

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 126000 B

Product name: CLARA

Chemical active substance:

Chlormequat chloride, 720 g/L

Southern Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Sharda Cropchem Ltd.

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When	What
05.2023	dRR ver. zRMS
August 2023	Applicant update
October 2023	Final version of RR after commenting period
03.2024	Corrected by zRMS

zRMS comments:

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

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6				7			8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: devel- opmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion											
					Method / Kind	Timing / Growth stage of crop & season	Max. num- ber a) per use b) per crop/ season	Min. inter- val between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants					
Zonal uses (field or outdoor uses, certain types of protected crops)																									
1	CEU	Winter wheat	F	Regulation of growth, prevention of lodging	Foliar Spray	BBCH 29- 32	a) 1 b) 1	-	a) 1.3-2.1 b) 1.3-2.1	a) 0.936- 1.51 b) 0.936- 1.51	200-300			A	A	A	A	A	A	A	A	A	A	A	A

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:	<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 (e.g. fumigation of a structure)(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application(5) Scientific names <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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zRMS comments:

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

9.1.1 Overall conclusions

9.1.1.1 Not relevant.

9.1.1.2 Effects on birds (KCP 10.1.1), According to the screening and first tier risk assessment for cereals, the TERA and TERLT values for Chlormequat chloride are lower than the Annex VI trigger of 10 and 5, respectively for the large herbivorous bird “goose”, indicating that CLARA (Chlormequat chloride 72% SL) presents unacceptable acute and long-term risk to birds according to the intended use in cereals. A refinement of the risk was done and the TERA and TERLT were above the triggers showing no risk.

Agree with the presented risk assessment.

9.1.1.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2), According to the first-tier risk assessment for cereals, the TERA values for the active substance Chlormequat chloride are lower than the Annex VI trigger of 10 for small omnivorous mammal ‘mouse’, indicating that CLARA (Chlormequat chloride 72% SL) presents an unacceptable acute risk to mammals. A refinement of the risk was done and the TERA were above the trigger showing no risk. The TERLT values for Chlormequat chloride are greater than the Annex VI trigger of 5 indicating that CLARA presents no unacceptable long-term risk to mammals.

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

- **Birds:**

According to the screening and first tier risk assessment for cereals, the TERA and TERLT values for Chlormequat chloride are lower than the Annex VI trigger of 10 and 5, respectively for the large herbivorous bird “goose”, indicating that CLARA (Chlormequat chloride 72% SL) presents unacceptable acute and long-term risk to birds according to the intended use in cereals. A refinement of the risk was done and the TERA and TERLT were above the triggers showing no risk

RMS 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 such as chlormequat chloride was confirmed based on TERA and TERLT above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

- **Mammals:**

According to the first-tier risk assessment for cereals, the TERA values for the active substance Chlormequat chloride are lower than the Annex VI trigger of 10 for small omnivorous mammal ‘mouse’, indicating that CLARA (Chlormequat chloride 72% SL) presents an unacceptable acute risk to mammals.

A refinement of the risk was done and the TERA were above the trigger showing no risk. The TER_{LT} values for Chlormequat chloride are greater than the Annex VI trigger of 5 indicating that CLARA presents no unacceptable long-term risk to mammals.

RMS 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 mammals such as chlormequat chloride was 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.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

Chlormequat chloride:

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

CLARA:

For the intended uses winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate acute as characterised by an EC₅₀ for *Daphnia magna* of 88.49 mg/L in connection with an assessment factor of 100) in all FOCUS Step 1scenarios. Therefore, no further assessment is necessary.

zRMS comment: The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters” (EFSA Journal 2013;11(7):3290).

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

No

9.1.1.6 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to CLARA (Chlormequat Chloride 72% SL) is expected according to the proposed intended uses on cereals.

zRMS comment:

Bees:

The risk assessment for bees was conducted in accordance with SANCO/10329/2002 rev. 2 final. The acute oral and contact toxicity data are available for the formulation **CLARA**. Based on the first-tier assessment results, the risk is acceptable (HQ values exceeded 50) for the product. In addition, the chronic study for adult bees and a study effects on honey bee development and other honey bee life stages ~~should be submitted~~ was submitted by Applicant. ~~The chronic studies were accepted by zRMS in updated RAR.~~ The risk assessment based on this studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

9.1.1.7 Effects on arthropods other than bees (KCP 10.3.2)

Studies on the toxicity to arthropods show that the active substance chlormequat chloride and the formulated product CLARA pose no in-field and off-field risk for non-target arthropods, since HQ values were below 2 and the PER_{in-field} and the corr. PER_{off-field} were below the rate with $\leq 50\%$ effect. Therefore, an application of CLARA in respect of the GAP does not present an unacceptable risk for arthropods other than bees.

zRMS comment:

Arthropods other than bees:

The Applicant used the available data for substance active chlormequat chloride to indicate acceptable risk for arthropods other than bees.

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

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

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

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

zRMS comment:

Earthworms:

The study on the effects of **CLARA** on earthworms was not provided by Applicant. In this case, the Applicant used the available data for substance active chlormequat chloride to indicate acceptable risk for earthworms. In opinion RMS this approach should be considered at MSs level. It was acknowledged that the active substance chlormequat chloride did not show a high toxicity to earthworms. The RMS noted that the risk assessment for chlormequat chloride indicated a very high margin of safety based on the currently available exposure assessment. In this case, the toxicity of the plant protection product CLARA can be predicted on the basis of the data for the active substance. Acceptable risk assessment could be conclude without the study for PPP and earthworms.

The risk assessment for earthworms should be considered at MSs level. The risk assessment for earthworms should be considered at MSs level. Perhaps at the level of national registrations in different countries, additional data will be required to elucidate the effects of CLARA on earthworms such as the study on the effects of CLARA on earthworms.

Other soil macro-organisms

In accordance with the data requirements of the (EU) Regulation 284/2013 data on *Folsomia candida* and *Hypoaspis aculeifer* should be submitted. No toxicity data are available for the PPP **CLARA**. However, the Applicant provided a justification indicating that the data requirements indicate that an assessment is not triggered since it is of low risk to NTAs. The justification was accepted by RMS.

The risk assessment for soil macro-organisms should be considered at MSs level.

- **Soil microorganisms:**

Risk assessments conducted with relevant PEC_{soil} for Chlormequat chloride in CLARA (Chlormequat chloride 72% SL) formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

zRMS comment: Agreed.

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

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for CLARA (Chlormequat chloride 72% SL) shows that Annex VI trigger of 5 is not exceeded, indicating that CLARA poses a low risk to non-target plants when applied according to the proposed use rates.

zRMS comment: Agreed.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

Not relevant.

9.1.3 Consideration of metabolites

Not relevant.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride.

However, the provision of further data on CLARA 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
<i>Coturnix japonica</i>	Chlormequat chloride	Oral 1 d Acute	LD₅₀ = 441 mg/kg bw	EFSA Scientific Report (2008) 179, 1-77
<i>Coturnix japonica</i>	Chlormequat chloride	Dietary 8 d Short-term	LDD ₅₀ > 310 mg/kg bw/d	
<i>Coturnix japonica</i>	Chlormequat chloride	Dietary Reproductive toxicity	NOEL = 54.8 mg/kg bw/d	

9.2.1.1 Justification for new endpoints

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

According to EFSA/2009/1438, ‘were it is lower than the acute LD₅₀, the dietary LD₅₀ should be used in the acute risk assessment’. The dietary endpoint in this case is a > value, the details for this study have been referred to in volume 3 of the DAR, the short-term dietary study was a limit test conducted only at 310 mg a.s./kg bw /day (5000ppm) with 0% mortality at this dose. Given the absence of mortality at this dose compared with an estimate of 50% via the oral route at 441 mg a.s./kg bw/day, it is likely that the dietary route is not of significantly higher toxicity than the oral route and hence it is acceptable to only consider the acute oral LD₅₀ in the risk assessment. Moreover, acute oral LD₅₀/10 is considered in the reproductive risk assessment. Therefore, NOEC of 44.1 mg/kg bw/d is used as a 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).

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 CLARA in winter wheat

Intended use	Winter wheat				
Active substance/product	Chlormequat chloride				
Application rate (g/ha)	1 x 1510				
Acute toxicity (mg/kg bw)	441				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a

Cereals early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose". Grass + cereals. 100% cereal shoots	30.5	1.0	46.06	9.58
Cereals BBCH 10-29	Small omnivorous bird "lark". Combination (invertebrates with interception). 25% crop leaves, 25% weed seeds, 50% ground arthropods	24.0	1.0	36.24	12.17
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves, 25% weed seeds, 50% ground arthropods	12.0	1.0	3.72	83.33
Reprod. toxicity (mg/kg bw/d)	44.1				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose". Grass + cereals. 100% cereal shoots	16.2	1.0 x 0.53	12.96	3.40
Cereals BBCH 10-29	Small omnivorous bird "lark". Combination (invertebrates with interception). 25% crop leaves, 25% weed seeds, 50% ground arthropods	10.9	1.0 x 0.53	8.72	5.06
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves, 25% weed seeds, 50% ground arthropods	5.4	1.0 x 0.53	4.32	10.20

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

The risk assessment at screening and Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

Safe use of active substance for birds such as chlormequat chloride were confirmed - except risk assessment for large herbivorous bird "goose" 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.

The refinement risk assessment for birds should be based on MS requirements.

9.2.2.2 Higher-tier risk assessment

An acute risk and long-term risk for large-herbivorous "goose" (cereals Early (shoots) winter BBCH 10-29) was observed. Therefore, a refinement of the risk is needed.

PT refinement

A 90th %ile PT of 0.67 is proposed. This value is relevant for the consumers only in cereal crops in winter according to Prosser, 2010. It must be, however, emphasized that individual geese visits to arable crops are rare, as it is also reflected in the abovementioned paper.

Table 9.2-3: Higher-tier assessment of the acute risk for birds due to the use of CLARA in winter wheat – refined parameters (*) are further described and justified in the text

Intended use		Winter wheat					
Active substance/product		Chlormequat chloride					
Application rate (g/ha)		1 x 1510					
Acute toxicity (mg/kg bw)		441					
TER criterion		10					
Focal species	Food category, % in diet	FIR/bw	RUD₉₀ × DF (mg/kg food)	MAF₉₀	PT	DDD_m (mg/kg bw/d)	TER_t
Goose BBCH 10-29	Early cereal shoots	0.30	102.3 x 1	1	0.67 ¹	31.05	14.20

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

¹ 90th %ile PT proposed in Consolidation of bird and mammal PT data for use in risk assessment (Prosser, 2010).

Table 9.2-4: Higher-tier assessment of the long-term/reproductive risk for birds due to the use of CLARA in winter wheat – refined parameters (*) are further described and justified in the text

Intended use		Winter wheat					
Active substance/product		Chlormequat chloride					
Application rate (g/ha)		1 x 1510					
Acute toxicity (mg/kg bw)		44.1					
TER criterion		5					
Focal species	Food category, % in diet	FIR/bw	RUD_m × DF (mg/kg food)	MAF_m × TWA*	PT	DDD_m (mg/kg bw/d)	TER_t
Goose BBCH 10-29	Early cereal shoots	0.30	54.2 x 1	1 x 0.53	0.67 ¹	8.72	5.06

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

¹ 90th %ile PT proposed in Consolidation of bird and mammal PT data for use in risk assessment (Prosser, 2010).

The refinement risk assessment at higher-tier is considered acceptable. The refinement risk assessment is based on refinement PT value (90th %ile PT proposed in Consolidation of bird and mammal PT data for use in risk assessment - Prosser, 2010).

Safe use of active substance for birds such as chlormequat chloride were confirmed based on TER_A and TER_{LT} above - the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

The refinement risk assessment for birds should be based on MS requirements.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is con-

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

Effective application rate (g/ha) =	1510			
Acute toxicity (mg/kg bw) =	441	quotient =		3.42
Reprod. toxicity (mg/kg bw/d) =	44.1	quotient =		34.2

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

Agree with the presented risk assessment.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Chlormequat chloride amounts from -3.07 to -3.47 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Agree with the presented risk assessment.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Agree with the presented risk assessment.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

Agree with the presented risk assessment.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

Agree with the presented risk assessment.

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

Not relevant.

9.2.4 Overall conclusions

According to the screening and first tier risk assessment for cereals, the TERA and TERIt values for Chlormequat chloride are lower than the Annex VI trigger of 10 and 5, respectively for the large herbivorous bird “goose”, indicating that CLARA (Chlormequat chloride 72% SL) presents unacceptable acute and long-term risk to birds according to the intended use in cereals. A refinement of the risk was done and the TERA and TERIt were above the triggers showing no risk.

Agree with the presented risk assessment.

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

9.3.1 Toxicity data

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

Effects on mammals of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride. However, the provision of further data on the formulation CLARA is not considered essential, because risk may be reliably assessed using the EU-agreed endpoints only and new studies should not be conducted in regards of animal welfare (EFSA Journal 2009; 7(12):1438).

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

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

Species	Substance	Exposure System	Results	Reference
Rabbit	Chlormequat chloride	Oral 1 d Acute	LD ₅₀ = 115 mg/kg bw	EFSA Scientific Report (2008) 179, 1-77
Rat	Chlormequat chloride	Dietary Reproductive toxicity Multigeneration study	NOAEL = 74 mg/kg bw/d reproduction NOAEL = 41 mg/kg bw/d offspring	

9.3.1.1 Justification for new endpoints

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

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment

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

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

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

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

Intended use		Winter wheat			
Active substance/product		Chlormequat chloride			
Application rate (g/ha)		1 x 1510			
Acute toxicity (mg/kg bw)		115			
TER criterion		10			
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	5.4	1.0	8.15	14.10
Cereals BBCH 10-29	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	17.2	1.0	25.97	4.43
Cereals BBCH 30-39	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	8.6	1.0	12.99	8.86
Reprod. toxicity (mg/kg bw/d)		41			
TER criterion		5			
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Growth stage					
Cereals BBCH > 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	1.9	1.0 x 0.53	1.52	26.96
Cereals BBCH 10-29	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	7.8	1.0 x 0.53	6.24	6.57
Cereals BBCH 30-39	Small omnivorous mammal "mouse". Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	3.9	1.0 x 0.53	3.12	13.14

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER:

toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.3.2.2 Higher-tier risk assessment

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

Endpoint refinement

In Confirmatory data (March 2014) it is specified:

In reference of the summary table given in the mammal toxicity DAR, the acute oral toxicity of Chlormequat chloride has been determined in four rat studies, three mice studies and on study in rabbits. It is possible to derive a geometric mean of the endpoints in the acute dietary assessment as different studies exist for one species, and furthermore different species have been tested. In accordance with the EFSA Guidance Document (2009), a geometric mean can be derived if the studies are considered to be equivalent in terms of guideline and, in particular, the vehicle/solvent used. A toxicology specialist has advised that all the rat and mouse studies comply with the minimum criteria for the OECD acute oral LD₅₀ study (the basics being 5 animals/sex/group and 3 or more dose levels). They all used water as the vehicle. Furthermore, the results between these studies are very similar. It is noted that the study by Munk and Freisberg (1975) is the only value which is noticeable different; however, as this value is within a factor of 2 of any other value, it is not considered to be an outlier. The rabbit study (1975) does not match the minimum requirement and gives the lowest toxicity value. Despite this, the RMS proposes that this study can still be used in the geometric mean calculations; the inclusion of the endpoint produces a lower LD₅₀ geometric mean and thus a more conservative risk assessment.

All differences between males and females in the acute oral toxicity studies have been calculated to be <25%, therefore the combined endpoints of males and females have been used in calculations of the geometric mean below. It is worth noting that some study summaries only stated a combined endpoint. Furthermore, the study on rabbits produces an LD₅₀ of 115 mg a.s./kg bw, it is not stated whether this study endpoints is based on combined sexes, reference is only made to the use of mixed breeds. Despite this no other study was considered with rabbits and so this endpoint will be used in the calculations of the geometric mean.

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

Using a stepwise approach, a geometric mean is first calculated for the acute oral toxicity endpoints derived from the studies with rats and mice, respectively. Next, this value is used to derive the geometric mean of the endpoints determined for the three different species. The LD₅₀ geometric mean of 343 mg/kg b.w. has been calculated based on these values in the summary table.

Table 9.3-3: Calculations of the relevant mammalian toxicity endpoints for the acute risk assessment of Chlormequat chloride (from section B.9.3.7 of the DAR of April 2007)

Species	Experimental LD ₅₀	Reference	LD ₅₀ (geometric mean single species)	LD ₅₀ (geometric mean all species)
Rat	522	Lowe C.A. (1990) RD#1990/10676	598	343

Rat	534	Suresh T.P. (1991a) Report ST959-AOR		
Rat	883	Munk R., Freisberg K.O.(1975) RD#1975/012		
Rat	520	Hattori K. (1981) RD#1981/10230		
Mouse	629	Suresh T.P. (1991b) Report ST960-AOM	586	
Mouse	589	Munk R., Freisberg K.O.(1975) RD# 1975/0072		
Mouse	544	Hattori K. (1981) RD#1981/10230		
Rabbit	115	Kirsch P. et al. (1975) RD#1975/091	115	

An LD₅₀ geomean, of 343 mg a.s./kg bw will be used in the acute mammal toxicity risk assessment.

Deposition factor

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

Table 9.3-4: Higher-tier assessment of the acute risk for mammals due to the use of CLARA in winter wheat – refined LD₅₀

Intended use		Winter wheat					
Active substance/product		Chlormequat chloride					
Application rate (g/ha)		1 x 1510					
Acute toxicity (mg/kg bw)		343					
TER criterion		10					
Focal species	Food category, % in diet	FIR/bw	RUD₉₀** × DF* (mg/kg food)	MAF₉₀	PT	DDD₉₀ (mg/kg bw/d)	TER_a
Wood mouse (<i>Apodemus sylvaticus</i>)	Combination (invertebrates with interception). 25% weeds, 50% weed seeds, 25% ground arthropods	0.27 ¹	64.5 ¹ × 0.8 ²	1.0	1.0	21.04	16.30

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

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

² Deposition factor according to FOCUS groundwater guidance.

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

The risk assessment at screening and Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of active substance for birds such as chlormequat chloride 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 for all scenario except RA for .

However, TER for first-tier assessment for of the acute risk for mouse for chlormequat chloride is **9.7**. In opinion RMS the acute risk assessment for small omnivorous mammal “mouse” with TER = 9.7 should be accepted without refinement. This value is very close to the trigger value of 10.

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

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

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

Effective application rate (g/ha) =	1510		
Acute toxicity (mg/kg bw) =	115	quotient =	13.13
Reprod. toxicity (mg/kg bw/d) =	41	quotient =	36.80

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

9.3.2.4 Effects of secondary poisoning

The $\log P_{ow}$ of Chlormequat chloride amounts to -3.07 to -3.47 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 first-tier risk assessment for cereals, the TERA values for the active substance Chlormequat chloride are lower than the Annex VI trigger of 10 for small omnivorous mammal 'mouse', indicating that CLARA (Chlormequat chloride 72% SL) presents an unacceptable acute risk to mammals.

A refinement of the risk was done and the TERA were above the trigger showing no risk. The TERIt values for Chlormequat chloride are greater than the Annex VI trigger of 5 indicating that CLARA presents no unacceptable long-term risk to mammals.

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

No data available.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Chlormequat chloride. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	Chlormequat chloride	96 h, f	LC ₅₀ > 100 mg a.s./L _{nom}	EFSA Scientific Report (2008) 179; 1-77
<i>Oncorhynchus mykiss</i>	Chlormequat chloride	21 d, ss	NOEC = 43.1 mg a.s./L _{nom}	
Aquatic invertebrate				
<i>Daphnia magna</i>	Chlormequat chloride	48 h, s	EC ₅₀ = 31.7 mg a.s./L _{nom}	EFSA Scientific Report (2008) 179; 1-77
<i>Daphnia magna</i>	Chlormequat chloride	21 d, ss	NOEC = 2.4 mg a.s./L _{nom}	
Algae				
<i>Pseudokirchneriella subcapitata</i>	Chlormequat chloride	72 h, s	E _b C ₅₀ > 100 mg a.s./L _{nom} E _r C ₅₀ > 100 mg a.s./L _{nom}	EFSA Scientific Report (2008) 179; 1-77
Higher plant				
<i>Lemna gibba</i>	Chlormequat chloride	7 d, s	E _b C ₅₀ = 5.3 mg a.s./L _{mm} E _r C ₅₀ = 28.0 mg a.s./L _{mm}	EFSA Scientific Report (2008) 179; 1-77
Higher-tier studies (micro- or mesocosm studies)				
No study submitted.				

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Chlormequat chloride 72% SL

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	CLARA	96 h, s	LC ₅₀ > 100 mg/L	KCP 10.2.1-01. XXXXXXX (2018). W/132/17
Aquatic invertebrate				
<i>Daphnia magna</i>	CLARA	48 h, s	EC ₅₀ = 88.49 mg/L	KCP 10.2.1-02. Nierzędska, E. (2019). W/133/17
Algae				
<i>Pseudokirchneriella subcapitata</i>	CLARA	72 h, s	E _r C ₅₀ >100 mg/L E _y C ₅₀ >100 mg/L	KCP 10.2.1-03. Nierzędska, E. (2019). W/134/17 W/133/17
Higher plant				
<i>Lemna gibba</i>	CLARA	7 d, s	Based on frond number: E _r C ₅₀ >1000 mg/L E _y C ₅₀ > 13.79 mg/L Based on dry weight: E _r C ₅₀ > 97.23 mg/L E _y C ₅₀ = 8.88 mg/L	KCP 10.2.1-04. Nierzędska, E. (2019). W/135/17
Higher-tier studies (micro- or mesocosm studies)				
No study submitted.				

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

9.5.1.1 Justification for new endpoints

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

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.

CLARA

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CLARA for each organism group based on drift calculations for winter wheat

Group		Fish acute	Inverteb. acute	Algae	Higher Plant
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>L. gibba</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀	E _r C ₅₀
(µg/L)		400000 97230*	88490	100000	1000000
AF		100	100	10	10
RAC (µg/L)		4000 972.30	884.9	10000	100000
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	21.953	0.022 0.023	0.025	0.002	< 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

*based on the lowest endpoint for dry weight

CHLORMEQUAT CHLORIDE

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Chlormequat chloride for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CLARA in winter wheat

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>P. subcapitata</i>	<i>L. gibba</i>
Endpoint (µg/L)		LC ₅₀ 100000	NOEC 43100	EC ₅₀ 31700	NOEC 2400	E _r C ₅₀ 100000	E _r C ₅₀ 28000
AF		100	10	100	10	10	10
RAC (µg/L)		1000	4310	317	240	10000	2800
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	453.2	0.453	0.105	1.430	1.888	0.045	0.162
Step 2							
S-Europe	135.65	0.136	0.031	0.428	0.565	0.014	0.048
N-Europe	72.1	0.072	0.017	0.227	0.300	0.007	0.026

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

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

9.5.3 Overall conclusions

Chlormequat chloride:

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

CLARA:

For the intended uses winter wheat, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for invertebrate acute as characterised by an EC₅₀ for *Daphnia magna* of 88.49 mg/L in connection with an assessment factor of 100) in all FOCUS Step 1 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 Chlormequat chloride. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride. New data submitted with this application are listed in Appendix 1 and

summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Chlormequat chloride	Oral	LD ₅₀ > 80.2 µg/bee	EFSA Scientific Report (2008) 179, 1-77
<i>Apis mellifera</i>	Chlormequat chloride	Contact	LD ₅₀ > 65.2 µg/bee	
<i>Apis mellifera</i>	CLARA	Oral	LD ₅₀ > 400 µg/bee	KCP 10.3.1.1.1. Glanas, A. (2017). B/100/16
<i>Apis mellifera</i>	CLARA	Contact	LD ₅₀ > 400 µg/bee	KCP 10.3.1.1.2. Glanas, A. (2017). B/101/16
<i>Apis mellifera</i>	CLARA	Chronic, 10d	LDD ₅₀ = 81.87 µg/bee/day NOEDD = 31.89 µg/bee/day	KCP 10.3.1.2 M. Mohanraj, 2023, 12354/2023
<i>Apis mellifera</i>	CLARA	Larvae, 22d	ED ₅₀ = 29.46 µg /larvae NOED = 9.5 µg /larvae	KCP 10.3.1.3 M. Mohanraj, 2023, 12355/2023
Higher-tier studies (tunnel test, field studies)				
Not required.				

9.6.1.1 Justification for new endpoints

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

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

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

Intended use	Winter wheat
Active substance	Chlormequat chloride
Application rate (g/ha)	1 x 1510 g a.s./ha

Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 80.2	1510	18.83
Contact toxicity	> 65.2		23.16
Product	CLARA (Chlormequat chloride 72% SL)		
Application rate (g/ha)	1 x 2.1 L/ha (2377.62 g/ha*)		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 400	2377.62	5.94
Contact toxicity	> 400		5.94

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

* Considering a density of 1.1322 g/cm³

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

First-tier assessments indicate that no unacceptable risk for bees exposed to CLARA (Chlormequat chloride 72% SL) is expected according to the proposed intended uses on cereals.

zRMS comment:

Bees:

The risk assessment for bees was conducted in accordance with SANCO/10329/2002 rev. 2 final. The acute oral and contact toxicity data are available for the formulation CLARA. Based on the first-tier assessment results, the risk is acceptable (HQ values exceeded 50) for the product. In addition, the chronic study for adult bees and a study effects on honey bee development and other honey bee life stages should be submitted was submitted by Applicant. The chronic studies were accepted by zRMS in updated RAR. The risk assessment based on this studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

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

Effects on non-target arthropods of Chlormequat chloride 72% SL were not evaluated as part of the EU assessment of Chlormequat chloride. 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>Typhlodromus pyri</i>	Chlormequat chloride	Laboratory test glass plates (2D)	LR ₅₀ > 2250 g a.s./ha	EFSA Scientific Report (2008) 179, 1-77
<i>Aphidius rhopalosiphi</i>	Chlormequat chloride	Laboratory test glass plates (2D)	LR ₅₀ > 2200 g a.s./ha	
<i>Poecilus cupreus</i> (carabid beetle)	STE 24371 W (Chlormequat chloride 720 g/L)	Silica sand 14 days	At 1512 g a.s./ha: 0% mortality -40% feeding	
<i>Poecilus cupreus</i> (carabid beetle)	Stabilan (Chlormequat chloride 465 g/L)	Quartz sand 14 days	At 1395 g a.s./ha: 0% mortality -1.3% feeding	
<i>Poecilus cupreus</i> (carabid beetle)	Stabilan (chlormequat chloride 465 g/L)	Quartz sand 14 days	At 1395 g a.s./ha: 0% mortality -15% feeding	
<i>Aleochara bilineata</i> (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Sand 5 day survival 10 day hatching	At 1395 g a.s./ha: 0% mortality No significant difference reproduction	
<i>Aleochara bilineata</i> (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Moist sand 55 days	At 1395 g a.s./ha: +4.3% parasitic capacity	
<i>Aleochara bilineata</i> (rove beetle)	Stabilan (chlormequat chloride 465 g/L)	Moist quartz sand 4 weeks	At 1395 g a.s./ha: +26% parasitisation	
<i>Chrysoperla carnea</i> (green lacewing)	BAS 062 03 W (Chlormequat chloride 765.8 g/L)	Glass plates 4-5 days after pupation 7 days hatching	At 2297.4 g a.s./ha: Slight reduction mortality No effect reproduction	
Laboratory studies – CLARA (Chlormequat chloride 72% SL)				
<i>Aphidius rhopalosiphi</i>	CLARA	Extended laboratory test (3D)	LR ₅₀ > 16.8 L/ha ER ₅₀ > 16.8 L/ha	KCP 10.3.2.2-01. Lemańska, N. (2018). B/102/16

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i>	CLARA	Extended laboratory test (2D)	LR ₅₀ > 2.069 L/ha equivalent to > 1500 g a.s./ha ER ₅₀ > 1.084 L/ha equivalent to 785.9 g a.s./ha	KCP 10.3.2.2-02, Lemańska, N. (2018), B/103/16
Field or semi-field tests				
Not required.				

9.7.1.1 Justification for new endpoints

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

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CLARA in winter wheat

Intended use		Winter wheat		
Active substance/product		Chlormequat chloride		
Application rate (g/ha)		1 x 1510 g a.s./ha		
MAF		1.0		
Test species Tier I	LR₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2	
<i>Typhlodromus pyri</i>	> 2250	1510	0.67	
<i>Aphidius rhopalosiphi</i>	2200	1510	0.69	
Test species Tier II	LR₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 1	
<i>Poecilus cupreus</i> [‡]	1395	1510	1.08	
<i>Aleochara bilineata</i> [‡]	1395	1510	1.08	
Active substance/product		CLARA (Chlormequat chloride 72% SL)		
Application rate (g/ha)		1 x 2.1 L/ha		
MAF		1.0		
Test species Higher-tier	ER₅₀ (L/ha)	PER_{in-field} (L/ha)	PER_{in-field} below rate with ≤ 50 % effect?	
<i>Aphidius rhopalosiphi</i>	> 16.8	2.1	yes	

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

* Studies were performed with other chlormequat chloride formulation containing 465 g s.a./L. Results of these studies were considered not acceptable for purposes of the risk assessment for CLARA, since difference in active substance content was significant.

9.7.2.2 Risk assessment for off-field exposure

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

Intended use		Winter wheat			
Active substance/product		Chlormequat chloride			
Application rate (g/ha)		1 x 1510 g a.s./ha			
MAF		1.0			
vdf		10 (2D) / 1 (3D)			
Test species Tier I	LR₅₀ (lab.) (g/ha)	Drift rate	PER_{off-field} (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	> 2250	0.0277	4.183	10	0.019
<i>Aphidius rhopalosiphi</i>	2200				0.019
<i>Poecilus cupreus</i> [‡]	1395				0.030

<i>Aleochara bilineata</i>*	1395				0.030
Active substance/product	CLARA (Chlormequat chloride 72% SL)				
Application rate (g/ha)	1 x 2.1 L/ha				
MAF	1.0				
vdf	10 (2D) / 1 (3D)				
Test species Higher-tier	ER₅₀ (L/ha)	Drift rate	PER_{off-field} (L/ha)	CF	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	> 16.80	0.0277	0.006 0.06	5	yes (0.017)

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.

* Studies were performed with other chlormequat chloride formulation containing 465 g s.a./L. Results of these studies were considered not acceptable for purposes of the risk assessment for **CLARA**, since difference in active substance content was significant.

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

Studies on the toxicity to arthropods show that the active substance chlormequat chloride and the formulated product CLARA pose no in-field and off-field risk for non-target arthropods, since HQ values were below 2 and the PER_{in-field} and the corr. PER_{off-field} were below the rate with ≤ 50 % effect. Therefore, an application of CLARA in respect of the GAP does not present an unacceptable risk for arthropods other than bees.

zRMS comment:

Arthropods other than bees:

The Applicant used the available data for substance active - chlormequat chloride to indicate acceptable risk for arthropods other than bees. The toxicity of chlormequat chloride to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. In addition - Tier II tests with *Poecilus cupreus*, *Aleochara bilineata* were presented by Applicant. However, studies were performed with other chlormequat chloride formulation containing 465 g s.a./L. Results of these studies were considered not acceptable for purposes of the risk assessment for **CLARA**, since difference in active substance content was significant. In EU DAR, the test for *Poecilus cupreus* and *Chrysoperla carnea* are available. Studies were performed with other chlormequat chloride formulation containing 720 g s.a./L (*P.cupreus*) and 765.8 g s.a./L (*Ch.carnea*). Results of these studies were considered acceptable for purposes of the risk assessment for **CLARA**, since difference in active substance content was not significant. The HQ values of the tier-I risk assessment for *A. rhopalosiphi* and *T. pyri* are below the trigger of 2, indicating that the risk to in-field and off-field non-target arthropods is acceptable following the use of **CLARA** according to the proposed use pattern. Additionally, acceptable

risk assessment for arthropods other than bees for *P.cupreus* and *Ch.carnea* could be conclude.

The study on the effects of **CLARA** on *A. rhopalosiphi* was also submitted by Applicant. HQ values was below 2 for *A.rhopalosiphi* and the $PER_{in-field}$ and the corr. $PER_{off-field}$ was below the rate with $\leq 50\%$ effect. Study on the toxicity to *T.pyri* for formulated product **CLARA** was also provided by Applicant. In general, the study was accepted as its validity criteria were met. However, RMS decided not to use the results of this study for risk assessment due to study limitations such as: 1. No clear dose-response for test concentrations for mortality and reproduction parameters. 2. For the dose of 2.069 L/ha, the LR_{50} value was determined. Therefore, an analysis of the effect of **CLARA** on reproduction at a dose of 2.069 L/ha should be performed. In this case, risk assessment for *T.pyri* was based on toxicity endpoints for substance active – chlormequat chloride.

RMS considered that a low risk from the formulation **CLARA** (containing 1 active substance) can be concluded, due to the margin of safety based on the exposure assessment for the active substance - chlormequat chloride for *A.rhopalosiphi* and *T.pyri* is sufficient.

Acceptable risk assessment for arthropods other than bees for **CLARA** could be conclude.

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

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

9.8.1 Toxicity data

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride.

However, the provision of further data on Chlormequat chloride 72% SL is not considered essential, because active substance toxicity data can be used.

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>	Chlormequat chloride	Mixed into substrate 14 d, acute	LC ₅₀ = 320 mg a.s./kg dw	EFSA Scientific Report (2008) 179, 1- 77
<i>Eisenia fetida</i>	Chlormequat chloride	Mixed into substrate 56 d, chronic	NOEC = 681 mg a.s./kg dw	
Other soil macro-organisms: Not required as DT ₉₀ is 105.1 days and NTA HQ, earthworm TER and effect on soil micro-organisms all below triggers.				
Field studies				
No data, not required.				
Litter bag test				
No data, not required.				

9.8.1.1 Justification for new endpoints

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

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

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 CLARA in winter wheat

Intended use	Winter wheat		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Chlormequat chloride	320	1.611	198.6
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)

Chlormequat chloride	681	1.611	422.7
Chronic effects on other soil macro- and mesofauna			
NR			

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

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

zRMS comment:

Earthworms:

The study on the effects of **CLARA** on earthworms was not provided by Applicant. In this case, the Applicant used the available data for substance active chlormequat chloride to indicate acceptable risk for earthworms. In opinion RMS this approach should be considered at MSs level. It was acknowledged that the active substance chlormequat chloride did not show a high toxicity to earthworms. The RMS noted that the risk assessment for chlormequat chloride indicated a very high margin of safety based on the currently available exposure assessment. In this case, the toxicity of the plant protection product **CLARA** can be predicted on the basis of the data for the active substance. Acceptable risk assessment could be conclude without the study for PPP and earthworms.

The risk assessment for earthworms should be considered at MSs level. Perhaps at the level of national registrations in different countries, additional data will be required to elucidate the effects of CLARA on earthworms such as the study on the effects of CLARA on earthworms.

Other soil macro-organisms

In accordance with the data requirements of the (EU) Regulation 284/2013 data on *Folsomia candida* and *Hypoaspis aculeifer* should be submitted. No toxicity data are available for the PPP **CLARA**. However, the Applicant provided a justification indicating that the data requirements indicate that an assessment is not triggered since it is of low risk to NTAs. This approach was accepted by RMS.

Justification:

As stated in Commission Regulation EU No 284/2013 of 1 March 2013, “For plant protection products applied as a foliar spray, data on the relevant two non-target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on *Folsomia candida* and *Hypoaspis aculeifer* shall be required.” The formulated product **CLARA** is applied as a foliar spray treatment. Acceptable risks are expected towards the earthworms and a low in-field and off-field risk is demonstrated for non-target arthropods - such as - *Typhlodromus pyri*, *Aphidius rhopalosiphi* (standard laboratory studies).

The risk assessment for soil macro-organisms should be considered at MSs level.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with Chlormequat chloride. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Chlormequat chloride	28 d, aerobic	No effect at day 28 at 18.6 mg a.s./kg dw soil	EFSA Scientific Report (2008) 179, 1-77
C-mineralisation	Chlormequat chloride	28 d, aerobic	No effect at day 28 at 18.6 mg a.s./kg dw soil	
N-mineralisation	CLARA	28 d, aerobic sandy clay loam soil	No significant effects (< 25% effect compared to untreated control) 2.8% at the applicataion rate of 6.38 mg test item/kg dw (4.06 mg chlormequat chloride/kg dw) 7.2% at the application rate of 31.90 mg test item/kg dw (20.30 mg chlormequat chloride/kg dw)	KCP 10.5-01 Gierbuszewska, A. (2020). G/195/17
C-mineralisation	CLARA	28 d, aerobic sandy clay loam soil	No significant effects (< 25% effect compared to untreated control) 8.2% at the applicataion rate of 6.38 mg test item/kg dw (4.06 mg chlormequat chloride/kg dw) 10.1% at the application rate of 31.90 mg test item/kg dw (20.30 mg chlormequat chloride/kg dw)	KCP 10.5-02 Gierbuszewska, A. (2020). G/194/17

9.9.1.1 Justification for new endpoints

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

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).

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

Intended use	Winter wheat		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Chlormequat chloride	20.30 (at 28 d)	1.611	yes
CLARA	31.90 (at 28 d)	2.536	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Chlormequat chloride	20.30 (at 28 d)	1.611	yes
CLARA	31.90 (at 28 d)	2.536	yes

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for Chlormequat chloride in CLARA (Chlormequat chloride 72% SL) formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

zRMS comments:

The risk assessment for soil micro-organism after exposure of **CLARA** has been accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of **CLARA**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **CLARA**.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of CLARA (Chlormequat chloride 72% SL) were not evaluated as part of the EU assessment of Chlormequat chloride. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Carrot and sunflower (4 other species also tested)	Chlormequat chloride	Vegetative vigour	ER ₅₀ plant weight > 3750 g a.s./ha	EFSA Scientific Report (2008) 179, 1-77
Oat (<i>Avena sativa</i>) Onion (<i>Allium cepa</i>) Sugar beet (<i>Beta vulgaris</i>) Rape (<i>Brassica napus</i>) Carrot (<i>Daucus carota</i>) Soy bean (<i>Glycine max</i>)	Chlormequat chloride	Vegetative vigour Seedling emergence	ER ₅₀ plant weight > 2100 g a.s./ha ER ₅₀ emergence > 2100 g a.s./ha	
Pea (<i>Pisum sativum</i>) Cabbage (<i>Brassica oleracea var. capitata</i>) Carrot (<i>Daucus carota</i>) Sunflower (<i>Helianthus annuus</i>) Onion (<i>Allium cepa</i>) Oats (<i>Avena sativa</i>)	CLARA	Vegetative vigour	ER ₅₀ > 4200.0 g/ha (>3045.0 g chlormequat chloride/ha)	KCP 10.6.2-01 Gierbuszewska, A. (2020). G/200/17
Pea (<i>Pisum sativum</i>) Cabbage (<i>Brassica oleracea var. capitata</i>) Carrot (<i>Daucus carota</i>) ¹⁾ Sunflower (<i>Helianthus annuus</i>) Onion (<i>Allium cepa</i>) Oats (<i>Avena sativa</i>)		Seedling emergence	¹⁾ ER ₅₀ = 4098.1 g/ha (2971.1 g chlormequat chloride/ha)	KCP 10.6.2-02 Gierbuszewska, A. (2020). G/199/17

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

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

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (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.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CLARA in winter wheat

Intended use	Winter wheat
Active substance/product	Chlormequat chloride

Application rate (g/ha)		1 x 1510 g a.s./ha		
MAF		1.0		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
Oat, onion, sugar beet, rape, carrot and soybean	> 2100	0.0277	41.827	50.2
Active substance/product		CLARA (Chlormequat chloride 72% SL)		
Application rate (g/ha)		1 x 2.1 L/ha (2377.62 g/ha*)		
MAF		1.0		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
Pea, cabbage, carrot, sunflower, onion,oats	> 4200.0	0.0277	46.620 65.86	252.7 63.77
Sunflower	4098.1			246.6 62.22

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

* Considering a density of 1.1322 g/cm³

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for CLARA (Chlormequat chloride 72% SL) shows that Annex VI trigger of 5 is not exceeded, indicating that CLARA poses a low risk to non-target plants when applied according to the proposed use rates.

zRMS comment:

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

The study on the effects of **CLARA** on non-target terrestrial plants for the vegetative vigour test (OECD 227 "Terrestrial Plant Test: Vegetative Vigour Test) and the study on the effects of MEPCY (SHA 126085 A) on non-target terrestrial plants in terms of seedling emergence and seedling growth test (OECD Guideline for the Testing of Chemicals No. 208 “Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test”) were provided by Applicant. In this case, the Applicant used also the available data for substance active to indicate acceptable risk for non-target plants. The RMS noted that the risk assessment for chlormequat chloride and formulation **CLARA** indicated a very high margin of safety based on the currently available exposure assessment.

Overall, the RMS considered that a low risk from the substance active such as chlormequat chloride and formulation **CLARA** can be concluded. No mitigation measures is needed.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	CLARA
Common name	Chlormequat chloride 72% SL
Classification and proposal labelling	
With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: - Code(s) for hazard pictogram(s): - Signal word: - Hazard statement(s): - EU specific statements: EUH401 Precautionary statement: -

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.
 MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-01	XXXXXXXX	2018	Chlormequat chloride 72% SL, Rainbow trout Acute toxicity test. Report No. W/132/17. XXXXXXXX GLP Unpublished	Y	SHARDA Cropchem Limited
KCP 10.2.1-02	Nierzędska, E.	2019	Chlormequat chloride 72% SL <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i>) SAG 61.81. Growth inhibition test. Report No. W/133/17. Institute of Industrial Organic Chemistry, Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-03	Nierzędska, E.	2019	Chlormequat chloride 72% SL <i>Daphnia magna</i> , Acute immobilisation test. Report No. W/134/17. Institute of Industrial Organic Chemistry, Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-04	Nierzędska, E.	2019	Chlormequat chloride 72% SL <i>Lemna gibba</i> CPCC 310, Growth inhibition test. Report No. W/135/17. Institute of Industrial Organic Chemistry, Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1.1	Glanas, A.	2017	Chlormequat chloride 72% SL Honeybees (<i>Apis mellifera L.</i>), Acute Oral Toxicity Test. Report No. B/100/16. Institute of Industrial Organic Chemistry, Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.1.2	Glanas, A.	2017	Chlormequat chloride 72% SL Honeybees (<i>Apis mellifera L.</i>), Acute Contact Toxicity Test. Report No. B/101/16. Institute of Industrial Organic Chemistry, Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.2	M Mohanraj	2023	Chronic Oral Toxicity Study of Chlormequat chloride 72% SL on honey bee (<i>Apis mellifera</i>). Report No. 12354/2023, Bioscience Research Foundation. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.3	M Mohanraj	2023	Effect of Chlormequat chloride 72% SL on larvae of honey bee, <i>Apis mellifera (L.)</i> following repeated exposure. Report No. 12355/2023, Bioscience Research Foundation. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.2.2- 01	Lemańska, N.	2018	An extended laboratory test for evaluating the effects of Chlormequat chloride 72% SL on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez). Report No. B/102/16. Institute of Industrial Organic Chemistry Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.5- 01	Gierbuszewska, A.	2020	Chlormequat chloride 72% SL Soil Microorganisms: Nitrogen Transformation Test. Report No. G/195/17, Institute of Industrial Organic Chemistry Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.5-02	Gierbuszewska, A.	2020	Chlormequat chloride 72% SL Soil Microorganisms: Carbon Transformation Test. Report No. G/194/17, Institute of Industrial Organic Chemistry Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.6.2-01	Gierbuszewska, A.	2020	Chlormequat Chloride 72% SL Terrestrial Plant Test: Vegetative Vigour Test. Report No. G/200/17. Institute of Industrial Organic Chemistry Branch Pszczyna. GLP Unpublished	N	SHARDA Cropchem Limited
KCP 10.6.2-02	Gierbuszewska, A.	2020	Chlormequat Chloride 72% SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. Report No. G/199/17. Institute of Industrial Organic Chemistry Branch Pszczyna. 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 was accepted by RMS. The validity criteria: - The mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used), - constant conditions are maintained throughout exposure, - dissolved oxygen concentrations were within the range of 92–99% of air saturation value (obligatory above 60% of air saturation value). Agreed endpoints based on nominal test concentrations: The LC ₅₀ /96 h value is higher than 100 mg formulation CLARA/L. The LC ₅₀ /96 h value is higher than 63.60 mg chlormequat chloride/L.
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Reference:	10.2.1-01
Report:	“Chlormequat chloride 72% SL, Rainbow trout Acute toxicity test xxxxxxxx MSc Eng., 2018. xxxxxxxx STUDY CODE: W/132/17.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 203 (1992).
Deviations:	Yes. The guideline recommended lower than 1.0 g of fish per liter, whereas

	in the definitive test was 1.1 g of fish per liter in the test aquaria. The deviation did not have impact on the results generated during the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat chloride 72% SL Batch number: SCL-23170 The content of chlormequat chloride is 72.5% (w/v) Manufacture date: January 04/2018 Expiry date: January 03/2020 Density: 1.14 g/mL at 20°C
Test organism:	Rainbow trout (<i>Oncorhynchus mykiss</i> Walb.) Age: approximately 4.5 months Average weight: 1.10 g ± 0.17 g Average body length: 4.69 cm ± 0.31 cm Supplier: 'The Culture of Salmonidae Fish in Zawoja', Poland.
Test design:	Static system (96 h of exposure) One replicate of each test item concentration and control Ten fish in each aquarium The ratio of fish weight per volume (10 L) was 1.1 g/L
Nominal test item concentration:	100 mg/L plus the control
Nominal concentration of chlormequat chloride:	63.60 mg/L plus the control
Test conditions:	Temperature of water: 14.1 – 14.8 °C pH of the control: 7.68 – 7.89 Dissolved oxygen concentration in the test item concentration and the control: 92 – 99% ASV Lighting daily cycle: 16 h light: 8 h dark No feeding Constant aeration
Endpoints:	LC ₅₀

Results

The concentration of the test item was determined using a validated spectrophotometric method. The concentration of the test item was chemically determined in samples of the test item concentration and the control collected at exposure initiation and at exposure termination.

At exposure initiation, the determined concentration of the test item was 93.65% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

At exposure termination, the determined concentration of the test item was 97.36% of the nominal concentration. Therefore, the concentration of the test item was stable under test conditions.

The endpoint values were determined on the basis of the nominal test item concentration and nominal concentration of chlormequat chloride and mortality of fish. Since in the test item concentration of 100 mg/L and the control the mortality is not observed, the statistical analysis is not needed. The endpoint value determined on the basis of the nominal test item concentration and mortality of fish: The LC₅₀/96 h value is higher than 100 mg/L.

The endpoint value determined on the basis of the nominal concentration of chlormequat chloride and mortality of fish: The LC₅₀/96 h value is higher than 63.60 mg/L.

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

Exposure time	Number of dead fish	Number of alive fish	Total mortality of fish [%]	Symptom category				
				Loss of balance	Nontypical swimming	Respiratory problems	Pigmentation change	
3h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms
6h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms
24h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms
48h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms
72h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms
96h	0	10	0	0	0	0	0	Number of fish with symptoms
				10	10	10	10	Number of fish without symptoms

Table 2. Concentration and stability of the test item, definitive test.

Nominal test concentration [mg/L]	Nominal concentration of chlormequat chloride [mg/L]	Average concentration (n=3) of the test item measured in samples collected [mg/L]			
		at exposure initiation	% of the nominal concentration	at exposure termination	% of the nominal concentration
Control	---	<LoD	---	<LoD	---
100	63.60	93.65	93.65	97.36	97.36

Validity criteria

The following validity criteria specified in the OECD Guideline No. 203 (1992) were met:

- The mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used),
- Constant conditions are maintained throughout exposure,
- Dissolved oxygen concentrations were within the range of 92 – 99% of air saturation value (obligatory above 60% of air saturation value).

Comments of zRMS:	<p>The study was accepted by RMS.</p> <p>The validity test:</p> <ul style="list-style-type: none"> - the biomass in the control increased by a factor of 120.0 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.3% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 17.9% (criterion: it must not exceed 35%). <p>Agreed toxicity endpoints:</p> <p>The endpoint values based on the nominal test item concentration:</p> <p>The concentration causing a 50% inhibition of the growth rate of <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i>) SAG 61.81, i.e. the $E_rC_{50/72}$ h value is higher than 100 mg/L.</p> <p>The concentration causing a 50% inhibition of yield of <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i>) SAG 61.81, i.e. the $E_yC_{50/72}$ h value is higher than 100 mg/L.</p> <p>The $E_rC_{20/72}$ h and $E_rC_{10/72}$ h values are higher than 100 mg/L.</p> <p>The endpoint values based on the nominal concentration of chlormequat chloride:</p> <p>The concentration causing a 50% inhibition of the growth rate of <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i>) SAG 61.81, i.e. the $E_rC_{50/72}$ h value is higher than 63.60 mg/L.</p> <p>The concentration causing a 50% inhibition of yield of <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i>) SAG 61.81, i.e. the $E_yC_{50/72}$ h value is higher than 63.60 mg/L.</p> <p>The $E_yC_{20/72}$ h and $E_yC_{10/72}$ h values are higher than 63.60 mg/L.</p>
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Reference: KCP 10.2.1-02

Report “Chlormequat chloride 72% SL *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*) SAG 61.81. Growth inhibition test”. Ewa Nierzędska, MSc Eng., 2019. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: W/133/17.

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

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication
(if vertebrate study) No

Materials and methods

Test item: Chlormequat chloride 72% SL
Batch number: SCL-23170
The content of chlormequat chloride is 72.5% (w/v)
Manufacture date: January 04/2018
Expiry date: January 03/2020
Density: 1.14 g/mL at 20°C

Test organism: The unicellular freshwater green algae, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*) SAG 61.81 (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz) cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.

Test design: 72 hours of exposure
Six replicates for the test item concentration and six for the control
A background for the control and the test item concentration
Initial algal cell density: 1×10^4 cells/mL.

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

Nominal concentration of chlormequat chloride: 63.60 mg/L

Test conditions: Temperature: 21.7 – 22.3°C
pH of the control: 7.37 – 8.84
Mean light intensity: 7206 – 7384 lux
Constant illumination and shaking
Medium: AAP

Chemical determinations: The test item concentrations were determined using validated spectrophotometric method

Statistic: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Two-sample t-test procedure

Endpoints: E_rC_{50} , E_yC_{50} , LOEC and NOEC

Results

The concentration of the test item was determined using a validated spectrophotometric method. Samples of the test item concentration and the control at exposure initiation and termination were spectrophotometrically determined.

At exposure initiation, the determined concentration of the test item was 104.0% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

At exposure, the determined concentration of the test item was 107.2% of the initial concentration. Therefore, the concentration of the test item was stable under test conditions during exposure.

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

In the test item concentration of 100 mg/L no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control.

The endpoint values determined based on the nominal test item concentrations

The concentration causing a 50% inhibition of the average specific growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the $E_rC_{50}/72$ h value is higher than 100 mg/L. The $E_rC_{20}/72$ h and $E_rC_{10}/72$ h values are higher than 100 mg/L.

Statistical tests based on the growth rate data were Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were homogeneous and Two-sample t-Test Procedure which did not show significant difference between the test item concentration of 100 mg/L and the control.

The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the $E_yC_{50}/72$ h value is higher than 100 mg/L. The $E_yC_{20}/72$ h and $E_yC_{10}/72$ h values are higher than 100 mg/L.

Statistical tests based on the yield data were Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were homogeneous and Two-sample t-Test Procedure which did not show significant difference between the test item concentration of 100 mg/L and the control.

The endpoint values determined based on the nominal concentrations of chlormequat chloride

The concentration causing a 50% inhibition of the average specific growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the $E_rC_{50}/72$ h value is higher than 63.60 mg/L. The $E_rC_{20}/72$ h and $E_rC_{10}/72$ h values are higher than 63.60 mg/L.

Statistical tests based on the growth rate data were Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were homogeneous and Two-sample t-Test Procedure which did not show significant difference between the nominal concentration of chlormequat chloride 63.60 mg/L and the control.

The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the $E_yC_{50}/72$ h value is higher than 63.60 mg/L. The $E_yC_{20}/72$ h and $E_yC_{10}/72$ h values are higher than 63.60 mg/L.

Statistical tests based on the yield data were Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) showed that the variances were homogeneous and Two-sample t-Test Procedure which did not show significant

difference between the nominal concentration of chlormequat chloride of 63.60 mg/L and the control.

Table 1. Endpoint values for growth rate, definitive test

Endpoint value [mg/L]	Based on nominal concentrations of the test item		
	24h	48h	72h
E _r C ₅₀	>100	>100	>100
E _r C ₂₀	>100	>100	>100
E _r C ₁₀	>100	>100	>100
Endpoint value [mg/L]	Based on nominal concentrations of chlormequat chloride		
	24h	48h	72h
E _r C ₅₀	>63.60	>63.60	>63.60
E _r C ₂₀	>63.60	>63.60	>63.60
E _r C ₁₀	>63.60	>63.60	>63.60

Table 2. Endpoint values for yield, definitive test

Endpoint value [mg/L]	Based on nominal concentrations of the test item		
	24h	48h	72h
E _y C ₅₀	>100	>100	>100
E _y C ₂₀	>100	>100	>100
E _y C ₁₀	>100	>100	>100
Endpoint value [mg/L]	Based on nominal concentrations of chlormequat chloride		
	24h	48h	72h
E _y C ₅₀	>63.60	>63.60	>63.60
E _y C ₂₀	>63.60	>63.60	>63.60
E _y C ₁₀	>63.60	>63.60	>63.60

Validity criteria

In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 120.0 within the 72-hour test period (criterion: at least a 16-fold growth)
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.3% (criterion: it must not exceed 7%)
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 17.9% (criterion: it must not exceed 35%).

Comments of zRMS:	<p>The study was accepted by RMS.</p> <p>The validity criteria:</p> <ul style="list-style-type: none"> ▪ the immobilisation of <i>Daphnia magna</i> in the control was 0% (criterion: not more than 10%), ▪ the dissolved oxygen concentrations in the test vessels were within the range of 7.9 – 8.5 mg/L (criterion: not less than 3 mg/L). <p>Agreed endpoints:</p> <p>The endpoint values determined based on the nominal test item concentrations: The EC₅₀ value after 48h of exposure is 88.49 mg/L (with 95% confidence limits: 65.71–119.08). The EC₂₀ value after 48 h of exposure is 39.19 mg/L (with 95% confidence limits: 24.97–53.81), and EC₁₀ value after 48 h of exposure is 25.60 mg/L (with 95% confidence limits: 14.39–37.16). The LOEC/48 h = 19.40 mg/L The NOEC/48 h = 8.82 mg/L</p> <p>The endpoint values determined based on the nominal concentrations of chlormequat chloride: The EC₅₀ value after 48 h of exposure is 56.28 mg/L (with 95% confidence limits:</p>
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	41.79 – 75.73). The EC ₂₀ value after 48 h of exposure is 24.92 mg/L (with 95% confidence limits: 15.88 – 34.23), and EC ₁₀ value after 48 h of exposure is 16.28 mg/L (with 95% confidence limits: 9.15 – 23.64). The LOEC/48 h is 12.34 mg/L. The NOEC/48 h is 5.61 mg/L.
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Reference:	KCP 10.2.1-03
Report	“Chlormequat chloride 72% SL <i>Daphnia magna</i> , Acute immobilisation test”. Ewa Nierzędska, MSc Eng., 2019. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: W/134/17.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 202 (2004).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat chloride 72% SL Batch number: SCL-23170 The content of chlormequat chloride is 72.5% (w/v) Manufacture date: January 04/2018 Expiry date: January 03/2020 Density: 1.14 g/mL at 20°C
Test organism:	<i>Daphnia magna</i> Straus (< 24 h old at exposure initiation) Not first brood progeny Neonates collected from a laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology
Test design:	Static test (exposure: 48 h) Four replicates per each test item concentration and the control Five <i>Daphnia magna</i> in each replicate.
Nominal test item concentration:	1000, 454.54, 206.61, 93.91, 42.69, 19.40, 8.82 mg/L plus the control
Nominal concentration of chlormequat chloride:	635.96, 289.07, 131.40, 59.72, 27.15, 12.34, 5.61 mg/L plus the control
Test conditions:	Temperature: 18.9 – 20.8°C pH of the control: 7.17 – 7.47 Dissolved oxygen concentration in the control: 8.1 – 8.3 mg/L Daily cycle: 16 h light: 8 h dark Fluorescent light source No feeding No aeration Medium: Elendt M7

Chemical determinations: The test item concentrations were determined using validated spectrophotometric method

Endpoints: EC₅₀

Results

Daphnia magna were observed for immobilization after 24 and 48 h of exposure. At exposure termination in the control and the test item concentration of 8.82 mg/L no immobilization of *Daphnia magna* was observed. In the test item concentrations: 1000, 454.54, 206.61, 93.91, 42.69, 19.40 mg/L, the immobilization was 100%, 100%, 75%, 45%, 25% and 10%, respectively.

The concentration of the test item was determined using a validated spectrophotometric method. The concentrations of the test item were chemically determined in samples of all test item concentrations and the control collected at exposure initiation and at exposure termination.

At exposure initiation, the determined test item concentrations were in the range of 83.7 – 102.4% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

At exposure termination, the determined test item concentrations were in the range 91.5 – 99.9% of the nominal concentration. Therefore, the test item concentrations were stable under test conditions.

The endpoint values were determined based on the nominal test item concentration and nominal concentrations of chlormequat chloride.

No immobilisation of *Daphnia magna* was observed during the period of exposure, neither in the control, nor in the test item concentration of 8.82 mg/L. In the test item concentrations: 1000, 454.54, 206.61, 93.91, 42.69, 19.40 mg/L, the immobilization was 100%, 100%, 75%, 45%, 25% and 10%, respectively.

Table 1. Immobilisation of *Daphnia magna*.

Nominal item concentration [mg/L]	Nominal concentration of chlormequat chloride	Number of alive fish	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
			24h				48h				24h	48h
			Replicates									
			A	B	C	D	A	B	C	D		
Control	---	20	0	0	0	0	0	0	0	0	0	0
8.82	5.61	20	0	0	0	0	0	0	0	0	0	0
19.40	12.34	20	0	0	0	0	1	0	1	0	0	10
42.69	27.15	20	0	1	1	1	1	2	1	1	15	25
93.91	59.72	20	0	1	0	2	2	2	1	4	15	45
206.61	131.40	20	3	2	0	4	4	3	4	4	45	75
454.54	289.07	20	3	3	3	3	5	5	5	5	60	100
1000	635.96	20	5	5	5	5	5	5	5	5	100	100

Time of exposure: 14.11.2018 –16.11.2018

The endpoint values are summarised in the table below.

Table 2. Endpoint values – definitive test

Endpoint value [mg/L]	24 h		48 h	
	Based on nominal test item concentration	Based on nominal concentrations of chlormequat chloride	Based on nominal test item concentration	Based on nominal concentrations of chlormequat chloride
EC ₅₀	231.79 (168.69 – 327.84)	147.41 (107.28 – 208.50)	88.49 (65.71 – 119.08)	56.28 (41.79 – 75.73)
EC ₂₀	91.50 (56.41 – 128.41)	58.19 (35.88 – 81.67)	39.19 (24.97 – 53.81)	24.92 (15.88 – 34.23)
EC ₁₀	56.29 (29.75 – 84.14)	35.80 (18.92 – 53.51)	25.60 (14.39 – 37.16)	16.28 (9.15 – 23.64)
LOEC	42.69	27.15	19.40	12.34
NOEC	19.40	12.34	8.82	5.61

Validity criteria

In the definitive test, the following validity criteria specified in the OECD Guidelines No. 202 (2004) were met:

- the immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 7.9 – 8.5 mg/L (criterion: not less than 3 mg/L).

Comments of zRMS:	<p>The study was accepted by RMS.</p> <p>Validity criteria:</p> <ul style="list-style-type: none"> ▪ The doubling time of frond number in the control was 2.0 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 10.7). ▪ The average specific growth rate in the control between day 0 and day 7 was 0.339 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹). <p>Deviations in the study: none.</p> <p>Agreed endpoints:</p> <p>The endpoint values based on the nominal test item concentrations:</p> <p>Endpoints based on the frond number:</p> <p>The E_rC₅₀/7 d value is 97.23 mg/L (95% confidence interval 60.93 – 162.25). The E_rC₂₀/7 d value is 6.47 mg/L (95% confidence interval 2.59 – 12.09). The E_rC₁₀/7 d value is 1.57 mg/L (95% confidence interval 0.42 – 3.66). The E_yC₅₀/7 d value is 8.88 mg/L (95% confidence interval 5.69 – 13.62). The E_yC₂₀/7 d value is lower than 0.32 mg/L. The E_yC₁₀/7 d value is lower than 0.32 mg/L. For growth rate and yield NOEC/7 d value is lower than 0.32 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.32 mg/L.</p> <p>Endpoints based on the dry weight:</p> <p>The E_rC₅₀/7 d value is higher than 1000 mg/L. The E_rC₂₀/7 d value is 19.75 mg/L (95% confidence interval 13.80 – 26.87). The E_rC₁₀/7 d value is 1.98 mg/L (95% confidence interval 1.07 – 3.25). For growth rate NOEC/7 d value is lower than 0.32 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.32 mg/L. The E_yC₅₀/7 d value is 13.79 mg/L (95% confidence interval 10.12 – 18.72). The E_yC₂₀/7 d value is 0.35 mg/L (95% confidence interval 0.18 – 0.59). The E_yC₁₀/7 d value is lower than 0.32 mg/L For yield the NOEC/7 d value is lower than 0.32 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.32 mg/L.</p> <p>The endpoint values based on the nominal concentrations of chlormequat chloride:</p>
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	<p>Endpoints based on the frond number: The $E_rC_{50}/7$ d value is 61.84 mg/L (95% confidence interval 38.75 – 103.21). The $E_rC_{20}/7$ d value is 4.12 mg/L (95% confidence interval 1.64 – 7.70). The $E_rC_{10}/7$ d value is 1.00 mg/L (95% confidence interval 0.27 – 2.33). The $E_yC_{50}/7$ d value is 5.64 mg/L (95% confidence interval 3.61 – 8.65). The $E_yC_{20}/7$ d value is lower than 0.20 mg/L. The $E_yC_{10}/7$ d value is lower than 0.20 mg/L. For growth rate and yield NOEC/7 d value is lower than 0.20 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.20 mg/L.</p> <p>Endpoints based on the dry weight: The $E_rC_{50}/7$ d value is higher than 635.96 mg/L. The $E_rC_{20}/7$ d value is 12.55 mg/L (95% confidence interval 8.77 – 17.07). The $E_rC_{10}/7$ d value is 1.26 mg/L (95% confidence interval 0.68 – 2.07). The $E_yC_{50}/7$ d value is 8.75 mg/L (95% confidence interval 6.43 – 11.88). The $E_yC_{20}/7$ d value is 0.22 mg/L (95% confidence interval 0.12 – 0.38). The $E_yC_{10}/7$ d value is lower than 0.20 mg/L. For growth rate and yield the NOEC/7 d value is lower than 0.20 mg/L, whereas the LOEC/7 d value is lower than or equal to 0.20 mg/L.</p>
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Reference:	KCP 10.2.1-04
Report	“Chlormequat chloride 72% SL <i>Lemna gibba</i> CPCC 310, Growth inhibition test”. Ewa Nierzędska, MSc Eng., 2019. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: W/135/17.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 221 (2006).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat chloride 72% SL Batch number: SCL-23170 The content of chlormequat chloride is 72.5% (w/v) Manufacture date: January 04/2018 Expiry date: January 03/2020 Density: 1.14 g/mL at 20°C
Test organism:	The freshwater aquatic plant, <i>Lemna gibba</i> CPCC 310 cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; the plants were obtained from the Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.
Test design:	Static system; 7 days of exposure; three replicates for each test item concentration and six replicates for control.
Nominal test item concentration:	1000, 200, 40, 8, 1.6, and 0.32 mg/L and control.

Nominal concentration of chlormequat chloride: 635.96, 127.19, 25.44, 5.09, 1.02, and 0.20 mg/L and control.

Test conditions: 20X AAP nutrient solution
pH of the control: 7.62 – 9.06
Mean light intensity: 8210 – 8562 lux
Constant illumination
Glass beakers containing 400 mL of a given test item concentration or control
Initial frond number: 9, i.e. 3 plants per 3 fronds
Temperature: 22.7 – 23.0°C.

Chemical determinations: The test item concentrations were determined using validated spectrophotometric method

Statistic: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh-t-test After Bonferroni Holm, Step-down Jonckheere-Tepstra Test Procedure.

Endpoints: E_rC_{10} , E_rC_{20} , E_rC_{50} , E_yC_{10} , E_yC_{20} , E_yC_{50} , LOEC and NOEC on frond number and dry weight.

Results

The test item concentrations were chemically determined using a validated spectrophotometric method. Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.

In samples at exposure initiation the determined test item concentration was in the range of 82.9 – 98.8% of nominal concentration. The results confirm that the test item concentrations were prepared correctly.

In samples at exposure termination the determined test item concentration was in the range of 80.6 – 103.4% of nominal concentration. Therefore, the test item concentrations were stable under test conditions during exposure.

The endpoint values were determined based on the nominal test item concentrations and nominal concentrations of chlormequat chloride.

After 2 days of exposure, in the test item concentrations from 0.32 – 40 mg/L no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of 200 mg/L, discoloration of fronds was observed. In the test item concentration of 1000 mg/L lighter fronds were observed.

After 5 days of exposure, in the test item concentrations from 0.32 to 8 mg/L, bending down of fronds was observed. Additionally, in the test item concentration of 8 mg/L, overlapping of colonies was observed. In the test item concentration of 40 mg/L, overlapping of colonies was observed. In the test item concentrations of 200 mg/L, discoloration of fronds was observed. In the test item concentration of 1000 mg/L, lighter fronds and break-up of colonies were observed.

At exposure termination, in the test item concentrations from 0.32 and 1.6 mg/L, bending down of fronds was observed. In the test item concentrations of 8 and 40 mg/L, overlapping of colonies was observed. In the test item concentration of 200 mg/L discoloration of fronds and overlapping of colonies were observed. In the test item concentration of 1000 mg/L, lighter fronds and break-up of colonies were observed.

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The endpoint values are summarised in the tables below.

Table 1. Endpoint values for growth rate, definitive test

Endpoint value [mg/L]	Based on nominal concentrations of the test item			
	FronD number Dry weight			FronD number Dry weight
	0-2 d	0-5 d	0-7 d	0-7 d
E_rC₅₀	197.66 (121.09 – 332.28)	82.32 (51.61 – 136.68)	97.23 (60.93 – 162.25)	> 1000
E_rC₂₀	40.34 (13.74 – 72.10)	5.03 (2.02 – 9.45)	6.47 (2.59 – 12.09)	19.75 (13.80 – 26.87)
E_rC₁₀	17.58 (3.82 – 37.39)	1.17 (0.32 – 2.74)	1.57 (0.42 – 3.66)	1.98 (1.07 – 3.25)
LOEC	1.60	≤ 0.32	≤ 0.32	≤ 0.32
NOEC	0.32	≤ 0.32	≤ 0.32	≤ 0.32
Endpoint value [mg/L]	Based on nominal concentrations of chlormequat chloride			
	FronD number Dry weight			FronD number Dry weight
	0-2 d	0-5 d	0-7 d	0-7 d
E_rC₅₀	125.71 (77.01 – 211.32)	52.36 (32.82 – 86.96)	61.84 (38.75 – 103.21)	> 635.96
E_rC₂₀	25.66 (8.74 – 45.86)	3.20 (1.28 – 6.01)	4.12 (1.64 – 7.70)	12.55 (8.77 – 17.07)
E_rC₁₀	11.18 (2.43 – 23.78)	0.74 (0.20 – 1.74)	1.00 (0.27 – 2.33)	1.26 (0.68 – 2.07)
LOEC	1.02	≤ 0.20	≤ 0.20	≤ 0.20
NOEC	0.20	≤ 0.20	≤ 0.20	≤ 0.20

Table 2. Endpoint values for yield, definitive test

Endpoint value [mg/L]	Based on nominal concentrations of the test item			
	FronD number Dry weight			FronD number Dry weight
	0-2 d	0-5 d	0-7 d	0-7 d
E_yC₅₀	119.61 (80.75 – 175.20)	9.47 (5.54 – 15.80)	8.88 (5.69 – 13.62)	13.79 (10.12 – 18.72)
E_yC₂₀	44.57 (19.60 – 68.28)	< 0.32	< 0.32	0.35 (0.18 – 0.59)
E_yC₁₀	26.61 (8.58 - 45.46)	< 0.32	< 0.32	< 0.32
LOEC	1.60	< 0.32	< 0.32	< 0.32
NOEC	0.32	< 0.32	< 0.32	< 0.32
Endpoint value [mg/L]	Based on nominal concentrations of chlormequat chloride			
	FronD number Dry weight			FronD number Dry weight
	0-2 d	0-5 d	0-7 d	0-7 d
E_yC₅₀	76.07 (51.35 – 111.43)	6.01 (3.51 – 10.03)	5.64 (3.61 – 8.65)	8.75 (6.43 – 11.88)
E_yC₂₀	28.34 (12.46 – 43.42)	< 0.20	< 0.20	0.22 (0.12 – 0.38)
E_yC₁₀	16.92 (5.46 - 28.91)	< 0.20	< 0.20	< 0.20
LOEC	1.02	< 0.20	< 0.20	< 0.20
NOEC	0.20	< 0.20	< 0.20	< 0.20

Validity criteria

In the definitive test, the following validity criteria specified in the OECD Guideline No. 221 (2006) were met:

- The doubling time of frond number in the control was 2.0 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 10.7).
- The average specific growth rate in the control between day 0 and day 7 was 0.339 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:	Acute oral toxicity test. The study are accepted by RMS. Validity criteria: <ul style="list-style-type: none">▪ the average mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%),▪ the 24-hour LD₅₀ of the reference item (dimethoate) was 0.11 µg/bee (criterion: 0.10-0.35 µg a.i./bee). <i>Apis mellifera</i> Agreed toxicity endpoints: 48-h LD ₅₀ > 400 µg formulation CLARA /bee 48-h LD ₅₀ > 254.39 µg chlormequat chloride /bee
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Reference: KCP 10.3.1.1.1

Report “Chlormequat chloride 72% SL Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test”. Aneta Glanas, MSc., 2017. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: B/100/16.

Guideline(s): OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C. 16. (2008)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Chlormequat chloride 72% SL
 The content of chlormequat chloride is 72.5% (w/v)
 Batch number: SCL-27012
 Manufacture date: February 20, 2016
 Expiry date: January 19, 2018

Test organism: The honeybee, *Apis mellifera* L., strain: carnica
 Source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna
 Age: approximately 3 weeks

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

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

Test item doses: 25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee (15.90; 31.80; 63.60; 127.19; and 254.39 µg chlormequat chloride/bee) and a control (0.0 µg/bee)

Reference item doses: 0.03, 0.06 and 0.12 µg a.i./bee.

Test conditions: temperature: 24 °C
 Relative air humidity: 67 - 68%
 Place: a dark room

Endpoints: - honeybee mortality after 48 hours of the exposure (LD₅₀)
 - the contact LD₅₀/24 h of the reference item (dimethoate).

Statistic method: Regression analysis using the log-probit method

Results

The acute contact toxicity study of the test item, Chlormequat chloride 72% SL on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Table 1. Endpoint values for mortality after 48h

Dose		Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD ₅₀	
[µg/bee] ^a	[µg/bee] ^b		Total		[µg/bee] ^a	[µg/bee] ^b
			[no.]	[%]		
0.0 (Control)		30	0	0.0	Above	Above
25.0	15.90	30	0	0.0	400.0	254.39

50.0	31.80	30	1	3.3		
100.0	63.60	30	1	3.3		
200.0	127.19	30	0	0.0		
400.0	254.39	30	0	0.0		

^a : µg test item/bee

^b : µg active ingredient/bee

Validity criteria

The following validity criteria were met during the test:

- the average mortality for the controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%),
- the 24-hour LD₅₀ of the reference item (dimethoate) was 0.11 µg a.i./bee (criterion: 0.10 - 0.35 µg a.i./bee).

Conclusions

The median lethal doses (LD₅₀/24 h and LD₅₀/48 h contact) are higher than the highest dose used in the test, i.e. 400.0 µg/honeybee (254.39 µg chlormequat chloride/bee).

With respect to the test results, it can be concluded that the test item, Chlormequat chloride 72% SL had no adverse effect on mortality of honeybees (*Apis mellifera* L.).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study are accepted by RMS. Validity criteria: <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 0.24 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee). Apis mellifera Agreed toxicity endpoints: 48-h LD ₅₀ > 400 µg formulation CLARA /bee 48-h LD ₅₀ > 254.39 µg chlormequat chloride /bee
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Reference:	KCP 10.3.1.1.2
Report	“Chlormequat chloride 72% SL Honeybees (<i>Apis mellifera</i> L.), Acute Contact Toxicity Test”. Aneta Glanas, MSc., 2017. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: B/101/16.
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C. 17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat chloride 72% SL The content of chlormequat chloride is 72.5% (w/v) Batch number: SCL-27012 Manufacture date: February 20, 2016 Expiry date: January 19, 2018
Test organism:	The honeybee, <i>Apis mellifera</i> L., strain: carnica Source: an apiary at the Institute of Industrial Organic Chemistry, Branch

Pszczyna
 Age: approximately 3 weeks

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

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

Test item doses: 25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee (15.90; 31.80; 63.60; 127.19; and 254.39 µg chlormequat chloride/bee) and a control (0.0 µg/bee)

Reference item doses: 0.1, 0.2, and 0.4 µg a.i./bee and a control (0.0 µg/bee)

Test conditions: temperature: 24 – 25.5°C
 Relative air humidity: 61 - 68%
 Place: a dark room

Endpoints: - honeybee mortality after 48 hours of the exposure
 - the contact LD50 of the test item after 24 and 48 hours of the exposure
 - the contact LD50/24 h of the reference item (dimethoate).

Statistic method: Regression analysis using the log-probit method

Results

The acute contact toxicity study of the test item, Chlormequat chloride 72% SL on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Table 1. Endpoint values for mortality after 48h

Dose		Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD ₅₀	
[µg/bee] ^a	[µg/bee] ^b		Total		[µg/bee] ^a	[µg/bee] ^b
			[no.]	[%]		
0.0 (Control)		30	0	0.0	Above 400.0	Above 254.39
25.0	15.90	30	0	0.0		
50.0	31.80	30	0	0.0		
100.0	63.60	30	0	0.0		
200.0	127.19	30	0	0.0		
400.0	254.39	30	0	0.0		

^a : µg test item/bee

^b : µg active ingredient/bee

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 0.24 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Conclusions

The median lethal doses (LD₅₀/24 h and LD₅₀/48 h contact) are higher than the highest dose used in the test, i.e. 400.0 µg/honeybee (254.39 µg chlormequat chloride/bee).

With respect to the test results, it can be concluded that the test item, Chlormequat chloride 72% SL had no adverse effect on mortality of honeybees (*Apis mellifera* L.).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study are accepted by RMS. Validity criteria: <ul style="list-style-type: none">There was no mortality in the group.The average mortality in the reference substance treated group is 73.33% at the end of the test (Test Guideline criteria: > 50%) <i>Apis mellifera</i> Agreed toxicity endpoints: 10 d LDD ₅₀ = 81.87 µg formulation/bee/day, equivalent to 51.99 µg chlormequat chloride/bee/day 10 d NOEDD = 31.89 µg/bee/day, equivalent to 20.25 µg chlormequat chloride/bee/day
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Reference: KCP 10.3.1.2

Report “Chronic Oral Toxicity Study of Chlormequat chloride 72% SL on honey bee (*Apis mellifera*)”. M. Mohanraj. 2023. Study code 12354/2023. BIO-SCIENCE RESEARCH FOUNDATION.

Guideline(s): Yes, OECD Guideline No. 245. Guideline for the Testing of Chemicals. Honeybees, Chronic Oral Toxicity Test (Adopted 9th October 2017).

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication NA
(if vertebrate study)

The mortality of honeybees exposed to Chlormequat chloride 72% SL was investigated during 10- days chronic oral toxicity test. Five doses of the test item were used. The nominal concentrations were 1481.5, 2222.2, 3333.3, 5000 and 7500 mg/kg of diet (corresponding to the nominal doses of 29.6, 44.4, 66.7, 100 and 150.0 µg/20 mg bee/day).

Each group of bees (3 replicates/group; 10 bees/replicate) was fed with 50% sucrose solution containing the test item at the concentrations of 1481.5, 2222.2, 3333.3, 5000 and 7500 mg/kg, or 50% sucrose solution alone (control group) for 10 days.

Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per concentration) was fed with a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.016 µg/bee/day). Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10.

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

Materials and methods

Test item:	Chlormequat chloride 72% SL Batch no.: SCL – 800410 Active substance: chlormequat chloride – 72.4 % w/v Date of manufacture: 6 th January 2023 Date of expiry: 5 th January 2025
Reference item:	Technical dimethoate (expiry date: December 2025; purity: 99.4% (CAS number: 60-51-5), which is a recommended reference item, was used to verify the precision of the test procedure and the sensitivity of the bees.
Test system :	Species: <i>Apis mellifera</i> L. strain carnica. Source: bee hive maintained at BRF test facility. Age: freshly emerged worker honeybees (max. 2 days old) from queen-right colonies.
Experimental design:	– the test item: number of concentrations: 5 number of replicates: 3 number of insects: 10 bees/replicate – control: number of replicates: 3 number of insects: 10 bees/replicate – the reference item: number of concentrations: 1 number of replicates: 3 number of insects: 10 bees/replicate exposure duration: 10 days
Nominal concentrations of the test item:	1481.5, 2222.2, 3333.3, 5000 and 7500 mg/kg
Nominal doses of the test item:	29.6, 44.4, 66.7, 100 and 150.0 µg/bee/day
Doses of the test item consumed by the bees (i.e. dietary doses):	31.89, 45.74, 69.96, 100.69 and 154.79 µg/bee/day
Nominal concentration of the reference item (dimethoate):	0.8 mg/kg
Nominal dose of the reference item (dimethoate):	0.016 µg/bee/day
Statistical method:	The LC ₅₀ , LDD ₅₀ were determined using probit analysis. The NOEC and NOEDD were determined by Turkey's multiple comparison test.
Endpoints:	LC ₅₀ , LDD ₅₀ , NOEC and NOEDD after 10 days of exposure

Chemical verification of nominal concentration

In order to verify the nominal concentration of the test item in sucrose solution medium, the analytical measurements were performed.

The validation of analytical method was performed according to SANTE/2020/12830, Rev.1. Method validation results and concentrations verification analysis of Chlormequat chloride are summarized in the table below.

Method validation parameters	Acceptance criteria	Obtained results		
Specificity/Selectivity	< 30% at the LOQ level	Solvent/Diluent: no interference with Chlormequat chloride peak at RT 3.2 min. Medium in diluent: no interference with Chlormequat chloride peak at RT 3.2 min. Hence < 30% at the LOQ level.		
Confirmatory method	In HPLC-MS by monitoring at least 2 additional fragment ions (preferably m/z > 100)	In HPLC-MS monitoring fragment ion was confirmed in 58 m/z.		
Limit of Detection (LOD)	-	0.05 mg/kg		
Limit of Quantification (LOQ)	-	0.1 mg/kg		
Linearity	Slope	25358		
	Intercept	-223.1		
	Correlation of determination (r ²)	1.0		
	Correlation Coefficient (r)	1.0		
Precision (RSD%) and Accuracy (%Recovery)	The mean % recovery for each fortification levels should be in the range of 70-110% with RSD% ≤ 20.00%	Fortification Level - 1	Fortification Level - 2	
		99.75% RSD% - 0.6787	100.24% RSD% - 0.3027	
Dose concentration verification analysis	The mean % recovery should be in the range of 80-120%	Concentration (mg/kg)	Recovery (%)	
		Day 1	1481.5	100.8
			2222.2	98.9
			3333.3	99.3
			5000.0	100.6
		Day 2	7500.0	100.4
			1481.5	101.0
			2222.2	99.2
			3333.3	99.4
		Day 3	5000.0	100.7
			7500.0	100.6
			1481.5	101.4
			2222.2	99.4
		Day 4	3333.3	99.7
			5000.0	101.2
			7500.0	100.8
			1481.5	102.3
		Day 5	2222.2	99.9
			3333.3	99.8
			5000.0	101.0
7500.0	100.7			
Day 5	1481.5	102.0		
	2222.2	99.7		
	3333.3	100.3		
	5000.0	101.4		

			7500.0	101.2
Day 6			1481.5	100.9
			2222.2	99.2
			3333.3	99.5
			5000.0	100.7
			7500.0	100.5
Day 7			1481.5	101.4
			2222.2	99.4
			3333.3	100.1
			5000.0	100.9
			7500.0	100.5
Day 8			1481.5	101.0
			2222.2	99.2
			3333.3	99.6
			5000.0	100.9
			7500.0	100.3
Day 9			1481.5	100.7
			2222.2	98.5
			3333.3	98.9
			5000.0	100.0
			7500.0	99.5
Day 10			1481.5	101.5
			2222.2	98.9
			3333.3	98.8
			5000.0	99.7
			7500.0	99.1

Results and discussions

Table: Mortality, NOEC, NOEDD, LC₅₀ and LDD₅₀

Initial		Consumed	No. of tested bees	Mortality total		LC ₅₀	LDD ₅₀
Concentration [mg test item/kg of food]	Dose [µg/20 mg/bee/day]	Dose [µg/bee/day]		No	[%]		
Chlormequat chloride 72% SL							
0.0 control			30	0	0.00	3974.53 (3822.98 – 4126.07) [mg test item /kg food]	81.87 (78.83 – 84.90) [µg test item/bee/day]
1481.5	29.6	31.89	30	2	6.67		
2222.2	44.4	45.74	30	6	20.00		
3333.3	66.7	96.96	30	9	30.00		
5000.0	100.0	100.69	30	15	50.00		
7500.0	150.0	154.79	30	30	100.00		
Dimethoate							
0.8 mg a.i./kg	0.016 µg a.i./bee	0.02 µg a.i./bee	30	22	73.33	Not determined	
NOEC		1481.5 [mg test item /kg food]					
NOEDD		31.89 [µg test item/bee/day]					

Conclusion

The following validity criteria were met during the test:

- There was no mortality in control groups.

- The mortality of reference substance 0.8 mg/kg was found to be 73.33% ($\geq 50\%$) between the stipulated range of 0.5 – 1.0 mg a.i./kg for 10 days exposure on *Apis mellifera*.

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

Comments of zRMS:	<p>The study are accepted by RMS.</p> <p>The following validity criteria were met during the test:</p> <ul style="list-style-type: none">Larval mortality in the controls: $\leq 15\%$ for larvae across all control replicates (between D3 and D8 – 2.78% (control A1).Adult emergence rate: $\geq 70\%$ for bees across all control replicates (between D3 and D22) – 91.67% (control A1).Larval mortality in the reference item: $\geq 50\%$ for larvae exposed to 7.39 $\mu\text{g/larva}$ across all reference replicates (between D3 and D8 – 60.00 % on D8). <p>Agreed toxicity endpoints:</p> <p>ED₅₀ = 29.46 μg formulation/larvae, equivalent to 18.71 μg chlormequat chloride/larvae</p> <p>NOED = 9.5 μg formulation/larvae, equivalent to 6.03 μg chlormequat chloride/larvae</p>
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Reference: KCP 10.3.1.3

Report: "Effect of Chlormequat chloride 72% SL on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure", M. Mohanraj, 2023, Study code 12355/2023. BIOSCIENCE RESEARCH FOUNDATION.

Guideline(s): Yes, Guidance Document on Honey Bee Larval Toxicity Test Following Repeated Exposure, Series on Testing and Assessment, No. 239, OECD (2016).

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The purpose of this study was to determine the chronic toxicity (e.g., ED₅₀, EC₅₀, NOED and NOEC, adult emergence up to day 22) of Chlormequat chloride 72% SL (Batch number: SCL-800410) applied to the honey bee, *Apis mellifera*, larvae in an in vitro test after repeated oral diet administration.

The test species was honey bee (*Apis mellifera* L.), synchronized first instar (L1) larvae originating from bee hive maintained at BRF test facility.

The test was conducted as a dose response test with a duration of 22 days from grafting on day 1 (D1) to the final assessment on day 22 (D22); from day 3 (D3) until day 6 (D6) of the test, test item and reference item (dimethoate) were dissolved in the appropriate larval diet and provided to larvae. The active substances contents were used to calculate the test item doses and dimethoate content was used to calculate the reference item dose.

The experimental groups were: one untreated control, 5 test item groups and 1 reference item group. The control group and treated groups were exposed for the same period of time under identical conditions. Each treatment group consisted of 3 different replicates and 12 larvae per replicate; mortality assessments

were performed on day 4 (D4), day 5 (D5), day 6 (D6), day 7 (D7) and day 8 (D8). Additionally, other observations were reported on D8 such as small body size or large quantities of remaining food. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22 respectively.

The dose of test item was 9.5, 17.1, 30.9, 55.6 and 100.0 µg/larva, the concentrations of the test item in the diet were 61.9, 111.5, 200.6, 361,1 and 650.0 mg/kg food. Additionally, honeybee larvae were treated with Dimethoate Technical as reference item at a dose of 7.39 µg dimethoate/larva (test concentration 48 mg/kg) of diet and with an untreated diet as control.

One day old honeybee larvae (D1) were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates before start of the treatment (3 days). On 4 successive days (D3 to D6) the larvae were repeatedly exposed to test item diluted in the larval food. After the applications no additional feedings provided to the larvae.

Chemical verification of nominal concentration

In order to verify the nominal concentration of the test item in medium, the analytical measurements were performed.

The validation of analytical method was performed according to SANTE/2020/12830, Rev.1.

Method validation results and concentrations verification analysis of Chlormequat chloride are summarized in the table below.

Method validation parameters	Acceptance criteria	Obtained results		
Specificity/Selectivity	< 30% at the LOQ level	Solvent/Diluent: no interference with Chlormequat chloride peak at RT 3.2 min. Medium in diluent: no interference with Chlormequat chloride peak at RT 3.2 min. Hence < 30% at the LOQ level.		
Confirmatory method	In HPLC-MS by monitoring at least 2 additional fragment ions (preferably m/z > 100)	In HPLC-MS monitoring fragment ion was confirmed in 58 m/z.		
Matrix effect	± 20	-9.03		
Limit of Detection (LOD)	-	0.05 mg/kg		
Limit of Quantification (LOQ)	-	0.1 mg/kg		
Linearity	Slope	25458		
	Intercept	551.8		
	Correlation of determination (r ²)	1.0		
	Correlation Coefficient (r)	1.0		
Precision (RSD%) and Accuracy (%Recovery)	The mean % recovery for each fortification levels should be in the range of 70-110% with RSD% ≤ 20.00%	Fortification Level - 1	Fortification Level - 2	
		99.61% RSD% - 0.6565	100.79% RSD% - 0.6010	
Dose concentration verification analysis	The mean % recovery should be in the range of 80-120%	Concentration (mg/kg)	Recovery (%)	
		Day 3	61.9	101.5
			111.5	100.8
			200.6	101.5
			361.1	99.8
650.0	101.3			

	Day 4	61.9	101.0
		111.5	100.4
		200.6	100.33
		361.1	98.4
	Day 5	650.0	100.0
		61.9	101.1
		111.5	101.5
		200.6	101.6
	Day 6	361.1	99.8
		650.0	101.4
		61.9	101.5
		111.5	101.3
	200.6	101.4	
	361.1	100.5	
	650.0	102.6	

Results and discussion

Table 1: Toxicity of test item to larvae of *Apis mellifera* L.

Treatment group	Test solution (ID)	Dose [$\mu\text{g}/\text{larva}$]	Conc. [$\text{mg}/\text{kg food}$]	On D8			On D15		On D22		
				Larval mortality D3 to D8		Mean OO	Pupae stage D8 to D15		Total mortality D3-D22		Adult emergence rate %
				mor. (%)	corr. (%)	(%)	mor. (%)	corr. (%)	mor. (%)	corr. (%)	(%)
Control	A1	-	-	2.78	-	0	2.78	-	8.33	-	91.67
Test Item	T1	9.5	61.9	5.56	2.86	0	2.78	0.00	13.89	6.06	86.11
	T2	17.1	11.5	11.11	8.57	0	5.56	2.86	38.89	33.33	61.11
	T3	30.9	200.6	22.22	20.00	0	13.89	11.43	58.33	54.55	41.67
	T4	55.6	361.1	33.33	31.43	0	16.67	14.29	72.22	69.70	27.78
	T5	100	650	47.22	45.71	0	22.22	20.00	94.44	93.94	5.56
Ref. Item	R1	7.39	48	61.11	60.00	0	13.89	11.43	88.89	87.88	11.11

Note: D-Day, Mor- Mortality, corr.-Corrected Mortality, OO-Other observation
 Results are averages based on 3 replicates, containing 12 larvae each

Table 2: Endpoint values

Endpoint [95 % CL]	Test item dose ($\mu\text{g}/\text{larva}$)	Test item dose ($\mu\text{g chlormequat chloride}/\text{larva}$)
22-Day NOED	9.5	6.03
22-Day ED ₁₀	9.60 (8.58-10.61)	6.10 (5.45-6.74)
22-Day ED ₂₀	14.11 (12.95-15.26)	8.96 (8.22-9.69)
22-Day ED ₅₀	29.46 (27.78-31.15)	18.71 (17.64-19.78)
Endpoint [95 % CL]	Test item concentrations (mg/kg food)	Test item concentrations ($\mu\text{g chlormequat chloride}/\text{larva}$)
22-Day NOEC	61.9	39.31
22-Day EC ₁₀	62.57	39.74

	(55.96-69.17)	(35.54-43.93)
22-Day EC ₂₀	91.88 (84.35-99.41)	58.35 (53.57-63.13)
22-Day EC ₅₀	191.63 (180.72-202.55)	121.70 (114.77-128.63)

The following **validity criteria** were met during the test:

- Larval mortality in the controls: ≤ 15% for larvae across all control replicates (between D3 and D8 – 2.78% (control A1).
- Adult emergence rate: ≥ 70% for bees across all control replicates (between D3 and D22) – 91.67% (control A1).
- Larval mortality in the reference item: ≥50% for larvae exposed to 7.39 µg/larva across all reference replicates (between D3 and D8 – 60.00 % on D8).

Conclusion

In a repeated exposure larval toxicity study with Chlormequat chloride 72% SL, the ED₅₀ (successful adult emergence up to D22) was calculated to be 29.46 µg/larva, which is equivalent to an EC₅₀ of 191.63 mg/kg food.

ED₁₀ was calculated to be 9.60 µg/larva, equivalent to an EC₁₀ of 62.57 mg/kg food.

ED₂₀ was calculated to be 14.11 µg/larva, equivalent to an EC₂₀ of 91.88 mg/kg.

The NOED was 9.5 µg/larva and the corresponding NOEC was 61.9 mg/kg food.

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 arthropods other than bees

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

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

Comments of zRMS:	The study was accepted by RMS. The validity criteria: – after 48 hours, mortality of the control group was 0% (criterion: a maximum of 10.0%), – after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 60.0% (criterion: a minimum of 50%), – all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity), – the mean number of mummies per female in the control group was 56.0 (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).
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Agreed endpoints:							
Study group (application rate)	Parameter (endpoint)						
	Mortality after 48 h of exposure			Fecundity			
Test item [L/ha]	Total	LR ₅₀		Mean no. of mummies/ female	Fecundity reduction Pr [%]	ER ₅₀	
	[%]	Test item [L/ha]	Active ingredient [kg/ha]			Test item [L/ha]	Active ingredient [kg/ha]
Control (0.0)	0.0	-		56.0	-	-	
Clomazone 48%EC							
2.1	3.3	> 16.8	> 12.2	46.3*	17.3	> 16.8	> 12.2
4.2	6.7			40.5*	27.6		
8.4	0.0			39.4*	29.6		
16.8	13.3			36.2*	35.4		
NOER _{mortality}		> 16.8	> 12.2	NOER _{fecundity}		< 2.1	< 1.5
Danadim 400 EC							
Reference item [mL/ha]	not determined / not assessed						
5.0	60.0	not determined		not assessed			

*: statistically significant difference

Reference:	KCP 10.3.2.2-01
Report	“An extended laboratory test for evaluating the effects of Chlormequat chloride 72% SL on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez)”. Natalia Lemańska PhD Eng., 2018. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: B/102/16
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the Joint Initiative of IOBC, BART, and EPPO (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat chloride 72% SL The content of chlormequat chloride is 72.5% (w/v) Batch number: SCL-23170 Manufacture date: 04.01.2018 Expiry date: January 03.01.2020
Test organism:	the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae – age: adult females (24 - 48 hours after emerging from mummies) – source: a laboratory-bred culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented by Katz Biotech AG (Baruth, Germany)
Experimental design:	6 study groups:

- a control group (0.0 L/ha)
 - Chlormequat chloride 72% SL at the rate of 2.1 L/ha (1.5 kg a.i./ha)
 - Chlormequat chloride 72% SL at the rate of 4.2 L/ha (3.0 kg a.i./ha)
 - Chlormequat chloride 72% SL at the rate of 8.4 L/ha (6.1 kg a.i./ha)
 - Chlormequat chloride 72% SL at the rate of 16.8 L/ha (12.2 kg a.i./ha)
 - Danadim 400 EC at the rate of 5.0 mL/ha (2.0 g a.i./ha)
- mortality assessment: 6 replicates/group; 5 females/replicate
 fecundity assessment: 15 replicates/group; 1 females/replicate

Test conditions: temperature: 19 – 22 °C
 Relative air humidity: 71 - 77%
 Photoperiod: 16 h light (mortality assessment: 1993 lx and oviposition: 8493 lx)
 8h dark

Endpoints: – wasp mortality after 48 hours of exposure
 – reduction in fecundity (Pr) of the surviving female wasps exposed to Chlormequat chloride 72% SL, 12 days after the oviposition period

Statistical analyses: Shapiro-Wilk’s test on normal distribution, Levene’s test on variance homogeneity, One-way ANOVA Analysis of Variance, Duncan test, Chi2 2x2-table test with Bonferroni Correction and Williams Multiple Sequential t-test Procedure

Results

The effects of the test item, Chlormequat chloride 72% SL on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Table 1. Effects of Chlormequat chloride 72% SL on mortality and reproduction of mortality and fecundity of *Aphidius rhopalosiphi* in the definitive test

Study group [application rate]	Parameter (endpoint)						
	Mortality after 48h of exposure			Fecundity			
Test item [L/ha]	Total [%]	LR ₅₀		Mean number of mummies/female (Rr) [no.]	Fecundity reduction Pr [%]	ER ₅₀	
		Test item [L/ha]	Active ingredient [Kg/ha]			Test item [L/ha]	Active ingredient [Kg /ha]
Control (0.0)	0.0	-	-	56.0	-	-	-
Chlormequat chloride 72% SL							
2.1	3.3	>16.8	>12.2	46.3 ⁺	17.3	>16.8	>12.2
4.2	6.7			40.5 ⁺	27.6		
8.2	0.0			39.4 ⁺	29.6		
16.8	13.3			36.2 ⁺	35.4		
NOER_{mortality}		>16.8	>12.2	NOER_{fecundity}		<2.1	<1.5
Reference item	Danadim 400 EC						
[mL/ha]							
5.0	60.0	not determined		not assessed			

⁺ : statistically significant difference

Validity criteria

The following validity criteria were met during the study:

- after 48 hours, mortality of the control group was 0% (criterion: a maximum of 10.0%)
- after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 60.0% (criterion: a minimum of 50%)
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity)

- the mean number of mummies per female in the control group was 56.0 (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).

Conclusions

The validity criterion concerning mortality was met, because mortality of the control group was 0% (criterion: a maximum of 10.0%) after 48 hours of exposure.

Mortality of the wasps after 48 hours of exposure to the test item at the rate of 2.1, 4.2, 8.4 and 16.8 L/ha was 3.3, 6.7, 0.0 and 13.3%, respectively. Mortality of the wasps exposed to Danadim 400 EC at the rate of 5.0 mL/ha was 60.0% after 48 hours. Therefore, the validity criterion was met. The results showed that the insects were sensitive to dimethoate.

There were statistically no significant differences in mortality between the groups treated with the test item at all rates and the control group.

On the basis of the obtained results the endpoints regarding mortality could not be determined. For Chlormequat chloride 72% SL the LR_{50} value is higher than 16.8 L/ha and $NOER_{mortality}$ value is higher than 16.8 L of Chlormequat chloride 72% SL/ha.

The fecundity assessment showed that the mean number of mummies per female in the control group was 56.0. The numbers of mummies/female for the wasps treated with Chlormequat chloride 72% SL at rates of 2.1, 4.2, 8.4 and 16.8 L/ha were 46.3, 40.5, 39.4 and 36.2, respectively. Fecundity reduction (Pr) caused by Chlormequat chloride 72% SL at the rates mentioned above was 17.3, 27.6, 29.6 and 35.4%, respectively.

At the significance level of 0.05, there were statistically significant differences in reproduction between all groups treated with the test item and the control group.

On the basis of the obtained results, the ER_{50} and the $NOER_{reproduction}$ value for Chlormequat chloride 72% SL could not be estimated. The ER_{50} is higher than 16.8 L/ha and the $NOER_{reproduction}$ value is lower than 2.1 L of Chlormequat chloride 72% SL/ha.

Comments of zRMS: In general, the study was accepted as its validity criteria were met. However, RMS decided not to use the results of this study for risk assessment due to study limitations such as: 1. No clear dose-response for test concentrations for mortality and reproduction parameters. 2. For the dose of 2.069 L/ha, the LR₅₀ value was determined. Therefore, an analysis of the effect of CLARA on reproduction at a dose of 2.069 L/ha should be performed.

The validity criteria:

- mortality of the control group was 5% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 86.0% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 8.2 (required: ≥ 4 eggs per female).

Study limitation:

1. No clear dose-response for test concentrations for mortality and reproduction parameters.
2. For the dose of 2.069 L/ha, the LR₅₀ value was determined. Therefore, an analysis of the effect of CLARA on reproduction at a dose of 2.069 L/ha should be performed.

Toxicity endpoints:

Study group [application rate]	Parameter (endpoint)							
	Mortality				Reproduction			
Test item	Total [%]	Corrected*	LR ₅₀		Mean number of eggs/ female (Rr) [no.]	Reproduction reduction Pr [%]	ER ₅₀	
[L/ha]			[L/ha]	[g a.i./ha]			[L/ha]	[g a.i./ha]
Control (0.0)	5.0	-	-	-	8.2	-	-	-
Chloromequat chloride 72% SL								
0.259	5.0	0.0	>2.069	>1500	6.6*	19.2	1.084	785.9
0.517	16.7	12.3			7.5	7.8		
1.035	10.0	5.3			5.1*	37.3		
2.069	53.3	50.9*			not assessed			
NOER_{mortality}			1.035	750.0	NOER_{reproduction}		n.d.	n.d.
Danadim 400 EC								
Reference item								
[mL/ha]								
9.0	86.7	86.0	not determined		not assessed			

*: mortality corrected using the Abbott equation
 †: statistically significant difference
 n.d.: not determined due to mathematical reasons or inappropriate data

The use of the toxicity endpoints from this study in risk assessment should be considered at MSs level.

Reference:

KCP 10.3.2.2-02

Report

“An extended laboratory test for evaluating the effects of Chloromequat chlo-

ride 72% SL on the predatory mite, *Typhlodromus pyri* (Sch.)". Natalia Lemańska PhD Eng., 2018. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: B/103/16

Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the laboratory test was to evaluate the effects of the test item, Chlormequat chloride 72% SL on mortality and reproduction of the predatory mite, *T. pyri* (Sch.). On the basis of the preliminary test results, it was decided to use four rates of the test item in the definitive test. These were 0.259, 0.517, 1.035 and 2.069 L/ha. The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (*Pinus sp.*). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and all groups treated with the test item were made after 8, 11, and 14 days of the treatment. Mortality of *T.pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints. To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Materials and methods

Test item:	Chlormequat chloride 72% SL; content: 72.5% (w/v) of Chlormequat Chloride as the active ingredient; batch no.: SCL-27012; manufacturing date: 20.02.2016; expiry date: 19.02.2018
Test organism:	the predatory mite, <i>Typhlodromus pyri</i> (Sch.) (Acari: Phytoseiidae) age: 24-hur-old protonymphs source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna, the culture was augmented by Katz Biotech AG (Baruth, Germany)
Experimental design:	6 study groups: – a control group (0.0 g/ha) – Chlormequat chloride 72% SL at the rate of 0.259 L/ha – Chlormequat chloride 72% SL at the rate of 0.517 L/ha – Chlormequat chloride 72% SL at the rate of 1.035 L/ha – Chlormequat chloride 72% SL at the rate of 2.069 L/ha – Danadim 400 EC at the rate of 9.0 mL/ha number of replicates: 3; number of mites in each replicate: 20
Test conditions:	temperature: 23–26°C Relative air humidity: 67-79% Photoperiod: 16 h light (672 lux) : 8 h dark
Endpoints:	– mite mortality after 7 days of the treatment – LR ₅₀ and NOER _{mortality} – reproduction reduction (Pr) after 14 days of the treatment – ER ₅₀ and NOER _{reproduction}

Statistical analyses: Multiple Sequentially – rejective Fisher Test After Bonferroni-Holm, $p > 0.05$

Results

In the definitive test, mortality of the control group after 7 days of exposure was 5.0%. After 7 days of exposure to Chlormequat chloride 72% SL at the rates 0.259, 0.517, 1.035 and 2.069 L/ha, the percentages of *T. pyri*, mortality corrected using the formula of Abbott, were 0.0, 12.3, 5.3 and 50.9%, respectively. There were statistically no significant differences in mortality between the groups treated with the test item at rates of 0.259, 0.517, 1.035 L/ha and the control group (Multiple Sequentially – rejective Fisher Test After Bonferroni-Holm, $p > 0.05$). Although, statically significant differences were noticed in the groups treated with Chlormequat chloride 72% SL at rate of 2.069 L/ha (Multiple Sequentially - rejective Fisher Test After Bonferroni-Holm, $p < 0.05$). On the basis of the obtained results the endpoints regarding mortality could not be utterly determined. The LR_{50} value is higher than 2.069 L/ha and NOER-mortality value is equal to 1.035 L/ha. After 7 days of exposure to Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha), mortality of the mites, corrected using the formula of Abbott, was 86.0 %. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate. The mean reproduction rate (Rr) in the control group was 8.2 eggs/female. Due to the mortality exceeding 50% the group treated with Chlormequat chloride 72% SL at rate of 2.069 L/ha was excluded from the reproduction test. The mean Rr after 14 days of exposure to Chlormequat chloride 72% SL at rates 0.259, 0.517 and 1.035 L/ha were 6.6, 7.5 and 5.1 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by at the rates of 0.259, 0.517 and 1.035 L/ha were 19.2, 7.8 and 37.3%, respectively. At the significance level of 0.05, there were no statistically significant differences in reproduction between the group treated with the test item at the rate of 0.517 L/ha and the control group (Multiple Sequentially- rejective t-test After Bonferroni-Holm, $p = 0.05$). On the other hand, there were statistically significant differences in reproduction between the group treated with the test item at rates 0.259 and 1.035 L/ha and the control group (Multiple Sequentially- rejective t-test After Bonferroni-Holm, $p < 0.05$). On the basis of the obtained results, the NOERreproduction value could not be estimated. Nevertheless, the ER_{50} was determined and it is equal to 1.084 L/ha.

Table 1. The effects of Chlormequat chloride 72% SL on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Study group [application rate]	Parameter (endpoint)							
	Mortality				Reproduction			
Test item	Total [%]	Corrected*	LR ₅₀		Mean number of eggs/ female (Rr) [no.]	Reproduction reduction Pr [%]	ER ₅₀	
			[L/ha]	[g a.i./ha]			[L/ha]	[g a.i./ha]
Control (0.0)	5.0	-	-	-	8.2	-	-	-
Chlormequat chloride 72% SL								
0.259	5.0	0.0	>2.069	>1500	6.6*	19.2	1.084	785.9
0.517	16.7	12.3			7.5	7.8		
1.035	10.0	5.3			5.1*	37.3		
2.069	53.3	50.9*			not assessed			
NOER_{mortality}			1.035	750.0	NOER_{reproduction}		n.d.	n.d.
Reference item	Danadim 400 EC							
[mL/ha]								
9.0	86.7	86.0	not determined		not assessed			

*: mortality corrected using the Abbott equation
 *: statistically significant difference
 n.d.: not determined due to mathematical reasons or inappropriate data

Validity criteria

The following validity criteria were met during the study:

- mortality of the control group was 5% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 86.0% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 8.2 (required: ≥ 4 eggs per female).

Conclusions

Based on the results it can be stated that Chlormequat chloride 72% SL at the rates of 0.259 0.517 and 1.035 L/ha has no adverse effect on mortality of the mites. However in rate 2.069 L/ha such effect is shown. On the basis of the obtained results the endpoints regarding mortality could not be utterly determined. The LR₅₀ value is higher than 2.069 L/ha and NOER_{mortality} value is equal to 1.035 L/ha. Slight adverse effect on the mites' fecundity was observed for the groups treated with Chlormequat chloride 72% SL at rates 0.259 and 1.035 L/ha. Based on the results received from the study, the ER₅₀ was determined and it is equal to 1.084 L/ha, but the NOER_{reproduction} could not be calculated.

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

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

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen/carbon transformation

Comments of zRMS:	<p>The study was accepted by RMS.</p> <p>Validity criteria: The coefficients of variation (CV) in the control group were 10.9, 3.2, 5.8 and 0.5%, after 0, 7, 14, and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than $\pm 15\%$.</p> <p>Deviations from the OECD Guideline No. 216 (2000), the EU Method C.21., SOP/G/32 : According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer.</p> <p>Deviations from the OECD Guideline No. 216 (2000), the EU Method C.21.: The predicted environmental concentration (PEC) was calculated assuming 2.5 cm of the soil depth according to the German conditions for the substances with the mobility in soil $K_{Foc} > 500$ mL/g. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000), EU Method C.21 and SOP/G/32, where the PEC is calculated by using 5 cm of the soil depth.</p> <p>These deviations did not affect the results of the study.</p> <p>Agreed toxicity endpoints: On the basis of the results, it was concluded that CLARA at the concentration corresponding to the PEC: 6.38 mg test item/kg dry soil (i.e. 4.06 mg of chlormequat chloride/kg dry soil) and 5 x PEC: 31.90 mg test item/kg dry soil (i.e. 20.30 mg of chlormequat chloride/kg dry soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.</p>
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Reference:	KCP 10.5-01
Report	“Chlormequat chloride 72% SL Soil Microorganisms: Nitrogen Transformation Test”. Aneta Gierbuszewska, MSc 2020. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: G/195/17.
Guideline(s):	According to the OECD Guideline No. 216 (2000) / EU Method C.21.
Deviations:	Yes. The predicted environmental concentration (PEC) was calculated assuming 2.5 cm of the soil depth according to the German conditions for the substances with the mobility in soil $K_{Foc} > 500$ mL/g. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000), EU Method C.21 and SOP/G/32, where the PEC is calculated by using 5 cm of the soil

depth. This deviation did not affect the results of the study.

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

Materials and methods

Test material: Chlormequat chloride 72% SL
The content of chlormequat chloride is 72.5% (w/v)
Batch number: SCL-23170

Soil: Agricultural soil taken from the area belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna.

Test design: Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days.

Concentrations of the test item: control, PEC: 6.38 mg of test item / kg of dry weight soil (i.e. 4.06 mg of chlormequat chloride / kg dry weight of soil) and 5 x PEC: 31.90 mg of test item / kg of dry weight soil (i.e. 20.30 mg of chlormequat chloride / kg dry weight of soil).

Test conditions: temperature: 20.9 – 22.0 °C
Soil moisture: 46.1% – 54.0 % of the maximum water holding capacity
Incubation in darkness

Statistical analysis: - Shapiro-Wilk's test on Normal Distribution
- Levene's Test on Variance Homogeneity (with Residuals)
- William's Multiple Sequential t-test Procedure.

Endpoints: The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation.
The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28 days.

Results

The difference in the soil respiration rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC and 5 x PEC did not exceed 25% on 28 day of analysis.

Table 1. Oxygen (O₂) consumption - deviations from the control [%]

Day	PEC	5 x PEC
0 – 7	19.5	21.4
0 – 14	11.8	17.2
0 – 28	2.8	7.2

– values of nitrate formation rate higher than the one obtained for the control group

Validity criteria

The coefficients of variation (CV) in the control group were 10.9, 3.2, 5.8 and 0.5%, after 0, 7, 14, and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than $\pm 15\%$.

Conclusions

On the basis of the results, it was concluded that Chlormequat chloride 72% SL at the concentration corresponding to the PEC: 6.38 mg test item/kg dry soil (i.e. 4.06 mg of chlormequat chloride/kg dry soil) and 5 x PEC: 31.90 mg test item/kg dry soil (i.e. 20.30 mg of chlormequat chloride/kg dry soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Comments of zRMS:	<p>The study is considered as additional source of information (According to Regulation 284/2013 the carbon transformation study this is not required). All validity criteria were met.</p> <p>The coefficient of variation in the control group was as follows 5.6, 2.5, 3.0 and 11.5% on 0, the 7th, 14th and 28th day of soil incubation, respectively. The criterion of validity: the variation between replicate samples in the control should be less than $\pm 15\%$.</p> <p>Toxicity endpoints as additional source of information: On the basis of the results, it was concluded that CLARA at the concentrations corresponding to PEC: 6.38 mg of test item / kg of dry weight soil (i.e. 4.06 mg of chlormequat chloride/kg dry weight of soil) and 5 x PEC: 31.90 mg of test item/kg of dry weight soil (i.e. 20.30 mg of chlormequat chloride/kg dry weight of soil), did not have any long-term adverse effects on the process of carbon transformation in aerobic surface soils.</p>
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Reference:	KCP 10.5-02
Report	“Chlormequat chloride 72% SL Soil Microorganisms: Carbon Transformation Test”. Aneta Gierbuszewska, MSc 2020. Institute of Industrial Organic Chemistry, Branch Pszczyna. STUDY CODE: G/194/17.
Guideline(s):	according to the OECD Guideline No. 217 (2000) / EU Method C.22.
Deviations:	Yes. The predicted environmental concentration (PEC) was calculated assuming 2.5 cm of the soil depth according to the German conditions for the active substances with the mobility in soil $K_{Foc} < 500$ mL/g. Thus, the applied soil depth is a deviation from OECD Guideline No. 217 (2000), the EU Method C.22 where the PEC is calculated by using 5 cm of the soil depth. This deviation did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material:	Chlormequat chloride 72% SL The content of chlormequat chloride is 72.5% (w/v) Batch number: SCL-23170
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Soil:	Agricultural soil taken from the area belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna.
Test design:	Three portions of soil weighing 1500 g each: one control group and two groups containing the test item. Every portion was divided into three replicates weighing 500 g each. Test duration: 28 days.
Concentrations of the test item:	control, PEC: 6.38 mg of test item / kg of dry weight soil (i.e. 4.06 mg of chlormequat chloride / kg dry weight of soil) and 5 x PEC: 31.90 mg of test item / kg of dry weight soil (i.e. 20.30 mg of chlormequat chloride / kg dry weight of soil).
Test conditions:	temperature: 20.9 – 22.0 °C Soil moisture: 44.3% – 51.8% of the maximum water holding capacity Incubation in darkness
Statistical analysis:	In order to determine significance in the soil respiration rate of differences between the control and the treated groups, Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity and Williams Multiple Sequential t-test Procedure
Endpoints:	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.

Results

The difference in the soil respiration rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC and 5 x PEC did not exceed 25% on 28 day of analysis.

Table 1. Oxygen (O₂) consumption - deviations from the control [%]

Day	PEC	5 x PEC
0	-1.6	-5.1
7	4.4	-2.4
14	5.3	4.6
28	8.2	10.1

– values of oxygen consumption higher than the one obtained for the control group

Validity criteria

On the basis of the obtained results, it may be stated that the validity criterion was met.

The coefficient of variation in the control group was as follows 5.6, 2.5, 3.0 and 11.5% on 0, the 7th, 14th and 28th day of soil incubation, respectively. The criterion of validity: the variation between replicate samples in the control should be less than $\pm 15\%$.

Conclusions

On the basis of the results, it was concluded that Chlormequat chloride 72% SL at the concentrations corresponding to PEC: 6.38 mg of test item / kg of dry weight soil (i.e. 4.06 mg of chlormequat chloride / kg dry weight of soil) and 5 x PEC: 31.90 mg of test item / kg of dry weight soil (i.e. 20.30 mg of chlormequat chloride / kg dry weight of soil), did not have any long-term adverse effects on the process of carbon transformation in aerobic surface soils.

A 2.6

KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	<p>The study was accepted by RMS. Validity criteria were met: - the seedling emergence of plants (validity criterion: at least 70%) was as follows: 95.2 – 100.0– sunflower, 95.2 – 100.0– cabbage, 92.9 – 100.0 – pea, 90.0 – 100.0 – carrot, 90.0 – 100.0 – onion, 95.0 – 100.0 – oats, - the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%), - the control plants did not exhibit any visible phytotoxic symptoms, - environmental conditions for all plants belonging to the same species were identical. Deviations in the study: According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between $90.0 - 149.8 \mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. Agreed endpoints: Toxicity endpoints expressed as mL of the test item CLARA/ha</p> <table border="1"> <thead> <tr> <th></th> <th>Sunflower <i>Helianthus annuus</i></th> <th>Cabbage <i>Brassica oleracea var. capitata</i></th> <th>Pea <i>Pisum sativum</i></th> <th>Carrot <i>Daucus carota</i></th> <th>Onion <i>Allium cepa</i></th> <th>Oats <i>Avena sativa</i></th> </tr> </thead> <tbody> <tr> <td colspan="7">Plant number at the end of the experiment</td> </tr> <tr> <td>ER₅₀</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> </tr> <tr> <td>NOER</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> </tr> <tr> <td colspan="7">Shoot length (plants without roots)</td> </tr> <tr> <td>ER₅₀</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> </tr> <tr> <td>NOER</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>155.6</td> <td>≥ 42000</td> <td>≥ 42000</td> </tr> <tr> <td colspan="7">Plant dry weight (plants without roots)</td> </tr> <tr> <td>ER₅₀</td> <td>> 42000</td> <td>> 42000</td> <td>> 42000</td> <td>> 4200</td> <td>> 4200 (3400.7 -> 4200)</td> <td>> 42000</td> </tr> <tr> <td>NOER</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>≥ 42000</td> <td>155.6</td> <td>466.7</td> <td>≥ 42000</td> </tr> </tbody> </table> <p>Toxicity endpoints expressed as g chlormequat chloride/ha</p>		Sunflower <i>Helianthus annuus</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Pea <i>Pisum sativum</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>	Plant number at the end of the experiment							ER ₅₀	> 42000	> 42000	> 42000	> 42000	> 42000	> 42000	NOER	≥ 42000	≥ 42000	≥ 42000	≥ 42000	≥ 42000	≥ 42000	Shoot length (plants without roots)							ER ₅₀	> 42000	> 42000	> 42000	> 42000	> 42000	> 42000	NOER	≥ 42000	≥ 42000	≥ 42000	155.6	≥ 42000	≥ 42000	Plant dry weight (plants without roots)							ER ₅₀	> 42000	> 42000	> 42000	> 4200	> 4200 (3400.7 -> 4200)	> 42000	NOER	≥ 42000	≥ 42000	≥ 42000	155.6	466.7	≥ 42000
	Sunflower <i>Helianthus annuus</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Pea <i>Pisum sativum</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>																																																																	
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Plant number at the end of the experiment						
ER ₅₀	> 3045	> 3045	> 3045	> 3045	> 3045	> 3045
NOER	≥ 3045	≥ 3045	≥ 3045	≥ 3045	≥ 3045	≥ 3045
Shoot length (plants without roots)						
ER ₅₀	> 3045	> 3045	> 3045	> 3045	> 3045	> 3045
NOER	≥ 3045	≥ 3045	≥ 3045	112.8	≥ 3045	≥ 3045
Plant dry weight (plants without roots)						
ER ₅₀	> 3045	> 3045	> 3045	> 3045	> 3045 (2465.5 - >3045)	> 3045
NOER	≥ 3045	≥ 3045	≥ 3045	112.8	338.3	≥ 3045

Phytotoxicity effect:
 During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The phytotoxic symptoms in cultivation of sunflower, pea, cabbage, carrot, onion and oats were not observed.

Reference: KCP 10.6.2-01

Report “Chlormequat Chloride 72% SL Terrestrial Plant Test: Vegetative Vigour Test”. Aneta Gierbuszewska, MSc., 2020. Study No: G/200/17. Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna.

Guideline(s): according to the OECD Guideline No. 227 (2006)

Deviations: Yes. According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 90.0 – 149.8 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Chlormequat Chloride 72% SL
 batch number: SCL – 23170
 active substances: Chlormequat Chloride – 72.5% (w/v)

Test species: Pea (*Pisum sativum*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), sunflower (*Helianthus annuus*), onion (*Allium cepa*), oats (*Avena sativa*).

Soil: Sandy loam

Study design: number of rates: 8 + control; number of replicates/rate: 4 (carrot, onion, oats) or 7 (sunflower, cabbage, pea).
 The total number of seeds per application rate – 20 (carrot, onion, oats) or 21

(cabbage, pea, sunflower)
 Test termination: 21 days after the spraying

Application rates: a control, 1.9, 5.8, 17.3, 51.9, 155.6, 466.7, 1400.0 and 4200.0 mL test item/ha (i.e. 1.4, 4.2, 12.5, 37.6, 112.8, 338.3, 1015.0 and 3045.0 g of chlormequat chloride /ha) volume of deionized water used to prepare the highest rate corresponded 300 L water/ha

Test conditions: temperature: 16.7 – 23.7°C
 Humidity: 48.8 – 93.7%
 Lighting: 16 h light : 8 h dark; light intensity: 90.0 – 149.8 µE/m²/s
 Carbon dioxide concentration: 318 – 356 ppm

Statistical analysis: ER₁₀, ER₂₅, ER₅₀ – probit analysis, logit analysis, the 4-parameter logistic.

In order to determine the NOER value for the plant number at the end of the experiment any computations had been performed because of no change in mortality of plants.

In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:

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

Endpoints: ER₁₀, ER₂₅, ER₅₀, NOER

Results and Conclusions

The results are summarized in the table below.

Table 1. Endpoint values – the impact of the Chlormequat chloride 72% SL on vegetative vigour of the plants tested based on test item concentration.

Endpoint value	Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment						
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	g/ha	≥4200.0	≥4200.0	≥4200.0	≥4200.0	≥4200.0
Shoot length (plants without roots)						
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	g/ha	≥4200.0	≥4200.0	155.6	≥4200.0	≥4200.0
Plant dry weight (plants without roots)						
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	g/ha	≥4200.0	≥4200.0	155.6	466.7	≥4200.0

Table 2. Endpoint values – the impact of the Chlormequat chloride 72% SL on vegetative vigour of the plants tested based on active substance concentration.

Endpoint value	Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment						
ER ₅₀	g/ha	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	g/ha	≥3045.0	≥3045.0	≥3045.0	≥3045.0	≥3045.0
Shoot length (plants without roots)						
ER ₅₀	g/ha	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0

NOER	g/ha	≥3045.0	≥3045.0	≥3045.0	112.8	≥3045.0	≥3045.0
Plant dry weight (plants without roots)							
ER₅₀	g/ha	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	g/ha	≥3045.0	≥3045.0	≥3045.0	112.8	338.3	≥3045.0

On the basis of the obtained results it was proved that the test item i.e. Chlormequat Chloride 72% SL had no influence on the plant number of the tested plant species at the end of the experiment.

On the basis of the obtained results it was proved that the test item i.e. Chlormequat Chloride 72% SL had no influence on the shoot length of sunflower, cabagge, pea, onion and oats at the end of the experiment.

On the basis of the obtained results it was proved that the test item i.e. Chlormequat Chloride 72% SL had influence on the shoot length of carrot.

On the basis of the obtained results it was proved that the test item i.e. Chlormequat Chloride 72% SL had no influence on the shoot dry weight of sunflower, cabagge, pea and oats at the end of the experiment.

On the basis of the obtained results it was proved that the test item i.e. Chlormequat Chloride 72% SL had influence on the shoot dry of carrot and onion.

The phytotoxic symptoms in cultivation of sunflower, pea, cabbage, carrot, onion and oats were not observed.

Validity criteria

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Chlormequat chloride 72% SL on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
 - 95.2 – 100.0 – sunflower
 - 95.2 – 100.0 – cabbage
 - 92.9 – 100.0 – pea
 - 90.0 – 100.0 – carrot
 - 90.0 – 100.0 – onion
 - 95.0 – 100.0 – oats
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%)
- the control plants did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Comments of zRMS:	<p>The study is accepted by RMS.</p> <p>Validity criteria are met: On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CLARA on seedling emergence and seedling growth of terrestrial plants were met: - the seedling emergence in the control (validity criterion: at least 70%) was as follows: 95.2% – pea, 100.0% – cabbage, 100.0% – carrot, 100% – sunflower, 100.0% – onion, 100.0% – oats, - the mean survival of the emerged control seedlings was 100% for all tested spe-</p>
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cies (validity criterion: at least 90%);
 - the control seedlings did not exhibit any visible phytotoxic effects;
 - environmental conditions for all plants of the same species were identical.

Deviations from OECD Guideline No. 208:

According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 73.17 and $191.6 \mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigor was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.

Agreed endpoints:

Toxicity endpoints expressed as mL of the test item CLARA/ha

	Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment						
ER ₅₀	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	≥4200.0	≥4200.0	≥4200.0	≥4200.0	≥4200.0	≥4200.0
Shoot length (plants without roots)						
ER ₅₀	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	≥4200.0	≥4200.0	1400.0	≥4200.0	≥4200.0	≥4200.0
Plant dry weight (plants without roots)						
ER ₅₀	>4200.0	>4200.0	>4200.0	4098.1 (3023.5 - >4200.0)	>4200.0	>4200.0
NOER	1400.0	≥4200.0	1400.0	1400.0	≥4200.0	1400.0

Toxicity endpoints expressed as g chlormequat chloride/ha

	Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment						
ER ₅₀	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	≥3045.0	≥3045.0	≥3045.0	≥3045.0	≥3045.0	≥3045.0
Shoot length (plants without roots)						
ER ₅₀	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	≥3045.0	≥3045.0	1015.0	≥3045.0	≥3045.0	≥3045.0
Plant dry weight (plants without roots)						
ER ₅₀	>3045.0	>3045.0	>3045.0	2971.1 (2192.0 - >3045.0)	>3045.0	>3045.0
NOER	1015.0	≥3045.0	1015.0	1015.0	≥3045.0	1015.0

Phytotoxicity effect:

The experiment finished 14 days after the emergence of 50% of the control seedlings. During the experiment, the plants were observed for emergence (every day

	and then every 2 – 3 days) and visual phytotoxicity (7 and 14 days after the emergence of 50% of the control seedlings). During the experiment the stunted growth was observed for sunflower plants. The damage did not exceed 10% in comparison for the control group. The damage was observed only for the highest application rate.
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Reference:	KCP 10.6.2-02
Report	“Chlormequat Chloride 72% SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test”. Aneta Gierbuszewska, MSc., 2020. Study No: G/199/17. Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna.
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	Yes. According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 73.17 and 191.6 $\mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Chlormequat Chloride 72% SL batch number: SCL – 23170 active substances: Chlormequat Chloride – 72.5% (w/v)
Test species:	Pea (<i>Pisum sativum</i>), cabbage (<i>Brassica oleracea var. capitata</i>), carrot (<i>Daucus carota</i>), sunflower (<i>Helianthus annuus</i>), onion (<i>Allium cepa</i>), oats (<i>Avena sativa</i>).
Soil:	Sandy loam
Study design:	number of rates: 8 + control; number of replicates/rate: 4 (carrot, onion, oats) or 7 (sunflower, cabbage, pea). The total number of seeds per application rate – 20 (carrot, onion, oats) or 21 (cabbage, pea, sunflower) Test termination: 14 days after the emergence of 50% of the control seedlings
Application rates:	a control, 4200.0, 1400.0, 466.7, 155.6, 51.9, 17.3, 5.8 and 1.9 mL test item/ha (i.e. 3045.0, 1015.0, 338.3, 112.8, 37.6, 12.5, 4.2 and 1.4 chlormequat chloride/ha) volume of deionized water used to prepare the highest rate corresponded 300 L water/ha
Test conditions:	temperature: 22.7 – 25.4 °C humidity: 42.8 – 62.2% lighting: 16 h light : 8 h dark light intensity: 73.17 – 191.6 $\mu\text{E}/\text{m}^2/\text{s}$ carbon dioxide concentration: 345 – 393 ppm

Statistical analysis: ER₁₀, ER₂₅, ER₅₀ –probit analysis,
In order to determine the NOER values for the emergence the following statistical tests were used:
Fisher’s Exact Binomial Test with Bonferroni Correction.
In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:
Shapiro-Wilk’s Test on Normal Distribution, Levene’s Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Endpoints: ER₁₀, ER₂₅, ER₅₀, NOER

Results and Conclusions

The results are summarized in the table below.

Table 1. Endpoint values – the impact of the Chlormequat chloride 72% SL on seedling emergence and seedling growth of the plants tested based on test item concentration.

Endpoint value		Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment							
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	g/ha	≥4200.0	≥4200.0	≥4200.0	≥4200.0	≥4200.0	≥4200.0
Shoot length (plants without roots)							
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0	>4200.0
NOER	g/ha	≥4200.0	≥4200.0	1400.0	≥4200.0	≥4200.0	≥4200.0
Plant dry weight (plants without roots)							
ER ₅₀	g/ha	>4200.0	>4200.0	>4200.0	4098.1 (3023.5 - >4200.0)	>4200.0	>4200.0
NOER	g/ha	1400.0	≥4200.0	1400.0	1400.0	≥4200.0	1400.0

Table 2. Endpoint values – the impact of the Chlormequat chloride 72% SL on seedling emergence and seedling growth of the plants tested based on active substance concentration.

Endpoint value		Pea <i>Pisum sativum</i>	Cabbage <i>Brassica oleracea var. capitata</i>	Carrot <i>Daucus carota</i>	Sunflower <i>Helianthus annuus</i>	Onion <i>Allium cepa</i>	Oats <i>Avena sativa</i>
Plant number at the end of the experiment							
ER ₅₀	g/ha	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	g/ha	≥3045.0	≥3045.0	≥3045.0	≥3045.0	≥3045.0	≥3045.0
Shoot length (plants without roots)							
ER ₅₀	g/ha	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0	>3045.0
NOER	g/ha	≥3045.0	≥3045.0	1015.0	≥3045.0	≥3045.0	≥3045.0
Plant dry weight (plants without roots)							
ER ₅₀	g/ha	>3045.0	>3045.0	>3045.0	2971.1 (2192.0 - >3045.0)	>3045.0	>3045.0
NOER	g/ha	1015.0	≥3045.0	1015.0	1015.0	≥3045.0	1015.0

The test item i.e. Chlormequat Chloride 72% SL had no significant impact on the seedling emergence of the test plant species. Seedling emergence of all tested species was not delayed in comparison to the control group. The death of plants was not observed, except for accidental death of one onion plant and two carrot plants at the highest application rate i.e. 4200.0 mL/ha. ER₁₀, ER₂₅, ER₅₀ and NOER values determined from the final number of plants proved that the test item did not inhibit the seedling emergence of all tested plant species.

On the basis of NOER and ER₁₀, ER₂₅, ER₅₀ determined from the dry shoot length it was proved that the test item slightly inhibited the process of growth of carrot and had no influence on the other test species.

On the basis of NOER and ER₁₀, ER₂₅, ER₅₀ determined from the dry shoot weight it was proved that the test item did not inhibited the process of growth of cabbage and onion. The test item slightly inhibited the process of growth of pea, oats and carrot. On the basis of dry weight results, sunflower plants proved to be the most sensitive among tested species.

During the experiment the stunted growth was observed for sunflower plants. The damage did not exceed 10% in comparison for the control group. The damage was observed only for the highest application rate. The following order of the test plant sensitivity was noticed:
sunflower > pea > carrot > oats > cabbage, onion

Validity criteria

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of **Chlormequat chloride 72% SL** on seedling emergence and seedling growth of terrestrial plants were met:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
 - 95.2% – pea,
 - 100.0% – cabbage
 - 100.0% – carrot
 - 100% – sunflower
 - 100.0% – onion
 - 100.0% – oats
- the mean survival of the emerged control seedlings was 100% for all tested species (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic effects.
- environmental conditions for all plants of the same species were identical.

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

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

A 2.8 KCP 10.8 Monitoring data