECD Guideline No. 201 (2006)

Deviations:

Yes, From study plan. The criteria for acceptance of analysis results of test concentration was 80 to 120 % of nominal concentration instead of claimed

concentration. This deviation did not have any impact on the outcome of the study.

GLP: Yes
Acceptability: Yes
Duplication (if vertebrate study) Not relevant

Materials and methods

The effect of Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL was tested on the growth of freshwater unicellular green alga *Raphidocelis subcapitata* for 72 hours.

The algae were exposed to the test item at the test concentrations of 6.25, 12.5, 25.0, 50.0, 100, 200 and 400 mg/L along with a negative control. Six replicates were maintained for the negative control and three replicates for each of the test concentrations. The initial cell density of algal cells at the start of exposure was 1×10^4 /mL. The cell growth was measured at 24, 48 and 72 hours after the initiation of the test using a haemocytometer (microscopic observation).

The test item was recoverable at the concentrations of 5.0 µg/L and 500 mg/L in the matrix. The active ingredient concentration analysis in all test concentrations showed that the recovery with the nominal concentration was from 92.174 to 97.124 % at the start of the test (RSD was 0.854 to 2.664 %), 87.869 to 96.262 % at 24 hour (RSD was 0.910 to 4.805 %), 90.351 to 100.399 % at 48 hour (RSD was 0.1466 to 4.178 %) and 108.89317 to 108.999 % at the end of the test (72 hour) (RSD was 0.215 to 3.985 %) indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

Results

Definitive test

At the end of the test (72 hours), the cell biomass in the test item solutions decreased with the increase in test concentration.

Table 1 Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	% Inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0	0
6.25	-1.32	-5.95*
12.5	-0.99	-4.42*
25	35.06	79.25
50	40.26	83.72
100	40.31	89.95
200	80.54	98.25
400	83.16	98.58

*: to be considered as 0

Validity criteria

- There was an increase in cell concentration of the negative control culture by a factor of 77.29, 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 22.35 %, which is within the required limit of 35%.
- The coefficient of variation of average growth rate between replicate cultures of negative control was 0.12 %, which is within the required limit of 7 %.

Conclusion

The endpoint values determined for growth rate and yield at 24, 48 and 72 hours are presented below based on nominal concentration.

Table 2 - Endpoint values for Growth rate and yield

Observations		EC values (µg test item/L)	EC values (µg Mepiquat chloride/L)
24 hours	E _r C ₅₀	55.99 (0.00 – 63.25)	2.82 (0.00 – 3.18)
	E _r C ₂₀	44.92 (36.14 – 0.00)	2.26 (1.82 – 0.00)
	E _r C ₁₀	39.48 (28.53 – 0.00)	1.99 (1.44 – 0.00)
	E _y C ₅₀	45.29 (38.09 – 53.19)	2.28 (1.92 – 2.68)
	E _y C ₂₀	28.62 (21.38 – 38.66)	1.44 (1.08 – 1.94)
	E _y C ₁₀	21.88 (14.74 – 33.33)	1.10 (0.74 – 1.68)
	LOEC	50.0	2.52
	NOEC	25.0	1.26
48 hours	E _r C ₅₀	56.86 (46.88 – 68.80)	2.86 (2.36 – 3.46)
	E _r C ₂₀	44.34 (27.25 – 53.84)	2.23 (1.37 – 2.71)
	E _r C ₁₀	38.34 (19.21 – 0.00)	1.93 (0.97 – 0.00)
	E _y C ₅₀	33.82 (26.85 – 42.57)	1.70 (1.35 – 2.14)
	E _y C ₂₀	18.22 (12.65 – 25.05)	0.92 (0.64 – 1.26)
	E _y C ₁₀	12.69 (7.70 – 19.14)	0.64 (0.39 – 0.96)
	LOEC	25.0	1.26
	NOEC	12.5	0.63
72 hours	E _r C ₅₀	75.83 (47.56 – 124.00)	3.81 (2.39 – 6.24)
	E _r C ₂₀	22.30 (9.54 – 42.92)	1.12 (0.48 – 2.16)
	E _r C ₁₀	10.90 (3.14 – 26.42)	0.55 (0.16 – 1.33)
	E _y C ₅₀	21.31 (0.00 – 0.00)	1.07 (0.00 – 0.00)
	E _y C ₂₀	18.04 (13.63 – 0.00)	0.91 (0.69 – 0.00)
	E _y C ₁₀	16.37 (8.83 – 0.00)	0.82 (0.44 – 0.00)
	LOEC	6.25	0.31
	NOEC	0.00 (<6.25)	<0.31

Note: 1) E_rC refers to growth rate, E_yC refers to yield.
2) Range mentioned after EC value refers to 95% fiducial limits.

Comments of zRMS:

The study is considered valid. 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.

Agreed endpoints:

Endpoint	Value [mg test item/L]	Mepiquat chloride [mg a.s./L]
EC ₅₀	203.6 (196.7 – 211.8) ^a	10.24
NOEC	88.0	4.43
LOEC	194	9.76

a: Fiducial limits at 95% based on nominal concentrations

Reference:	KCP 10.2.1-03
Report	“Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL: <i>Daphnia magna</i> , Acute Immobilization Test”, Saiqa Nazhath (2021), Report No. G14246. Eurofins Advinus Limited
Guideline(s):	OECD Guideline No. 202 (2004)
Deviations:	Yes, From study plan. The criteria for acceptance of analysis results of test concentration was 80 to 120 % of nominal concentration instead of claimed concentration. This deviation did not have any impact on the outcome of the study
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

The acute immobilization effect of the test item Mepiquat chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL was studied on *Daphnia magna* for 48 hours.

In definitive test, *Daphnia magna* less than 24 hours old were exposed to the nominal concentrations of 40.0, 88.0, 194, 427, 939 mg test item/L along with a negative control. The number of daphnia immobilized was recorded at 24 and 48 hours exposure.

All the test concentrations along with the negative control were analysed for the test item concentration at the beginning and end of test. For analysis, single composite sample was drawn from prepared test concentrations. The stability test results concluded that the test item was stable in the test medium at 97 h at 5.0 and 1000 mg/L nominal concentrations. The active ingredient concentration analysis in all test concentrations showed that the recovery with the nominal concentration was 88.451 to 103.413 % at the start of the test (RSD was 0.478 to 1.870 %) and 89.322 to 102.684 % at the end of the test (48 hour) (RSD was 0.511 to 5.243 %) indicating that the results were within the acceptable limit (80 to 120 % of the nominal concentration with an RSD of < 20%).

Results

Main test

There was no immobilization of daphnia in the negative control and at the tested concentrations of 40.0 and 88.0 mg/L at 24 and 48 hours of exposure. The immobilization of daphnia was 20, 30 and 50% at 24 h and 45, 95 and 100 % at 48 h exposure at 194, 427 and 939 mg/L.

Table 1 - Immobilization of *Daphnia magna*, definitive test

Treatment [mg/L]	Number of <i>Daphnia</i> immobilized (5 <i>Daphnia</i> per replicate)								% Immobilization	
	24 h				48 h					
	Replicates									
	R1	R2	R3	R4	R1	R2	R3	R4	24 h	48 h
Negative control	0	0	0	0	0	0	0	0	0	0
40.0	0	0	0	0	0	0	0	0	0	0
88.0	0	0	0	0	0	0	0	0	0	0
194	1	0	2	1	2	2	3	2	20	45*
427	2	1	1	2	4	5	5	5	30	95*
939	3	2	3	2	5	5	5	5	50	100*

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

Table 2 – Immobilization. Endpoint values at 48 hours

Endpoint	Value [mg test item/L]	Mepiquat chloride [mg a.s./L]
EC ₅₀	203.6 (196.7 – 211.8) ^a	10.24
NOEC	88.0	4.43
LOEC	194	9.76

a: Fiducial limits at 95% based on nominal concentrations

Comments of zRMS:

The study is considered valid. All validity criteria were met.

The results are considered valid because the following criteria were satisfied

- The doubling time of frond number in the control was 1.87 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 13.4).
- The average specific growth rate in the control between day 0 and day 7 was 0.371 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹)

Agreed endpoints:

Endpoint	Test item mg/L (based on nominal concentrations)	Mepiquat chloride mg/L (based on nominal concentrations)	Mepiquat ion mg/L (based on nominal concentrations ^a)
Yield—based on frond number			
E ₁₀	2.0223 (0.9761 – 1.5967)	0.0849 (0.0644 – 0.1054)	0.0775 (0.0665 – 0.0885)
E ₂₀	3.2351 (2.2711 – 3.2771)	0.1831 (0.1499 – 0.2163)	0.1240 (0.1100 – 0.1380)
E ₅₀	7.9474 (0.8571 – 12.2753)	0.7964 (0.2166 – 0.8762)	0.3046 (0.2825 – 0.3267)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198
Yield—based on dry weight			
E ₁₀	1.5417 (1.2675 – 1.8159)	0.0988 (0.0762 – 0.1214)	0.0594 (0.0486 – 0.0696)
E ₂₀	2.7084 (2.2302 – 3.0866)	0.2106 (0.1746 – 0.2465)	0.1038 (0.0802 – 0.1283)
E ₅₀	7.9596 (7.2875 – 8.6317)	0.8950 (0.8084 – 0.9819)	0.3054 (0.2703 – 0.3300)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198

^aCalculated on the basis of the content in the test item declared by the Sponsor in the Certificate of Analysis

Endpoint values – impact of the test item on *Lemna gibba* growth rate – main test

Endpoint t	Test item mg/L (based on nominal concentrations)	Mepiquat chloride mg/L (based on nominal con- centrations*)	Mepiquat ion mg/L (based on nominal con- centrations#)
Growth rate – based on frond number			
E_rC₁₀	4.2655 (3.7743 – 4.7567)	0.2146 (0.1898 – 0.2393)	0.1635 (0.1447 - 0.1823)
E_rC₂₀	6.8331 (6.2109 – 7.4553)	0.3437 (0.3124 – 0.3750)	0.2619 (0.2381 - 0.2858)
E_rC₅₀	16.8312 (15.7099 – 17.9525)	0.8466 (0.7902 – 0.9030)	0.6451 (0.6022 – 0.6881)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198
Growth rate – based on dry weight			
E_yC₁₀	3.2987 (2.7835 – 3.8139)	0.1659 (0.1400 – 0.1918)	0.1264 (0.1067- 0.1462)
E_yC₂₀	6.1593 (5.4314 – 6.8872)	0.3098 (0.2732 - 0.3464)	0.2361 (0.2082 - 0.2640)
E_yC₅₀	20.3396 (18.6711 – 22.0081)	1.0231 (0.9392 – 1.1070)	0.7796 (0.7157 - 0.8436)
NOEC	3.125	0.1572	0.1198
LOEC	6.25	0.3144	0.2396

*Calculated on the basis of the content in the test item declared by the Sponsor in the Certificate of Analysis

Endpoint values – impact of the test item on *Lemna gibba* yield – main test

Endpoint t	Test item mg/L (based on nominal concentrations)	Mepiquat chloride mg/L (based on nominal con- centrations*)	Mepiquat ion mg/L (based on nominal concen- trations#)
Yield – based on frond number			
E_rC₁₀	2.0223 (0.9761 – 1.5967)	0.0849 (0.0644 – 0.1054)	0.0775 (0.0665 - 0.0885)
E_rC₂₀	3.2351 (2.2711 – 3.2771)	0.1831 (0.1499 – 0.2163)	0.1240 (0.1100 - 0.1380)
E_rC₅₀	7.9474 (10.8571 – 13.2753)	0.7964 (0.7166 – 0.8762)	0.3046 (0.2825 - 0.3267)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198
Yield – based on dry weight			
E_yC₁₀	1.5417 (1.2675 – 1.8159)	0.0988 (0.0763 – 0.1214)	0.0591 (0.0486 - 0.0696)
E_yC₂₀	2.7084 (2.3302 – 3.0866)	0.2106 (0.1746 – 0.3125)	0.1038 (0.0893 - 0.1183)
E_yC₅₀	7.9596 (7.2875 – 8.6317)	0.8950 (0.8081 – 0.9819)	0.3051 (0.2793 - 0.3309)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198

*Calculated on the basis of the content in the test item declared by the Sponsor in the Certificate of Analysis

Report “Study of *Lemna gibba* growth inhibition with Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL”. S. Radha. 2019. Study code: 6090/2019. Bioscience Research Foundation

Guideline(s): OECD Guideline No. 221 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication No
(if vertebrate study)

Materials and methods

Test item: Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL
Batch no.: SCL-58304
Active substance content: Chloride content, 51.05 (g/L), Ion content, 38.9 (g/L)

Reference item : 3,5-dichlorophenol

Test medium: 20X AAP medium

Biological test system : *Lemna gibba* obtained from a standard laboratory culture at BRF
Colonies cultured for 8 days before exposure initiation

Test doses: A control, 3.125, 6.25, 12.5, 25, 50 and 100 mg test item/L. Equivalent to 0.157, 0.314, 0.629, 1.257, 2.515 and 5.030 mg mepiquat chloride/L and 0.120, 0.24, 0.479, 0.958, 1.916 and 3.833 mg mepiquat ion/L. There were 3 replicates of each test concentration and 6 replicates for the control. 3 colonies with 3 fronds each were introduced into each replicates.

Test conditions: temperature: 22.0 – 23.9°C;
pH at the beginning of the test: 7.4 – 7.7;
pH at the end of the test: 7.4 – 8.3;
lighting: 16 h light and 8h dark;
light intensity: 7000 - 7990 lux

Endpoints: EC₁₀, EC₂₀, EC₅₀, NOEC, LOEC
LC₁₀, LC₂₀, LC₅₀, NOEC, LOEC

Chemical analysis

In fresh samples of the test item concentrations collected at exposure initiation, the determined concentrations of Mepiquat chloride were between 99.5 and 100.7% 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 Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL were between 98.8 and 100.2% of the nominal concentrations, respectively. Therefore, the concentrations of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL were stable during 7 days under test conditions.

Results

No distinctive changes from the normal development of plants in the test item concentrations of 3.125 mg/L and in the control group, whereas in the test item concentrations of 6.25, 12.5, 25, 50 and 100 mg/L smaller fronds, spots of chlorosis and/or separated roots were observed during the 7-day experiment.

Table 3 - Endpoint values – impact of the test item on *Lemna gibba* growth rate – main test

Endpoint	Test item mg/L (based on nominal concentrations)	Mepiquat chloride mg/L (based on nominal concentrations*)	Mepiquat ion mg/L (based on nominal concentrations#)
Growth rate – based on frond number			
E_rC₁₀	4.2655 (3.7743 – 4.7567)	0.2146 (0.1898 – 0.2393)	0.1635 (0.1447 - 0.1823)
E_rC₂₀	6.8331 (6.2109 – 7.4553)	0.3437 (0.3124 – 0.3750)	0.2619 (0.2381 - 0.2858)
E_rC₅₀	16.8312 (15.7099 – 17.9525)	0.8466 (0.7902 – 0.9030)	0.6451 (0.6022 – 0.6881)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198
Growth rate – based on dry weight			
E_yC₁₀	3.2987 (2.7835 – 3.8139)	0.1659 (0.1400 – 0.1918)	0.1264 (0.1067 - 0.1462)
E_yC₂₀	6.1593 (5.4314 – 6.8872)	0.3098 (0.2732 - 0.3464)	0.2361 (0.2082 - 0.2640)
E_yC₅₀	20.3396 (18.6711 – 22.0081)	1.0231 (0.9392 – 1.1070)	0.7796 (0.7157 - 0.8436)
NOEC	3.125	0.1572	0.1198
LOEC	6.25	0.3144	0.2396

*Calculated on the basis of the content in the test item declared by the Sponsor in the Certificate of Analysis

Table 4 - Endpoint values – impact of the test item on *Lemna gibba* yield – main test

Endpoint	Test item mg/L (based on nominal concentrations)	Mepiquat chloride mg/L (based on nominal concentrations*)	Mepiquat ion mg/L (based on nominal concentrations#)
Yield – based on frond number			
E_rC₁₀	2.0223 (0.9761 – 1.5967)	0.0849 (0.0644 – 0.1054)	0.0775 (0.0665 - 0.0885)
E_rC₂₀	3.2351 (2.2711 – 3.2771)	0.1831 (0.1499 – 0.2163)	0.1240 (0.1100 - 0.1380)
E_rC₅₀	7.9474 (10.8571 – 13.2753)	0.7964 (0.7166 – 0.8762)	0.3046 (0.2825 - 0.3267)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198
Yield – based on dry weight			
E_yC₁₀	1.5417 (1.2675 – 1.8159)	0.0988 (0.0763 – 0.1214)	0.0591 (0.0486 - 0.0696)
E_yC₂₀	2.7084 (2.3302 – 3.0866)	0.2106 (0.1746 – 0.3125)	0.1038 (0.0893 - 0.1183)
E_yC₅₀	7.9596 (7.2875 – 8.6317)	0.8950 (0.8081 – 0.9819)	0.3051 (0.2793 - 0.3309)
NOEC	<3.125	<0.1572	<0.1198
LOEC	3.125	0.1572	0.1198

*Calculated on the basis of the content in the test item declared by the Sponsor in the Certificate of Analysis

Validity Criteria

The results are considered valid because the following criteria were satisfied

- The doubling time of frond number in the control was 1.87 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 13.4).
- The average specific growth rate in the control between day 0 and day 7 was 0.371 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹)

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:	<p>The study is considered valid. All validity criteria were met.</p> <ul style="list-style-type: none"> • No mortality was observed in control (criterion: it must not exceed 10%). • The LD₅₀/24 h of the reference item (dimethoate) was 0.13 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee). <p>Agreed endpoint: LD₅₀ > 100 µg product/bee</p>
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Reference: KCP 10.3.1.1.1

Report “Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL: acute oral toxicity test in honey bees”. Saiqa Nazhath, 2019, Study code G14247. Eurofins Advinus Limited.

Guideline(s): OECD Guideline for the Testing of Chemicals No. 213 (1998)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The acute oral toxicity study of Mepiquat chloride 5.105% w/v (equivalent to mepiquat ion 3.89% w/v) SL (batch No. SCL-22147) was conducted to determine the LD₅₀ values for honeybees. Five doses of the test item were used. These included: 6.25, 12.5, 25.0, 50.0 and 100.0 µg/honeybee. The range of doses was selected on the basis of the range finding test results. Each group of 10 bees (3 replicates/group, 10 bees/replicate) was fed with 200 µL of a 50% sucrose solution, containing the test item at the doses enu-

merated above, via glass feeding tubes (4 tubes with 50 µL each per cage). During the entire experiment, the insects were caged in groups of 10.

The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure.

After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made at 4 h after the beginning of the test and thereafter at 24 h and 48 h. The acute oral toxicity test ended after the 48-hour exposure.

Results

Table 1: Acute oral toxicity on honeybees (*Apis mellifera* L.)

Table 1: Acute oral toxicity on honey bees (<i>Apis mellifera</i> L.)				
Dose	Number of tested bees	Mortality after 48 h		LD ₅₀
		Total		
[µg test item/bee]		[no.]	[%]	[µg test item/bee]
0.0 (control)	30	0	0.0	>100
6.25	30	0	0.0	
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	0	0.0	
Toxic standard (µg dimethoate/bee)				
0.075	30	5	16.67	0.13
0.15	30	18	63.33	
0.30	30	28	96.67	

Findings

- The mortality in the test item treatments after 48 hours was lower than 50% when compared to the control.
- The median lethal doses of Mepiquat chloride 5.105% SL (LD₅₀) after 24 and 48 hours of the exposure are higher than the highest dose used in the study, i.e. 100 µg test item/bee.
- No sublethal toxicity effects (behavioural abnormalities) with respect to the test item and the control were observed over the 48 hours exposure.
- The reduction in food consumption (sucrose solution) during 48 h ranged from 185.67 to 189.60 µL per group of 10 treated bees 190.82 µL in the control.

Validity criteria

The following validity criteria were met during the test:

- No mortality was observed in control (criterion: it must not exceed 10%).
- The LD₅₀/24 h of the reference item (dimethoate) was 0.13 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee).

Conclusion

The LD₅₀ value of the test item, Mepiquat chloride 5.105% w/v (equivalent to mepiquat ion 3.89% w/v) SL at 48 h is higher than 100 µg/bee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	<p>The study is considered valid. All validity criteria were met.</p> <ul style="list-style-type: none"> The average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%). The 24 hour LD₅₀ of the reference item (dimethoate) was 0.13 µg/bee (criterion: 0.10 - 0.30 µg a.i./bee). <p>Agreed endpoint: LD₅₀ > 100 µg product/bee</p>
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Reference:	KCP 10.3.1.1.2
Report	“Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL: acute contact toxicity test in honey bees”, Saiqa Nazhath, 2019, Study code G14248. Eurofins Advinus Limited.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The acute contact toxicity study of Mepiquat chloride 5.105% w/v (equivalent to mepiquat ion 3.89% w/v) SL (batch No. SCL-22147) was conducted to determine the effects on honeybees. Five doses of the test item were used. These included: 6.25, 12.5, 25.0, 50.0 and 100.0 µg/honeybee. The range of doses was selected on the basis of the range finding test results.

A sample of 1 µL each of the control, test item and toxic standard was applied on to the dorsal thorax of each bee of the respective group using Hamilton microliter syringe. The treated bees were then transferred to respective test cages and were provided with 50% w/v sucrose solution in Milli-Q water throughout the test. During the entire experiment, the insects were caged in groups of 10 under controlled conditions of the temperature and the humidity.

The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure.

After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24, and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

Results

Table 1: Acute contact toxicity on honeybees (*Apis mellifera* L.)

Dose	Number of tested bees	Mortality after 48 h		LD ₅₀
		Total		
[µg test item/bee]		[no.]	[%]	[µg test item/bee]
0.0 (control)	30	0	0.0	>100
6.25	30	0	0.0	

12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	0	0.0	
Toxic standard (µg dimethoate/bee)				
0.075	30	5	16.67	0.13
0.15	30	18	60.00	
0.30	30	28	93.33	

Findings

- Mortality of the control group after 48 hours of exposure was 0%.
- Mortality of the treated groups was lower than 50% when compared to the control.
- No sublethal toxicity effects (behavioural abnormalities) with respect to the test item and the control were observed over the 48 hours exposure.

Validity criteria

The following validity criteria were met during the test:

- The average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%).
- The 24 hour LD₅₀ of the reference item (dimethoate) was 0.13 µg/bee (criterion: 0.10 - 0.30 µg a.i./bee).

Conclusion

The LD₅₀ value of the test item, Mepiquat chloride 5.105% w/v (equivalent to mepiquat ion 3.89% w/v) SL at 48 h is higher than 100 µg/bee.

A 2.3.1.2	KCP 10.3.1.2.	Chronic toxicity to bees
A 2.3.1.3	KCP 10.3.1.3	Effects on honey bee development and other honey bee life stages
A 2.3.1.4	KCP 10.3.1.4	Sub-lethal effects
A 2.3.1.5	KCP 10.3.1.5	Cage and tunnel tests
A 2.3.1.6	KCP 10.3.1.6	Field tests with honeybees
A 2.3.2	KCP 10.3.2	Effects on non-target arthropods other than bees

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

Comments of zRMS:	<p>The study is considered valid. All validity criteria were met.</p> <ul style="list-style-type: none"> • after 48 hours, mortality in the control group was 0.0% (criterion: a maximum of 10.0%), • after 24 hours, mortality in the group treated with the reference item at a rate of 5 mL/ha was 56.6 (criterion: minimum of 50%) • the mean number of mummies per female in the control was 28.0 (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). <p>Agreed endpoints: $LR_{50} > 3.8$ Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL L/ha, i.e., (> 194g Mepiquat Chloride/ha + 147.8g Mepiquat ion/ha). $NOER_{mortality} = 1.5$ L/ha, i.e., (76g Mepiquat Chloride + 58.4g Mepiquat ion/ha). $ER_{50} =$</p>
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Reference: KCP 10.3.2.1-01

Report: “A laboratory test for evaluating the effects of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez)”. G Sonali, 2020, 6050/2019. Bioscience Research Foundation

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 from Guidelines
 From the Study Plan: each group was divided into six replicates with five wasps in each replicate instead four replicates with ten wasps in each replicate as it had been planned.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

SUMMARY

The laboratory test involved the evaluation of the effects of the test item, Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on mortality and fecundity of the parasitic wasp, *A. rhopalosiphi*. In the definitive test, five rates of the test item were used. These were 0.6, 0.9, 1.5, 12.4 and 3.8 L/ha (30.6, 45.9, 76.6, 122.5 and 194.0 g Mepiquat chloride/ha and 23.3, 35.0, 58.4, 93.4 and 147.8 g Mepiquat ion/ha).

Adult wasps were exposed in exposure units to glass plates treated with the test item. The parasitoids were confined for 48 h and their condition was assessed after 2, 24, and 48 hours. Then, females which survived the 48-hour exposure to the test item and the ones from the control group were subjected to fecundity assessments. To allow the oviposition, 15 female wasps from the groups treated with the test item and the ones from the control group were individually introduced into the fecundity units containing the barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which the wasp pupae were developing) was recorded.

Mortality of the wasps after 48 hours of the exposure and the percentage of fecundity reduction (Pr) relative to the control group recorded 12 days after the oviposition were the endpoints.

To assess the susceptibility of the test system and the sensitivity of the test method, an insecticide, ROGOHIT (30% dimethoate, w/w) was used as a reference item. The rate of the reference item was 5 mL/ha (1.5 g dimethoate/ha). The control group was comprised of wasps having contact with glass plates sprayed with deionised water.

Materials and methods:

Test item:

Name: Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL; content: 51.05 g/L of Mepiquat Chloride (CAS no.: 24307-26-4) and 38.9 g/L of Mepiquat ion (CAS no.: 15302-91-7) as an active ingredient; batch no.: SCL-58304; manufacturing date: May 29, 2019; expiry date: May 28, 2021.

Biological test system:

the parasitic wasp, *Aphidius rhopalosiphii* (De Stefani-Perez); Hymenoptera: Braconidae

– age:

Larvae (24 - 48 hours after emerging from mummies)

– source:

BRF Insectary

Experimental design:

7 test groups:

– a control group (0.0 L/ha)

– Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rate of 0.6 L/ha (30.6 g Mepiquat Chloride/ha and 23.3 g Mepiquat ion/ha)

– Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rate of 0.9 L/ha (45.9g Mepiquat Chloride /ha and 35.0 g Mepiquat ion/ha)

– Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rate of 1.5 L/ha (76.6g Mepiquat Chloride /ha and 58.4 g Mepiquat ion/ha)

– Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rate of 2.4 L/ha (122.5g Mepiquat Chloride /ha and 93.4 g Mepiquat ion/ha)

– Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rate of 3.8 L/ha (194.0g Mepiquat Chloride /ha and 147.8 g Mepiquat ion/ha)

– ROGOHIT at the rate of 5 mL/ha (1.5 g a.i./ha)

6 replicates/group

5 wasps/replicate

Test conditions:

– temperature:

18.6-20.4°C

– relative air humidity:

64-72%

– photoperiod:

16 hours light (mortality assessment and oviposition: 570 lx; fecundity assessment: 3500 lx) : 8 hours dark

Statistical analyses:

Endpoints values for mortality and reproduction were determined by using Probit analysis in the NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphpad Prism 8.0. The means and standard deviations were calculated using validated Excel Sheets

Endpoints:

– LR₅₀ and ER₅₀value

– NOER

RESULTS AND DISCUSSION:

Mortality of the control wasps was 0.0% after 48 hours of the exposure. Mortality of *A. rhopalosiphi* after 48 hours of the exposure Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rates of 0.6, 0.9, 1.5, 12.4 and 3.8 L/ha (30.6, 45.9, 76.6, 122.5 and 194.0 g Mepiquat chloride/ha and 23.3, 35.0, 58.4, 93.4 and 147.8 g Mepiquat ion/ha) was 0.0, 0.0, 10.0, 20.0 and 30.0%, respectively.

The median lethal rate, LR₅₀ (the application rate at which 50% mortality of wasps is observed) with 95% confidence intervals after 48 hours of the exposure was > 3.8 Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL L/ha, i.e., (> 194g Mepiquat Chloride/ha + 147.8g Mepiquat ion/ha). The NOER_{mortality} value is 1.5 L/ha, i.e., (76g Mepiquat Chloride + 58.4g Mepiquat ion/ha).

Wasp mortality after 42 hours of the exposure to ROGOHIT was 87%. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

The wasps treated with the test item at the rates of 0.6, 0.9, 1.5, 12.4 and 3.8 L/ha produced 27.5, 26.3, 25.6, 20.2, and 18.0 mummies per female, respectively. The control group produced 28.0 mummies per female.

The percentages of fecundity reduction (Pr) after the exposure to Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL at the rates of 0.6, 0.9, 1.5, 12.4 and 3.8 L/ha (30.6, 45.9, 76.6, 122.5 and 194.0 g Mepiquat chloride/ha and 23.3, 35.0, 58.4, 93.4 and 147.8 g Mepiquat ion/ha) were 1.8, 6.1, 8.6, 27.9 and 35.7%, respectively. There were statistically significant differences in fecundity between the group treated with the test item at rates of 2.4 and 3.8 L/ha and the control group (one-way ANOVA).

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

- after 48 hours, mortality in the control group was 0.0% (criterion: a maximum of 10.0%),
- after 24 hours, mortality in the group treated with the reference item at a rate of 5 mL/ha was 56.6 (criterion: minimum of 50%)
- the mean number of mummies per female in the control was 28.0 (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).

Comments of zRMS:	The study is considered valid. All validity criteria were met.					
	<ul style="list-style-type: none"> 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 5.0 mL/ha was 95.0% on day 7 of exposure (criterion: from 50 to 100%), the mean number of eggs per female in the control group was 4.65 (required: ≥ 4 eggs per female). 					
	Agreed endpoints:					
	Mortality and reproduction of <i>T. pyri</i> in the laboratory test					
	Study group (application rate) [L/ha]	Parameter (endpoint)				
		Mortality after 7 days		Reproduction		
		Total [%]	LR ₅₀	Mean no. of eggs/female (Rr) [No]	Reproduction reduction Pr [%]	ER ₅₀
	Control	0.0	-	4.65	-	-
	Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v					
	0.6	0	>3.8 L/ha	4.20	9.67	2.399 L/ha
	0.9	0		3.06	34.21 ⁺	

	1.5	1	>(194 ^a + 174 ^b g a.i./ha)	2.62	43.77 ⁺	(122.5 ^a + 93.4 ^b g a.i./ha)
	2.4	3		2.43	47.83 ⁺	
	3.8	6		1.92	58.67 ⁺	
	NOER _{mortality}		2.4 L/ha (122.5 ^a + 93.4 ^b g a.i./ha)	NOER _{reproduction}		0.6 L/ha (30.6 ^a + 23.3 ^b g a.i./ha)
	Reference item	ROGOHIT				
	5.0 mL/ha	95.0 %	-	-	-	
	+ - statistically significant differences at $p < 0.05$ a: Mepiquat Chloride b: Mepiquat ion					

Reference: KCP 10.3.2.1-02

Report “A laboratory test for evaluating the effects of Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v on the predatory mite, *Typhlodromus pyri* (Scheuten)”. P. Bala, 2020, 6051/2019. BIOSCIENCE RESEARCH FOUNDATION

Guideline(s): ESCORT 1 Guidance Document (Barrett K.L. et al., 1994)
ESCORT 2 Guidance Document (Candolfi M.P. et al., 2001)
Guidelines developed by the IOBC, BART and EPPO Joint Initiative (Blumel S. et al., 2000)

Deviations: No from Guidelines. One deviation from the study plan: the study finished in February 2020, not in January 2020 as it had been planned. This deviation did not affect the study results.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) Not relevant

Materials and methods

The laboratory test for evaluating the effects of Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v on mortality and reproduction of the predatory mite, *T. pyri* (Sch.) was conducted for Sharda Cropchem Ltd, India at Bioscience Research Foundation.

The study was carried out based on the Sponsor recommended rates for the test item as the definite test. There were 0.6, 0.9, 1.5, 2.4 and 3.8 L/ha. A 24 hours old (protonymphal stage) of predatory mites *T. pyri* were exposed to the test item applied to bean leaf discs and fed with pine pollen (*Pinus* sp.) during the experimental period.

To verify the sensitivity of the mites and the precision of the test procedure, the insecticide, ROGOHIT (30% dimethoate) was used as a reference item. The rate of the reference item was 5.0 mL/ha (1.5 g a.i./ha). The control group was treated with distilled water.

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.

Endpoints based on mortality of *T. pyri* was 7 days and reproduction reduction (Pr) was 14 days post-test

item treatment.

Results

The effects of Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Mortality and reproduction of *T. pyri* in the laboratory test

Study group (application rate) [L/ha]	Parameter (endpoint)				
	Mortality after 7 days		Reproduction		
	Total [%]	LR ₅₀	Mean no. of eggs/female (Rr) [No]	Reproduction reduction Pr [%]	ER ₅₀
Control	0.0	-	4.65	-	-
Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v					
0.6	0	>3.8 L/ha >(194 ^a + 174 ^b g a.i./ha)	4.20	9.67	2.399 L/ha (122.5 ^a + 93.4 ^b g a.i./ha)
0.9	0		3.06	34.21 ⁺	
1.5	1		2.62	43.77 ⁺	
2.4	3		2.43	47.83 ⁺	
3.8	6		1.92	58.67 ⁺	
NOER _{mortality}		2.4 L/ha (122.5 ^a + 93.4 ^b g a.i./ha)	NOER _{reproduction}		0.6 L/ha (30.6 ^a + 23.3 ^b g a.i./ha)
Reference item	ROGOHIT				
5.0 mL/ha	95.0%	-	-	-	-

+ - statistically significant differences at $p < 0.05$

a: Mepiquat Chloride

b: Mepiquat ion

Findings

- Mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v at rates of 0.6, 0.9, 1.5, 2.4 and 3.8 L/ha, the percentages of *T. pyri* mortalities 0, 0, 1.0, 3.0 and 6.0%, respectively.
- There were statistically significant differences in mortality between group treated with the test item at rates of 3.8 L/ha, and the control group.
- On the basis of the obtained mortality results, the LR₅₀ value >3.8 L test item/ha, i.e. >(194 g Mepiquat Chloride/ha and 147.8g Mepiquat ion/ha). The NOER_{mortality} value is 2.4 L test item/ha, i.e., (122.5 g Mepiquat Chloride/ha and 93.4g Mepiquat ion/ha).
- For the reference item Rogohit (Dimethoate 30% EC, w/w), the mortality of mites after 7 days of exposure at the rate of 5.0 mL/ha, was 95.0%, 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 4.65 eggs/female. The mean reproduction rates (Rr) after 14 days of exposure to Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v at rates 0.6, 0.9, 1.5, 2.4 and 3.8 L/ha were 4.20, 3.06, 2.62, 2.43 and 1.92 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by rates of 0.6, 0.9, 1.5, 2.4 and 3.8 L/ha were 9.67, 34.21, 43.77, 47.83 and 58.67%, respectively.
- There were statistically significant differences in reproduction between group treated with the test item at rates of 0.9, 1.5, 2.4 and 3.8 L/ha and the control group.
- On the basis of the obtained reproduction results, the ER₅₀ value is equal to 2.399 L test item/ha, i.e., (122.5g Mepiquat Chloride/ha and 93.4g Mepiquat ion/ha). The NOER_{reproduction} value is 0.6 L test item/ha, i.e., (30.6g Mepiquat Chloride/ha and 23.3g Mepiquat ion/ha).

Conclusion

On the basis of the obtained results it can be concluded that Mepiquat Chloride 5.105% equivalent to Mepiquat ion 3.89 w/v had no adverse effects on mortality of the predatory mite *T. pyri* at rates of 0.6, 0.9, 1.5, 2.4 and 3.8 L/ha; and an ER₅₀ of 2.399 L test item/ha was obtained for reproduction.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Comments of zRMS:	The study is considered valid. All validity criteria were met.			
	<ul style="list-style-type: none"> • Mean adult mortality: 0.0% (criterion: $\leq 10\%$). • The mean number of juveniles per vessel at the end of the test: 111.9 (criterion: ≥ 30 juveniles at the end of the test) • The coefficient of variation calculated for the number of juveniles: 4.8 (criterion: $\leq 30\%$). • Reference substance (carbendazim) group exhibited statistically significant reduction in juvenile production at 2.25 mg a.i./kg dry soil as compared with the control. 			
	Agreed endpoints:			
	Endpoint	Value [mg test item/kg dry weight of artificial soil]	Value [mg of Mepiquat chloride/kg dry weight of artificial soil]	Value [mg of Mepiquat ion/kg dry weight of artificial soil]
	EC ₁₀	89.70 (75.28 – 104.13)	4.51 (3.79 – 5.24)	3.44 (2.88 – 3.99)
	EC ₂₀	275.19 (234.84 – 315.54)	13.84 (11.81 – 15.87)	10.55 (9.00 – 12.09)
	EC ₅₀	> 1000 (n.d.)	> 50.30 (n.d.)	> 38.33 (n.d.)
	NOEC	52.92	2.66	2.03

Reference:

KCP 10.4.1.1

Report

“Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on reproduction of the earthworms (*Eisenia fetida*) in artificial soil”. K. Murali. (2020), Study code: 9549/2021. BIOSCIENCE RESEARCH FOUNDATION

Guideline(s):

OECD Guideline No. 222 (2016)

Deviations:

No

GLP:

Yes

Acceptability:

Yes

Duplication
(if vertebrate study)

Not relevant

Materials and methods

The toxic effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL was studied on the earthworm, *Eisenia fetida* by artificial soil test.

The test was carried with the test concentrations of 5.04, 9.07, 16.33, 29.40, 52.92, 171.47, 308.64, 555.56 and 1000 mg/kg dry soil along with a control (deionized water) and reference substance (Carbendazim). Each of them was divided into four replicates. There were also untreated control group (with deionised water and without test item) divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all adult earthworms were removed from the test containers and observed. All changes in their 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.

Dose concentration verification analysis

The exposure concentrations revealed that the exposed concentrations (5.04, 95.26 and 1000 mg sample/kg soil) were analysed under analytical method (HPLC) and the average detected concentration of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL in artificial soil was between 100 - 100.1% (day 0), 99.6 - 100.9% (day 28) and 99.7 - 100.2% (day 56); compared to nominal concentrations.

Results and discussions

❖ Observation of the earthworms

No pathological and behavioral symptoms were observed in juveniles in the control and treated groups.

❖ Mortality data

The impact of the test item on mortality of the earthworms is presented in the table below.

Table 1 - Mortality of the adult earthworms (*Eisenia fetida*) after 4 weeks of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Number of tested earthworms [no.]	Total Mortality	
		No.	%
0 (control)	80	0	0
5.04	40	0	0
9.07	40	0	0
16.33	40	0	0
29.40	40	0	0
52.92	40	1	2.5
95.26	40	5	12.5 ⁺
171.47	40	6	15.0 ⁺
308.64	40	8	20.0 ⁺
555.56	40	11	27.5 ⁺
1000	40	14	35.0 ⁺

+: statistically significant difference between the control and the treatment group at $p < 0.05$;

❖ Body weight

Table 2 - Body weight change in the adult earthworms (*Eisenia fetida*) after 4 weeks.

Concentration [mg/kg dry weight of]	Number of tested earthworms [no.]	Mean body weight increase	
		mg	%

the artificial soil]			
0 (control)	80	12.93	3.55
5.04	40	12.23	3.50
9.07	40	9.78	2.58
16.33	40	8.76	2.43
29.40	40	7.38	2.09
52.92	40	6.21	1.67
95.26	40	6.13	1.66
171.47	40	5.40	1.47
308.64	40	4.24	1.16
555.56	40	-2.5	-0.68
1000	40	-9.12	-2.43

❖ **Impact of the test item on reproduction of the earthworms**

The results concerning the impact of the test item on reproduction are shown in the table below.

Table 3 - Number of juvenile worms (*Eisenia fetida*) after 8 weeks of the experiment

Concentration [mg/kg dry weight of the artificial soil]	Mean \pm SD	Reduction in juvenile production in comparison to the control [%]	CV [%]
0 (control)	111.9 \pm 5.41	—	4.8
5.04	110.0 \pm 3.56	1.68	3.2
9.07	109.5 \pm 2.89	2.12	2.6
16.33	109 \pm 2.58	2.57	2.4
29.40	108 \pm 1.83	3.46	1.7
52.92	107.25 \pm 3.20	4.13	3.0
95.26	99.75 ⁺ \pm 2.06	10.84	2.1
171.47	96.5 ⁺ \pm 4.43	13.74	4.6
308.64	85.25 ⁺ \pm 3.30	23.80	3.9
555.56	80.5 ⁺ \pm 6.86	28.04	8.5
1000	69.5 ⁺ \pm 10.28	37.88	14.8

CV: Coefficient of variation

+: statistically significant difference between the control and the treatment group at $p < 0.05$;

Validity criteria

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

- Mean adult mortality: 0.0% (criterion: $\leq 10\%$).
- The mean number of juveniles per vessel at the end of the test: 111.9 (criterion: ≥ 30 juveniles at the end of the test)
- The coefficient of variation calculated for the number of juveniles: 4.8 (criterion: $\leq 30\%$).

Reference substance (carbendazim) group exhibited statistically significant reduction in juvenile production at 2.25 mg a.i./kg dry soil as compared with the control. Hence the test has met the validity acceptance criteria that significant effects should be observed between 1 and 5 mg a.i./kg dry soil in a test. This result infers that the obtained results during this test are valid and hence test is acceptable.

Table 4 – Endpoint values on reproduction and survival of adult earthworms *Eisenia fetida*

Endpoint	Value [mg test item/kg dry weight of artificial soil]	Value [mg of Mepiquat chloride/kg dry weight of artificial soil]	Value [mg of Mepiquat ion/kg dry weight of artificial soil]
LC ₁₀	129.03 (111.04 – 147.02)	6.49 (5.58 – 7.39)	4.95 (4.26 – 5.63)
LC ₂₀	324.32 (282.84 – 365.80)	16.31 (14.23 – 18.40)	12.43 (10.84 – 14.02)
LC ₅₀	> 1000 (n.d.)	> 50.30 (n.d.)	> 38.33 (n.d.)
NOEC	52.92	2.66	2.03
LOEC	95.26	4.79	3.65

Table 5 – Endpoint values on reproduction adult earthworms *Eisenia fetida*

Endpoint	Value [mg test item/kg dry weight of artificial soil]	Value [mg of Mepiquat chloride/kg dry weight of artificial soil]	Value [mg of Mepiquat ion/kg dry weight of artificial soil]
EC ₁₀	89.70 (75.28 – 104.13)	4.51 (3.79 – 5.24)	3.44 (2.88 – 3.99)
EC ₂₀	275.19 (234.84 – 315.54)	13.84 (11.81 – 15.87)	10.55 (9.00 – 12.09)
EC ₅₀	> 1000 (n.d.)	> 50.30 (n.d.)	> 38.33 (n.d.)
NOEC	52.92	2.66	2.03

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study is considered valid. All validity criteria were met.			
	<ul style="list-style-type: none"> mean adult mortality: 0.0% (criterion: ≤ 20%), the mean number of juveniles per vessel at the end of the test: 799.1 (criterion: ≥ 100 juveniles at the end of the test), the coefficient of variation calculated for the number of juveniles: 2.08 (criterion: ≤ 30%). 			
	Agreed endpoints:			
	Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of active substances /kg dry weight of the artificial soil]	
			a	b
	EC ₁₀	208.49 (167.39 – 249.59)	10.49 (8.42 – 12.55)	7.99 (6.42 – 9.57)
	EC ₂₀	824.45 (592.92 – 1055.97)	41.47 (29.82 – 53.11)	31.60 (22.72 – 40.47)
	EC ₅₀	> 1000 (n.d.)	> (50.30)	> (38.33)
	NOEC	171.47	8.62	6.57
	LOEC	308.64	15.52	11.83
a – Mepiquat Chloride b – Mepiquat ion n.d. – not determined				

Reference: KCP 10.4.2.1-01

Report “Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the collembolans (*Folsomia candida*) in artificial soil”. K. Murali. 2020. Study code: 6091/2019. BIOSCIENCE RESEARCH FOUNDATION

Guideline(s): OECD Guideline No. 232 (2016)

Deviations: Yes. At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016). The deviations did not affect the study results.

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

Materials and methods

Test item: Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v
Batch no.: SCL-58304
Active substance: Mepiquat Chloride
Mepiquat ion

Artificial soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

Biological test system : the collembolan, *Folsomia candida* obtained from Bioscience Research Foundation

Test design: The test item in form of aqueous emulsion was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. Test duration: 28 days

Test doses: A control, 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 dry weight of artificial soil (i.e. 0.25, 0.46, 0.82, 1.48, 2.66, 4.79, 8.62, 15.52, 27.94, 50.30 mg Mepiquat Chloride/ kg dry weight of artificial soil and 0.19, 0.35, 0.63, 1.13, 2.03, 3.65, 6.57, 11.83, 21.29 and 38.33 mg Mepiquat ion/ kg dry weight of artificial soil). There were 4 replicates of each test concentration and a concurrent control group divided into eight replicates. Ten 9-12-day-old collembolans were introduced into each test container.

Test conditions: temperature: 19.2 – 20.9°C;
pH at the beginning of the test: 5.89 – 6.45;
pH at the end of the test: 5.91 - 6.39;
soil moisture content at the beginning of the test: 13.05 – 13.15% (45.92 – 49.65% of the maximum water holding capacity);
soil moisture content at the end of the test: 13.09 – 13.15% (46.05 – 49.76% of the maximum water holding capacity);
lighting: 16 h light and 8h dark;
light intensity: 575 - 665 lux
The collembolans were fed at the beginning of the experiment and after 2 weeks of incubation

Endpoints: EC₁₀, EC₂₀, EC₅₀, NOEC, LOEC
LC₁₀, LC₂₀, LC₅₀, NOEC

Results and discussions

After the application of the test item at the concentrations ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, mortality was between 0% - 22.5%. As for the control group, it was equal to 0.0%. Endpoints are given below:

a – Mepiquat Chloride
b – Mepiquat ion
n.d. – not determined

a – Mepiquat Chloride
b – Mepiquat ion
n.d. – not determined

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- mean adult mortality: 1.25% (criterion: $\leq 20\%$),
- the mean number of juveniles per replicate at the end of the test: 130.00 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 0.92 (criterion: $\leq 30\%$).

Agreed endpoints:

Endpoints	[mg t.s./kg sdw]	[mg a.i./kg sdw]
NOEC _{mortality}	308.64	15.52 ^a + 11.83 ^b
LOEC _{mortality}	555.56	27.94 ^a + 21.29 ^b
NOEC _{reproductive output}	308.64	15.52 ^a + 11.83 ^b
LOEC _{reproductive output}	555.56	27.94 ^a + 21.29 ^b
EC ₁₀	248.22 (193.10 – 303.33)	12.48 ^a + 9.51 ^b (9.71 – 15.26) + (7.40 –

			11.63)
	EC ₂₀	>1000 (n.d.)	> (50.30 ^a + 38.33 ^b) (n.d.)
	EC ₅₀	> 1000 (n.d.)	> (50.30 ^a + 38.33 ^b) (n.d.)
: Mepiquat Chloride b: Mepiquat ion n.d. – not determined +: Statistically significant difference between the control and the treatment group at $p < 0.05$			

Reference: KCP 10.4.2.1-02

Report “Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on the Reproductive Output of the Predatory Soil Mite *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae) in Artificial Soil”. S. Rajeshwari. 2020. Study code: 6092/2019. BIOSCIENCE RESEARCH FOUNDATION

Guideline(s): OECD 226 (2016): OECD Guidelines for the testing of chemicals, No. 226; Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.

Deviations: No deviations from the OECD Guideline 226 (2016)
Deviations from the study plan: The study finished in October 2019, not in September 2019 as it had been planned. This deviation did not affect the study result.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL
batch no.: SCL-58304
active substance: Mepiquat Chloride: 51.05 g/L
Mepiquat ion: 38.9 g/L

Artificial soil 5% sphagnum peat (air-dried and finely ground); 20% kaolin clay (kaolinite content preferably > 30 %); 75% air-dried industrial sand (predominantly fine sand with more than 50 % of the particles between 50 and 200 microns)

Biological test system : *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari, Laelapidae), from in-house culture, adult mites (33 days after starting of the egg-laying for synchronisation).

Test design: Adult females were exposed to the test substance in artificial soil. After 14 days, the surviving individuals were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving adult females were determined. The reproductive output and the mortality in each test item group were compared to that of the control group. A Dose-response test with 10 different test substance concentrations and 4 replicates each as well as a water control (without test substance) with eight replicates; 10 adult females were exposed per replicate.

Test doses: 0 (control), 5.04, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and

1000.00 mg test substance/kg soil dry weight. Equivalent to: 0.25, 0.46, 0.82, 1.48, 2.66, 4.79, 8.62, 15.52, 27.94 and 50.30 mg Mepiquat Chloride/kg soil dry weight and 0.19, 0.35, 0.63, 1.13, 2.03, 3.65, 6.57, 11.83, 21.29 and 38.33 mg Mepiquat ion/kg soil dry weight.

Test conditions: Temperature during exposure: 20.5 °C to 21.08 °C
pH at the beginning of the test: 5.78 to 6.35
pH at the end of the test: 5.70 to 6.14
Soil moisture content at the beginning of the test: 20.28 % to 21.78 % (corresponding to 51.49 – 52.73 % of the WHCmax)
Soil moisture content at the end of the test: 19.35 % to 19.88 % (corresponding to 49.52 – 50.73 % of the WHCmax)
Lighting: 16 h light and 8 h dark (long day conditions); light intensity: 550 lux to 690 lux

Endpoints: LOEC and NOEC for mortality and reproductive output; EC₁₀, 20, 50 for reproductive output, where possible.

Results and discussions

Mortality after 14 days of experiment at the concentrations of the test item ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, mortality was between 0.00 and 32.50% (corrected mortality: between 0.00 and 31.65%). As for the control group, it was 1.25%.

No behavioural abnormalities or any pathological symptoms of the test organisms could be observed in the control group and in any of the test substance groups.

After the application of the test item at the concentration ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 97.00 and 129.25 per replicate. As for the control group, the number of juveniles was equal to 130.00 per replicate.

The toxic reference item (a.i. dimethoate) was conducted between 26.09.2019 and 10.10.2019. The EC₅₀ for reproductive output was determined to be 3.05 mg./kg soil dry weight. This is within the target range of 3.0 to 7.0 mg a.i./kg soil dry weight given by the OECD guideline 226 (2016) and hence acceptable sensitivity of the test system was assured.

Mortality and reproductive output of *H. aculeifer* after exposure to artificial soil treated with test item

Treatment group	Test substance Concentration [mg t.s./kg sdw]	Mean Mortality [%]	Mean n° of juveniles per replicate	Coefficient of Variation [%]	Reduction in reproductive output [%] ^a
Control	0	1.25	130.00	0.92	-
	5.04	0.00	129.25	0.97	0.58
	9.07	0.00	128.00	1.69	1.54
Mepiquat	16.33	2.50	126.00	1.71	3.08
Chloride	29.40	2.50	125.75	2.09	3.27
5.105% w/v	52.92	2.50	123.75	2.13	4.81
equivalent to	95.26	5.00	122.75	1.54	5.58
Mepiquat ion	171.47	5.00	122.50	0.47	5.77
3.89 w/v SL	308.64	7.50	122.00	0.95	6.15
	555.56	25.00	112.00	9.56	13.85 ⁺
	1000.00	32.50	97.00	5.77	25.38 ⁺
Endpoints		[mg t.s./kg sdw]		[mg a.i./kg sdw]	

NOEC _{mortality}	308.64	15.52 ^a + 11.83 ^b
LOEC _{mortality}	555.56	27.94 ^a + 21.29 ^b
NOEC _{reproductive output}	308.64	15.52 ^a + 11.83 ^b
LOEC _{reproductive output}	555.56	27.94 ^a + 21.29 ^b
EC ₁₀	248.22 (193.10 – 303.33)	12.48 ^a + 9.51 ^b (9.71 – 15.26) + (7.40 – 11.63)
EC ₂₀	>1000 (n.d.)	> (50.30 ^a + 38.33 ^b) (n.d.)
EC ₅₀	> 1000 (n.d.)	> (50.30 ^a + 38.33 ^b) (n.d.)

a: Mepiquat Chloride

b: Mepiquat ion

n.d. – not determined

+: Statistically significant difference between the control and the treatment group at $p < 0.05$

Conclusion

All validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study is considered valid. All validity criteria were met.</p> <p>The test results were evaluated based on the difference between treated and control samples and the difference should be within $\pm 25\%$ (average value). The variation between results of replicate control samples were less than $\pm 15\%$.</p> <p>Agreed endpoints:</p> <p>The difference in the nitrate formation rate between the control soil and the one treated with the test item at the low: 10.14 mg test item/kg soil; and high concentration: 33.80 mg test item/kg soil, did not exceed 25% on 28 day of analysis.</p>
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Reference Report

KCP 10.5.1

“Soil microorganisms: nitrogen transformation test of mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL”, H. S. Anand, 2020, G14252. Eurofins Advinus Limited.

Guideline(s)

OECD Guideline No. 216 (2000)

Deviations

The predicted environmental concentration (PEC) was calculated assuming 1 cm of the soil depth according to the German conditions for the active substances with the mobility in soil K_{Foc} > 500 mL/g, instead of 5 cm soil depth.

GLP

Yes

Acceptability

Yes

Duplication

No

(if vertebrate study)

Material and methods

Test material	Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL
Soil	Agro-Forestry Division, Dry Land Research Centre, GKVK, Bengaluru, Karnataka state. Area where no protection products have been applied for a mini-

	<p>for at least six months before.</p>
Test design	<p>Three replicates for each of the both treated and untreated soils were taken (each containing 25 g dry weight). The soil samples were incubated as a series of individual (for each sampling interval) flasks (each containing 25 g (dry weight) for sampling up to 28 days and for samples beyond 28 days, soil samples were incubated in bulk. The test item was applied using Milli-Q water as a carrier. Test duration: 28 days.</p>
Concentrations of the test material	<p>Control; low concentration: 10.14 mg test item/kg soil (0.510 mg mepiquat chloride/kg soil and 0.388 mg mepiquat/kg soil) and high concentration: 33.80 mg test item/kg soil (1.70 mg mepiquat chloride/kg soil and 1.29 mg mepiquat/kg soil).</p>
Test conditions	<p>Temperature: 19.7 – 20.4°C, soil moisture: 46.6 ± 0.2% of MWHC, incubation in darkness.</p>
Endpoints	<p>The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation. The nitrate formation rate [mg/kg dry weight of soil/day] for selected time points of soil incubation, i.e. 0, 7, 14 and 28 days. Percent deviation from the control in nitrate formation rate calculated for selected time points i.e. 0, 7, 14 and 28 days.</p>
Statistical analysis	<p>The statistical analysis of the experimental data of day 28 was carried out using licensed copies of SYSTAT Statistical Package Ver.12.0. The variable (CO₂, mg/kg dry weight of soil/h) was tested using ANOVA. Comparison of means between treatment groups and control group was done using F-test. All analyses and comparisons were evaluated at the 5% (p < 0.05) level.</p>

Study design

The aim of the study was to detect long-term adverse effects of Mepiquat chloride 5.105% w/v on the processes of nitrogen transformation in aerobic surface soils.

Soil was manually cleared of large objects and sieved to a particle size less than or equal to 2 mm.

The concentrations of the test item were low: 10.14 mg test item/kg soil (0.510 mg mepiquat chloride/kg soil and 0.388 mg mepiquat/kg soil) and high concentration: 33.80 mg test item/kg soil (1.70 mg mepiquat chloride/kg soil and 1.29 mg mepiquat/kg soil). The treated and the control soils were divided into three replicates.

On days 0, 7, 14, and 28 of incubation, soil samples were collected to determine the quantities of nitrate. The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using 0.1 M KCl and measured its absorbance.

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

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the low: 10.14 mg test item/kg soil; and high concentration: 33.80 mg test item/kg soil, did not exceed 25% on 28 day of analysis.

Day	Low dose 10.14 mg test item/kg soil (0.388 mg mepiquat/kg soil)	High dose 33.80 mg test item/kg soil (1.29 mg mepiquat/kg soil)
0	1.89	5.32
7	3.10	8.35
14	3.17	7.94

28	3.98	7.65
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Conclusions

On the basis of the results, it was concluded that Mepiquat chloride 5.105% w/v (equivalent to mepiquat ion 3.89% w/v) SL at the concentration corresponding to 10.14 mg test item/kg soil (0.388 mg mepiquat/kg soil) and 33.80 mg test item/kg soil (1.29 mg mepiquat/kg soil), did not have long-term influence on nitrogen transformation in the soil microorganisms.

Comments of zRMS:	<p>The study is considered valid. All validity criteria were met.</p> <p>Agreed endpoints:</p> <p>On the basis of the results, it was concluded that Mepiquat chloride 5.105% w/v (mepiquat ion 3.89% w/v) SL at the concentrations corresponding to 10.14 mg test item/kg soil (0.388 mg mepiquat/kg soil) and 33.80 mg test item/kg soil (1.29 mg mepiquat/kg soil), does not have long-term influence on carbon transformation in soil microorganisms.</p>
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Reference:	KCP 10.5.2
Report	“Soil microorganisms: carbon transformation test of mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL”, H. S. Anand, 2020, G14251. Eurofins Advinus Limited.
Guideline(s):	OECD Guideline No. 217 (2000)
Deviations:	The predicted environmental concentration (PEC) was calculated assuming 1 cm of the soil depth according to the German conditions for the active substances with the mobility in soil K _{Foc} > 500 mL/g, instead of 5 cm soil depth.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Materials

Test item:	
Description:	Mepiquat chloride 5.105% w/v equivalent to mepiquat ion 3.89% w/v SL
Production batch:	SCL – 22147
Active ingredients content:	Chloride content – 51.05 g/L Ion content – 38.9 g/L
Vehicle and control:	Milli-Q water
Test system:	
Species:	Microorganisms
Source:	Agro-Forestry Division, Dry Land Research Centre, GKVK, Bengaluru, Karnataka state. Area where no 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:	19.7 – 20.4°C

Humidity: 46.6 ± 0.2% of MWHC
Air changes: -
Light and photoperiod: Dark (24/24h)

Study design and methods

Experimental period: 22/05/2020 – 25/06/2020
Test design and treatment: Three replicates for each of the both treated and untreated soils were taken (each containing 25 g dry weight). The soil samples were incubated as a series of individual (for each sampling interval) flasks (each containing 25 g (dry weight) for sampling up to 28 days and for samples beyond 28 days, soil samples were incubated in bulk. The test item was applied using Milli-Q water as a carrier. Test duration: 28 days.

Concentrations of the test material:

Control; low concentration: 10.14 mg test item/kg soil (0.510 mg mepiquat chloride/kg soil and 0.388 mg mepiquat/kg soil) and high concentration: 33.80 mg test item/kg soil (1.70 mg mepiquat chloride/kg soil and 1.29 mg mepiquat/kg soil).

The mean 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 of day 28 was carried out using licensed copies of SYSTAT Statistical Package Ver.12.0. The variable (CO₂, mg/kg dry weight of soil/h) was tested using ANOVA. Comparison of means between treatment groups and control group was done using F-test. All analyses and comparisons were evaluated at the 5% (p < 0.05) level.

Results

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.

Percent deviation of glucose induced respiration rate of treated from control:

Day	Low dose 10.14 mg test item/kg soil (0.388 mg mepiquat/kg soil)	High dose 33.80 mg test item/kg soil (1.29 mg mepiquat/kg soil)
0	20.05	33.34
7	20.72	31.58
14	18.70	31.63
28	10.49	23.84

Conclusion

On the basis of the results, it was concluded that Mepiquat chloride 5.105% w/v (mepiquat ion 3.89% w/v) SL at the concentrations corresponding to 10.14 mg test item/kg soil (0.388 mg mepiquat/kg soil) and 33.80 mg test item/kg soil (1.29 mg mepiquat/kg soil), does not have long-term influence on carbon

transformation in soil microorganisms.

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

Comments of zRMS:	The study is considered valid. All validity criteria were met.						
	Agreed endpoints:						
	Endpoint Value (L/ha ^a)	Soybean (G. max)	Corn (Z. mays)	Pea (P. sativum)	White mustard (S. alba)	Radish (R. sativus)	Tomato (S. lycopersicon)
	Plant number at the end of the experiment						
	ER ₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
	NOER	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
	Shoot length (plants without roots)						
	ER ₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
	NOER	0.2 (10.2 ^b – 7.8 ^c)	0.2 (10.2 ^b – 7.8 ^c)	0.2 (10.2 ^b – 7.8 ^c)	0.4 (20.4 ^b – 15.6 ^c)	<0.2 (<10.2 ^b – <7.8 ^c)	0.2 (10.2 ^b – 7.8 ^c)
	Plant dry weight (plants without roots)						
	ER ₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
	NOER	0.2 (10.2 ^b – 7.8 ^c)	0.4 (20.4 ^b – 15.6 ^c)	0.2 (10.2 ^b – 7.8 ^c)	0.2 (10.2 ^b – 7.8 ^c)	0.2 (10.2 ^b – 7.8 ^c)	<0.2 (<10.2 ^b – <7.8 ^c)
a: value for the test item. Expressed as L/ha							
b: Value for the active substance, mepiquat chloride as g/ha							
c: Value for the active substance, mepiquat ion as g/ha							

a: value for the test item. Expressed as L/ha

b: Value for the active substance, mepiquat chloride as g/ha

c: Value for the active substance, mepiquat ion as g/ha

Reference:

KCP 10.6.2-01

Report

“Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL on seedling emergence and seedling growth of terrestrial plants”. Dr. S. Radha, 2020. Study code: 6093/2019. Bioscience Research Foundation

Guideline(s):

OECD Guideline No. 208 (2006)

Deviations:

Yes, from study plan: The study finished in February 2020, not in September 2019 as it had been planned. This deviation did not affect the study results.

GLP:

Yes

Acceptability:

Yes

Duplication

No

(if vertebrate study)

Materials and methods

Test item:

Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89% w/v SL; Batch Number SCL - 58304; active substance: Chloride content – 51.05 g/L, Ion con-

	tent – 38.9 g/L
Test species:	Soybean (<i>Glycine max</i>), tomato (<i>Solanum lycopersicon</i>), radish (<i>Raphanus sativus</i>), pea (<i>Pisum sativum</i>), white mustard (<i>Sinapis alba</i>), corn (<i>Zea mays</i>)
Soil:	Sandy loam
Study design:	Number of concentrations: 5 application rates + a control Number of replicates: 7 replicates of each application rate and the control Number of seeds: 3 seeds/replicate Test termination: 14 days after the emergence of 50% of the control seedlings
Application rates:	Control, 0.2, 0.4, 0.8, 1.5 and 3 kg/ha (10.2, 20.4, 40.8, 76.6 and 153.2 g Mepiquat chloride/ha and 7.8, 15.6, 31.1, 58.4 and 116.7 g Mepiquat ion/ha). Volume of distilled water used to prepare the highest rate: 300 L water/ha.
Test conditions:	Temperature: 22.1– 23.4 °C; humidity: 52.5 – 63.5%; lighting: 16 h light : 8 h dark; light intensity: 336 – 400 µE/m ² /s; carbon dioxide concentration: 341 – 360 ppm
Statistical analysis:	ER ₁₀ , ER ₂₅ , ER ₅₀ and NOER – Probit in the NCSS and one-way ANOVA using GraphPad Prism 8.0, respectively
Endpoints:	ER ₁₀ , ER ₂₅ , ER ₅₀ , NOER

Results and Conclusions

The application of the test item at the rates ranging from 0.2 to 3 kg/ha had a varied impact on seedling emergence and seedling growth of all the plant species tested.

After the application of the test item, seedling emergence was not delayed for all the species including soybean, corn, pea, radish, tomato and white mustard in comparison with the control. However all the plant species emerged after the application of the test item at rates ranging from 0.2 to 3 L/ha. The phytotoxic symptoms were not observed for all the six plant species at rates of 0.2 to 3 kg/ha. The following symptoms were not observed:

- Chlorosis, wilting, necrosis, leaf deformation, stem deformation or stunted growth

Table 5 - Endpoint values

Endpoint Value (L/ha ^a)	Soybean (<i>G. max</i>)	Corn (<i>Z. mays</i>)	Pea (<i>P. sativum</i>)	White mustard (<i>S. alba</i>)	Radish (<i>R. sativus</i>)	Tomato (<i>S. lycopersicon</i>)
Plant number at the end of the experiment						
ER₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
NOER	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
Shoot length (plants without roots)						
ER₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
NOER	0.2 (10.2 ^b - 7.8 ^c)	0.2 (10.2 ^b - 7.8 ^c)	0.2 (10.2 ^b - 7.8 ^c)	0.4 (20.4 ^b - 15.6 ^c)	<0.2 (<10.2 ^b - <7.8 ^c)	0.2 (10.2 ^b - 7.8 ^c)
Plant dry weight (plants without roots)						
ER₅₀	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)	>3 (>153.2 ^b - >116 ^c)
NOER	0.2 (10.2 ^b - 7.8 ^c)	0.4 (20.4 ^b - 15.6 ^c)	0.2 (10.2 ^b - 7.8 ^c)	0.2 (10.2 ^b - 7.8 ^c)	0.2 (10.2 ^b - 7.8 ^c)	<0.2 (<10.2 ^b - <7.8 ^c)

a: value for the test item. Expressed as L/ha

b: Value for the active substance, mepiquat chloride as g/ha

c: Value for the active substance, mepiquat ion as g/ha

Comments of zRMS:	The study is considered valid. All validity criteria were met.						
	Agreed endpoints:						
	Endpoint Value (L/ha ^a)	Soybean (<i>G. max</i>)	Corn (<i>Z. mays</i>)	Pea (<i>P. sativum</i>)	White mustard (<i>S. alba</i>)	Radish (<i>R. sativus</i>)	Tomato (<i>S. lycopersicon</i>)
	Plant number at the end of the experiment						
	ER ₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
	NOER	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
	Shoot length (plants without roots)						
	ER ₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
	NOER	0.2 (10.2 ^b + 7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)	0.4 (20.4 ^b + 15.56 ^c)	0.4 (20.4 ^b + 15.6 ^c)	<0.2 (<10.2 ^b + <7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)
	Plant dry weight (plants without roots)						
	ER ₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
	NOER	0.2 (10.2 ^b + 7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)	0.4 (20.4 ^b + 15.6 ^c)	0.4 (20.4 ^b + 15.6 ^c)	<0.2 (<10.2 ^b + <7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)
	a: value for the test item. Expressed as kg/ha b: Value for the active substance, i.e Mepiquat chloride expressed as g/ha c: Value for the active substance, i.e Mepiquat ion expressed as g/ha						

Reference:

KCP 10.6.2-02

Report

“Effect of Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL on vegetative vigour of terrestrial plants”. Dr. S. Radha. 2020. Study code: 6094/2019. Bioscience Research Foundation.

Guideline(s):

OECD Guideline No. 227 (2006)

Deviations:

Yes. The study finished in February 2020, not in October 2019 as it had been planned. This deviation did not affect the study results.

GLP:

Yes

Acceptability:

Yes

Duplication

No

(if vertebrate study)

Materials and methods

Test item:

Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL; Batch

	Number: SCL-58304; active substance: Chloride content – 51.05 g/L, Ion Content – 38.9 g/L
Test species:	Soybean (<i>Glycine max</i>), tomato (<i>Solanum lycopersicon</i>), Radish (<i>Raphanus sativus</i>), Pea (<i>Pisum sativum</i>), white mustard (<i>Sinapsis alba</i>), Corn (<i>Zea mays</i>).
Soil:	Sandy loam
Study design:	Number of rates: 5 application rates + control; number of replicates: 7 replicates/rate. The total number of plants per application rate – 21. Test termination: 21 days after the spraying.
Application rates:	Control, 0.2, 0.4, 0.8, 1.5 and 3 kg/ha (10.2, 20.4, 40.8, 76.6 and 153.2 g Mepiquat chloride/ha and 7.8, 15.6, 31.1, 58.4 and 116.7 g mepiquat ion/ha). Volume of distilled water used to prepare the highest rate: 300 L water/ha.
Test conditions:	Temperature: 22.1 – 23.4°C, humidity: 52.5 – 63.5%, controlled light – dark cycles (16h:8h), light intensity: 336 – 400 µE/m ² /s, carbon dioxide concentration: 341 – 360 ppm.
Statistical analysis:	ER ₁₀ , ER ₂₅ , ER ₅₀ and NOER values were determined by using a Probit analysis in the NCSS and one-way ANOVA using Graph Pad Prism 8.0
Endpoints:	ER ₁₀ , ER ₂₅ , ER ₅₀ , NOER

Results and Conclusions

The test item Mepiquat Chloride 5.105% w/v equivalent to Mepiquat ion 3.89 w/v SL applied at rates ranging from 0.2 to 3 kg/ha had a varied impact on vegetative vigour of all plant species.

There was mortality observed for all the plant species at rates ranging from 0.2 to 3 kg test item/ha. The phytotoxic symptoms for all the plant species tested were observed at all the rates of the test item used. The following symptoms were not observed on 21 days after the test item application: Chlorosis, necrosis, wilting, leaf deformation, stem deformation or death.

Table 6 - Endpoint values

Endpoint Value (L/ha ^a)	Soybean (<i>G. max</i>)	Corn (<i>Z. mays</i>)	Pea (<i>P. sativum</i>)	White mustard (<i>S. alba</i>)	Radish (<i>R. sativus</i>)	Tomato (<i>S. lycopersicon</i>)
Plant number at the end of the experiment						
ER₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
NOER	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
Shoot length (plants without roots)						
ER₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
NOER	0.2 (10.2 ^b + 7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)	0.4 (20.4 ^b + 15.6 ^c)	0.4 (20.4 ^b + 15.6 ^c)	<0.2 (<10.2 ^b + <7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)
Plant dry weight (plants without roots)						
ER₅₀	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)	>3 (>153.2 ^b + >116.7 ^c)
NOER	0.2 (10.2 ^b + 7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)	0.4 (20.4 ^b + 15.6 ^c)	0.4 (20.4 ^b + 15.6 ^c)	<0.2 (<10.2 ^b + <7.8 ^c)	0.2 (10.2 ^b + 7.8 ^c)

a: value for the test item. Expressed as kg/ha

b: Value for the active substance, i.e Mepiquat chloride expressed as g/ha

c: Value for the active substance, i.e Mepiquat ion expressed as g/ha

A 2.6.1	KCP 10.6.1	Summary of screening data
A 2.6.2	KCP 10.6.2	Testing on non-target plants
A 2.6.3	KCP 10.6.3	Extended laboratory studies on non-target plants
A 2.7	KCP 10.7	Effects on other terrestrial organisms (flora and fauna)
A 2.8	KCP 10.8	Monitoring data