

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: 3AEY

Product name(s): Mevalone

Chemical active substances:

Eugenol 33 g/L

Geraniol 66 g/L

Thymol 66 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Eden Research plc

Submission date: 15/07/2021

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MS Finalisation date: July 2022 (initial Core Assessment)

November 2022 (final Core Assessment)

Version history

When	What
July 2021	Authorization of marketing in Central Zone of the plant protection product Mevalone on grapes and pome fruits
December 2021	Update of the GAP table
March 2022	Update of the GAP table due to typographical error
May 2022	Update of risk assessment due to surface water exposure
July 2022	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency .
November 2022	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is struck through and shaded .

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9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicological studies for the product Mevalone (3AEY) containing the active substances eugenol, geraniol and thymol, which were evaluated under Directive 91/414/EEC and approved under Commission Implementing Regulations (EU) 546/2013 of 14 June 2013, 570/2013 of 17 June 2013 and 568/2013 of 18 June 2013 respectively. In accordance with Regulation (EC) No 1107/2009 active substances included on Annex I of Council Directive 91/414/EEC of July 1991 concerning the placing of plant protection products on the market are deemed to be approved under Regulation 540/2011.

Where appropriate this document refers to the conclusions of the EU reviews of eugenol, geraniol and thymol. This will be where:

- the active substance data is relied upon in the risk assessment of the formulation; or when
- the EU review concluded that additional data/information should be considered at national re-registration.

Note: this Part B document only reviews data (active substance or product) and additional information that has not previously been considered within the EU review process, as part of the first EU review of eugenol, geraniol and thymol. New active substance data are only included if they are considered essential for the evaluation and in this case a full study summary is provided. Note, in some cases these new data include studies that have been submitted as part of the active substance renewal dossiers, submitted in February 2021, and currently under EU review. However, it is intended that this product registration is evaluated prior to the EU renewal of the active substances; existing EU-agreed endpoints therefore apply, unless further justification has been provided.

This product was the representative formulation for evaluation in the EU review of eugenol, geraniol and thymol. The product has also previously been evaluated in Southern Europe according to Uniform Principles and is authorised in Southern Europe for use on grapes.

Eugenol:

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on eugenol (SANCO/10577/2013 rev 3, 17 May 2013), the Conclusion on the peer review of the pesticide risk assessment of the active substance eugenol (EFSA Journal 2012;10(11):2914) and Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for eugenol in light of confirmatory data (EFSA Supporting publication 2017:EN-1165) shall be taken into account.

Geraniol:

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on geraniol (SANCO/10579/2013 rev 3, 17 May 2013), the Conclusion on the peer review of the pesticide risk assessment of the active substance geraniol (EFSA Journal 2012;10(11):2915) and Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for geraniol in light of confirmatory data (EFSA Supporting publication 2017:EN-1163) shall be taken into account.

Thymol:

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on thymol (SANCO/10581/2013 rev 3, 17 May 2013), the Conclusion on the peer review of the pesticide risk assessment of the active substance thymol (EFSA Journal 2012;10(11):2916) and Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for thymol in light of confirmatory data (EFSA Supporting publication 2017:EN-1162) shall be taken into account.

In this overall assessment:

In the review report for eugenol (SANCO/10577/2013) Member States shall pay particular attention to the:

- risk to aquatic organisms
- risk to insectivorous birds

In the review report for geraniol (SANCO/10579/2013) and thymol (SANCO/10581/2013) Member States shall pay particular attention to the:

- risk to aquatic organisms
- risk to birds and mammals

These concerns have been addressed within the current submission.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document details any new studies submitted for this evaluation.

Information on the detailed composition of Mevalone (3AEY) can be found in the confidential dossier of this submission (Registration Report - Part C).

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No.*	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g 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 arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Central Zone IE, GB, NL, BE, LU, DE, CZ, AT, SI, SK, HU, PL	Grape (<i>Vitis vinifera</i> VITVI)	F	Grey mould (<i>Botrytis cinerea</i> BOTRCI)	Foliar. Tractor- mounted air blast sprayer. Hand-held knapsack sprayer.	BBCH 60- 89	a) 1 b) 4	7	a) 1.6 – 4.0 L/ha b) 6.4 – 16 L/ha	a) 52.8 - 132 (E) 106 - 264 (G) 106 - 264 (T) b) 211 – 528 (E) 422 – 1056 (G) 422 – 1056 (T)	400-1000	7	The product is applied so that the concentration in g a.s./hL is kept constant at 13.2 (eugenol), 26.4 (geraniol), 26.4 (thymol) g a.s / hectolitre of spray water volume. Therefore, the higher application rate is diluted in the higher water volume. Apply at 3.0 - 3.2 L/ha LWA	C	C	R D3, D5, D6, R2, R3 A D4, R1, R4	C	A	A	A
2	Central Zone IE, GB, NL, BE, LU, DE, CZ, AT, SI, SK, HU, RO, PL	Apple <i>Malus domestica</i> MABSD, pear <i>Pyrus communis</i> PYUCO, quince <i>Cydonia oblonga</i> CYDOB, crab-apple	F	Post-harvest storage diseases	Foliar. Tractor- mounted air blast sprayer. Hand-held knapsack sprayer.	BBCH 75- 89	a) 1 b) 4	7	a) 2.4 – 4.0 L/ha b) 9.6 – 16 L/ha	a) 79.2- 132 (E) 158 - 264 (G) 158 - 264 (T) b) 317 – 528 (E) 634 – 1056 (G) 634 – 1056 (T)	600-1000	1	The product is applied so that the concentration in g a.s./hL is kept constant at 13.2 (eugenol), 26.4 (geraniol), 26.4 (thymol) g a.s / hectolitre of spray water	C	C	R All scenarios	C	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
		<i>Malus sylvestris</i> MABSY, loquat <i>Eryobotria japonica</i> EIOJA, medlar <i>Mespilus germanica</i> MSPGE, Nashi pear <i>Pyrus pyrifolia</i> var. <i>culta</i> PYUPC											volume. Therefore, the higher application rate is diluted in the higher water volume. Apply at 3.0 – 3.2 L/ha LWA Example or post-harvest storage diseases: <i>Phytophthora</i> spp. PHYTSP (mainly <i>P. cactorum</i> PHYTCC or <i>P. syringae</i> PHYTSY), <i>Alternaria</i> spp. ALTESP, <i>Botrytis cinerea</i> BOTRCI								

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

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

Explanation for column 15 – 21 “Conclusion”

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

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

9.1.1 Overall conclusions

zRMS comments:

Conclusions of the Applicant presented in this point were amended accordingly or changed entirely, depending on the outcome of the evaluation for particular groups of non-target species. Unlike in other points of this report, not agreed information provided by the Applicant has been removed instead of being struck through in order to present overall conclusions in a most transparent way.

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

Birds

Acceptable acute and long-term risk to birds is concluded at the screening step from the proposed uses of Mevalone in vineyards and pome fruit. The risk from secondary poisoning and drinking water is also considered to be acceptable.

It should be, however, noted that in absence of the EU agreed avian reproductive toxicity studies, the long-term risk assessment was performed with consideration of the surrogate LD₅₀/10 value and should be rather considered as illustrative. Nevertheless, in opinion of the zRMS, based on results of the performed calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of birds, no unacceptable risk to birds is anticipated from uses of Mevalone in line with the Central Zone GAP. Further evaluation will be performed once final and firm conclusions are taken at the EU level following the ongoing renewal process of all three active compounds.

Concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

Mammals

Acceptable acute and long-term risk to mammals is concluded at the screening step or first-tier for the proposed uses of Mevalone in vineyards and pome fruit. The risk from secondary poisoning and drinking water is also considered to be acceptable.

It should be, however, noted that in absence of the EU agreed mammalian reproductive toxicity studies for geraniol and thymol, the long-term risk assessment was performed with consideration of the provisional long-term toxicity endpoints derived by the zRMS with consideration of information available in the DAR (May 2011) for both active compounds. Nevertheless, in opinion of the zRMS, based on results of the performed calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of mammals, no unacceptable risk to mammals is anticipated from uses of Mevalone in line with the Central Zone GAP. Further evaluation will be performed once final and firm conclusions are taken at the EU level following the ongoing renewal process of all three active compounds.

Concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

An acceptable risk to aquatic organisms following the proposed uses of Mevalone (including the three active substances eugenol, geraniol and thymol) is concluded based on the available data when mitigation measures are considered. For the intended use orchards, the risk is acceptable with a vegetative filters strip of 10 m in scenarios D3, D4, D5, R1, R2 and R4 and 20 m in scenario R3. For the intended uses on

vines, the risk is acceptable considering a vegetative filter strip of 10 m in scenarios D3, D5, D6, R2 and R3. No risk mitigation measures are deemed necessary for uses in vines in scenarios D4, R1 and R4. Concerned Member States must decide on applicability of proposed mitigation measures in their countries.

It should be noted that due to measured concentrations of geraniol in aged test solutions being <LOD/LOQ, the evaluation performed for geraniol is provisional and further evaluation will be performed once decision on acceptability of the study is taken at the EU level following the ongoing renewal process.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation Mevalone, which was performed in line with the EU agreed methodology.

“The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk assessment conducted according to “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) indicated acceptable acute oral and contact risk to bees. The risk assessment conducted according to EFSA/2013/3295 indicated acceptable acute oral and contact risk to bees and acceptable risk *via* exposure of contaminated water. The Tier 1 chronic oral adult risk assessment indicated acceptable risk to bees for orchards (all intended BBCH stages) and vineyards at BBCH ≥ 70 . The Tier 1 chronic oral adult risk assessment is above the conservative trigger value of 0.03 only for the treated crop scenario in the intended uses on vineyards at BBCH 60-69. Due to the characteristics of the active substances (extremely short half-lives, high volatility and natural occurrence) and the low attractiveness of grapevines to bees for the collection of nectar, the chronic oral exposure to adult honey bees in treated vineyards at BBCH 60-69 is unlikely. Therefore, the intended uses in vineyards at BBCH 60-69 are also considered to be acceptable. However, this will have to be dealt with at the product authorisation by the CMS that performed bee risk assessment in line with EFSA (2013) at the national level, since at the zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and the off-field risk assessment indicated acceptable risk for non-target arthropods following the intended uses of product Mevalone in vineyards and orchards without the need for mitigation measures. All HQ values were below the trigger of 2 at Tier 1 risk assessment.

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

The risk to earthworms and *Folsomia candida* from exposure of product Mevalone was assessed and demonstrated to be acceptable when the maximum predicted concentration in soil was used. All TER_{LT} values were above the trigger of 5.

No significant effects (<25%) on soil microorganisms were shown for Mevalone at concentrations greater than the predicted maximum soil concentrations. Therefore, the risk to soil micro-organism was

considered to be acceptable.

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

The risk assessment for non-target plants was considered to be acceptable using the maximum application rate of Mevalone and the screening data reported in DAR. No adverse effects are expected at 4 L/ha x 4 applications of Mevalone. No risk mitigation measures are deemed necessary.

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

Further studies on other terrestrial organism are not required, as the risk to the standard organisms has been shown to be acceptable.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of Mevalone grouped according to crop type

Group	Intended uses	Maximum rate per application	Maximum number of applications	Minimum interval between applications	Maximum total rate per season
Vineyards	BBCH 60-89	4.0 L product/ha 4.12 kg product/ha* 132 g eugenol/ha 264 g geraniol/ha 264 g thymol/ha	4	7	16 L product/ha 16.5 kg product/ha* 528 g eugenol/ha 1056 g geraniol/ha 1056 g thymol/ha
Orchards	Pome fruit (BBCH 75-89)	4.0 L product/ha 4.12 kg product/ha* 132 g eugenol/ha 264 g geraniol/ha 264 g thymol/ha	4	7	16 L product/ha 16.5 kg product/ha* 528 g eugenol/ha 1056 g geraniol/ha 1056 g thymol/ha

* Based on Mevalone nominal density of 1.029 g/mL

zRMS comments:

zRMS agrees with grouping of the intended uses of Mevalone proposed in Table 9.1-2 above. The risk assessment for non-target species will be performed with consideration of the maximum intended application rate for each crop group.

9.1.3 Consideration of metabolites

The occurrence and risk from potentially ecotoxicologically relevant metabolites have been considered. No metabolites were identified at levels that would require evaluation for their effects on wildlife.

Methyleugenol is a relevant impurity present in eugenol. Its presence is limited to 0.1% in the active substance. Therefore, the levels present after application of the product would be 1/1000th of those predicted for eugenol. Its toxicity may be predicted to be comparable to that of eugenol, but using a default of 10 times more toxic would not result in any concerns due to the extremely low concentrations. No data on methyleugenol were presented in the DAR and none were requested in the Commission Review Report. No further consideration is required.

zRMS comments:

No relevant metabolites occurring at >5% in various environmental compartments were identified for geraniol and thymol.

With regard to eugenol, formation of relevant metabolite methyleugenol could not be excluded in the course of the EU review and the environmental risk assessment for this compound was identified as an issue that could be not finalised in EFSA Journal 2012;10(11):2914. In the text above the Applicant is not right claiming that no data on methyleugenol were requested in the Commission Review Report, since in the Review Report for eugenol (SANCO/10577/2013 rev 3 of May 2013) the following data gap relevant for the assessment in area of ecotoxicology was identified:

- data comparing natural background exposure situations of eugenol and methyl eugenol in relation to exposure from the use of eugenol as a plant protection product. This data shall cover human exposure as well as exposure of birds and aquatic organisms.

After Annex I inclusion additional data were generated and evaluated by the RMS in the Addendum for Confirmatory Data (2016). On the basis of the performed assessment it was concluded that methyleugenol is not formed in soil. Based on that no specific risk assessment for soil organisms is required. However, available data were not sufficient to establish the background concentration of methyleugenol in aquatic system and its formation

from uses as plant protection products. Taking this into account, potential exposure of aquatic organisms could not be excluded. The available data were also considered to be not sufficient to address the potential natural exposure of birds representative for feeding guilds other than frugivorous species..

The following conclusion is provided in the Addendum for Confirmatory Data (2016):

Overall, the RMS considers that the above confirmatory data requirements have been satisfactorily addressed, except with respect to establishing background exposure to birds and aquatic organisms

Furthermore, in EFSA Supporting publication 2017:EN-1165 it was concluded by EFSA that:

The available data are not sufficient to confirm the background exposure to methyl eugenol in grapes. EFSA noted that methyl eugenol is not formed in soil, but it was not excluded that it could be formed on plant materials after application of eugenol (targeted plant metabolism studies are not available). The confirmatory data requirements are not considered to have been addressed.

In the same document the RMS indicated that:

The relevance of methyl-eugenol to the risk assessment can be considered by individual member states based on the information in the confirmatory data evaluation. Open point – Issue to be dealt with at Member State level.

Since issue of potential exposure and risk assessment to methyleugenol was addressed neither during the first EU review, nor during the evaluation of the confirmatory data, respective assessment should have been provided by the Applicant within this submission. From the information provided above it seems that issue of methyleugenol was ignored by the Applicant, therefore respective discussion and risk assessments (if possible) will be performed by the zRMS in the points below. Nevertheless, the zRMS is of the opinion that such issues should be addressed at the EU level, since at the zonal level the evaluators should make use of the EU agreed endpoints and not generate new active substance data.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the product Mevalone containing the active substances eugenol, geraniol and thymol. Full details of these studies are provided in the respective EU DAR and related documents.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. For completeness, as a conservative worst-case a long-term risk assessment has also been conducted using the acute toxicity values LD₅₀/10 as a surrogate. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Northern bobwhite quail <i>Colinus virginianus</i>	Mevalone (3AEY)	Oral Acute	LD ₅₀ > 10000 mg product/kg bw (corresponding to > 320 mg eugenol/kg bw ^a ; corresponding to > 640 mg geraniol/kg bw ^a ; corresponding to > 640 mg thymol/kg bw ^a)	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916

^a Based on nominal active substance contents of 3.2% w/w eugenol; 6.4% w/w geraniol and 6.4% w/w thymol
 Endpoints in bold are used in the risk assessment

zRMS comments:

Avian toxicity data provided in Table 9.2-1 above are in line with EU agreed endpoint reported in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

No studies on reproductive toxicity of eugenol, geraniol and thymol were performed in the course of the EU review and a data gap in this area has been identified in EFSA reports for all three substances. However, all three active compounds were authorised for uses in plant protection products within the EU despite the fact that the long-term risk assessment could not be finalised and confirmatory data were not sufficient to demonstrate that application of Mevalone will not result with concentrations of eugenol, geraniol and thymol higher than the background exposure. Therefore, in opinion of the zRMS, responsibility for requesting submission of additional vertebrate studies should not be shifted to the zonal level, but should be dealt with during the ongoing renewal process, especially in the course of the peer-review it may be concluded that performance of the avian reproductive studies is not required based on WoE approach. In absence of the EU agreed avian reproductive toxicity data, at the zonal level the informative long-term risk assessment based on LD₅₀/10 values is considered acceptable until EU agreed vertebrate data become available.

Concentration of particular active compounds in Mevalone considered in conversion of the formulation endpoint to active compounds is in line with information provided in Part C of this submission. Endpoints expressed in term of the active compounds may be used for derivation of LD₅₀/10, to be used in the evaluation of the risk of secondary poisoning.

9.2.1.1 Justification for new endpoints

It is noted that a data gap for further information to address the long-term risk to birds was identified during the first EU review of eugenol, geraniol and thymol (EFSA Journal 2012; 10(11):2914; 2915; 2916). Further information on natural background levels of eugenol, geraniol and thymol was provided as confirmatory data, but this was not considered sufficient by EFSA to enable a comparison between the natural background exposure and the exposure due to the use of the plant protection product (EFSA Supporting publication 2017:EN-1165 ; EN-1163; EN-1162).

Eugenol, geraniol and thymol are naturally occurring terpene oils that can be found in a wide variety of plant species, fruit, foods and herbs.

Eugenol is an oil found in a wide variety of plant species from 0.02 to 180000 mg/kg, for blueberry and clove respectively (see eugenol Addendum – Confirmatory Data Table B.7.1.1).

Geraniol is found in a wide variety of fruits, vegetables, herbs and spices and can be found in concentrations varying between 30000 ppm to 1.26 ppm. Geraniol is also found in bergamot orange, carrot, coriander, lavender, lemon, lime, nutmeg, orange, rose, blueberry, blackberry and geranium (see geraniol Addendum – Confirmatory Data Table B.7.1).

Thymol is found in a variety of herbs and foods, particularly citrus fruit. Thymol is present in a variety of herbs including bergamot, thyme and crops such as blackberry, grapefruit, liquorice and celery seed oil. A comprehensive list of the concentrations of thymol in various edible plant species is presented in the thymol Addendum – Confirmatory Data Table B.7.1, with concentrations ranging from 1 mg/kg in the leaves of bitter orange to 24100 mg/kg in common thyme and 111000 mg/kg in lemon).

In accordance with Regulation (EU) No 283/2013, a test for the effects on reproduction in birds is currently requested if adult birds or nest sites are likely to be exposed during the breeding season. Following field application of the formulated product, Mevalone, initial environmental exposure of eugenol, geraniol and thymol will decline rapidly in relation to the applied dose due to volatilisation and degradation. It is observed that the DT₅₀ values in soil for eugenol, geraniol and thymol are less than one day. The DT₅₀ values in air obtained from the Atkinson model are 1.975 hours for eugenol, 0.713 hours for geraniol and 1.197 hours for thymol, respectively (see document B8, section 8.10, EFSA Journal 2012;10(11):2914; 2915; 2916 for details).

Consequently, the duration of exposure under typical conditions will be very limited, particularly in relation to background levels of eugenol, geraniol and thymol in the environment. This is also confirmed by the results of the residue trials conducted with Mevalone on grapevines and apples (please see section B7, Appendix 2, studies KCP 8.3/01 and KCP 8.3/02 for further details of 2020 trials). A total of 11 trials in grapes were conducted in Northern EU countries (Austria, Germany and Northern France) in 2006 and 2020. All 2020 trials were conducted according to the critical GAP to support the use of Mevalone in the CEU. In the 2020 season trials in grapes, residues of eugenol were not detected in the untreated control samples and not detected or detected up to 0.02 mg/kg in the treated samples. All residues of eugenol in grapes had declined to not detectable by 1 day after the last application.

In the 2020 season trials in grapes, residues of thymol were not detected or detected up to 0.01 mg/kg in the untreated control samples and not detected or detected up to 0.06 mg/kg in the treated samples. All residues of thymol in grapes had declined to the background levels found in the control samples by 7 days after the last application.

In the 2020 season trials in grapes, the mean residues of geraniol were below the limit of quantification (<0.01 mg/kg) to 0.04 mg/kg in the untreated control samples and <0.01 mg/kg to 0.07 mg/kg in the treated samples. All residues of geraniol in grapes had declined to the background levels found in the control samples by 7 days after the last application.

Furthermore, a total of 6 trials in apples were conducted in Northern EU countries (Austria, Germany and Northern France) in 2020 with an LOQ of 0.01 mg/kg. All trials were conducted according to the critical GAP to support the use of Mevalone in the CEU. No residues of eugenol and geraniol were detected at or above the LOQ of 0.01 mg/kg in any of the treated samples even on the day of the last application of Mevalone according to the critical GAP. Mean residues of thymol in the treated samples were <0.01 to 0.02 mg/kg on the day of application and not detected or below the LOQ of <0.01 mg/kg by 7 days after the last application of Mevalone according to the critical GAP.

This indicates that even the acute exposure will be significantly less than that estimated by the shortcut value (SV). There is thus a clear pattern of exposure with very low acute levels and very short duration so

that the long-term residue burden resulting from dietary exposure after application of Mevalone will be very limited.

Mevalone is of low acute toxicity to birds (please see Table 9.2-2). Two studies with the product Mevalone were conducted to address the acute and short-term toxicity. In the avian acute toxicity study with Mevalone and bobwhite quail (please see eugenol, geraniol and thymol DAR, Volume 3, Annex B.9, 2011, B.9.1.1.1) the acute oral LD₅₀ value was >10000 mg product/kg bw (corresponding to >320 mg eugenol/kg bw; >640 mg geraniol/kg bw; >640 mg thymol/kg bw, based on the nominal content of 3.2% w/w, 6.4% w/w and 6.4% w/w eugenol, geraniol and thymol respectively). In an 8-day dietary toxicity study (see eugenol, geraniol and thymol DAR, Volume 3, Annex B.9, 2011, B.9.1.2) with Mevalone there were no deaths or reductions in feed consumption or body weight at the maximum dose tested. The dietary LD₅₀ value was equivalent to 5866 mg product/kg bw/day (corresponding to > 187.7 mg eugenol/kg bw; >375.4 mg geraniol/kg; >375.4 mg thymol/kg based on the nominal content of 3.2% w/w, 6.4 w/w and 6.4 % w/w of eugenol, geraniol and thymol respectively).

Given the characteristics of the active substances, it is highly unlikely that the short-lived substances would result in any effects on reproduction. In the interests of minimising vertebrate testing, it is not justified to conduct a new reproductive avian toxicity study for active substances that are ubiquitous in the environment and degrade rapidly following application as a plant protection product, and of known low acute oral avian toxicity. As stated in Section 4.3 of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7 (12): 1438), the lowest of the acute LD₅₀/10 and the lowest NOAEL from avian reproduction studies should be used for the long-term screening assessment. Therefore, as a conservative worst-case a long-term risk assessment has been conducted using the acute toxicity values LD₅₀/10 as a surrogate. The long-term endpoints proposed are 32 mg/kg bw for eugenol and 64 mg/kg bw for geraniol and thymol (corresponding to 1000 mg product/kg bw).

zRMS comments:

As already indicated in point 9.2.1 above, the zRMS is not in the position to request additional studies on reproductive toxicity of the active compounds to any of the vertebrate species, since this issue – being a basic data requirement – should be dealt with at the EU level. However, despite obvious data gaps not resolved in the confirmatory data package, all three active compounds were authorised for uses in plant protection products within the EU with no restrictions resulting from not finalised long-term risk assessment for birds, mammals and aquatic species. Taking this into account, only provisional long-term risk assessment may be performed at the zonal level, at least until this issue is resolved in the course of the ongoing renewal process of all three active compounds.

In general, the zRMS agrees with discussion provided by the Applicant above. It should be noted that it was also agreed in the course of evaluation of Mevalone in the Southern Zone.

Eugenol, geraniol and thymol are natural essential oils found in various parts of multiple plant species which are constituents of the herbivorous and omnivorous birds diet. Although no specific trials investigating level of residues of particular compounds in plants stem and leaves were performed, the available public literature data clearly indicate that the natural concentrations of eugenol, methyl-eugenol, geraniol and thymol in plant tissues are in range of 0.02-180 000 ppm, 0-14 650 ppm, 1.26-30 000 ppm and 1.0-111 000 ppm, respectively. Although some concerns regarding the data base used to retrieve the information on the natural occurrence of the active substances in plant tissues were expressed by the RMS in the course of the confirmatory data evaluation, it has to be noted that the literature data rarely contain information sufficient for the proper validation. However, similar information on the background concentrations of eugenol, methyl-eugenol, geraniol and thymol may be found in publications of various authors and concentration of the essential oils (also other than compounds contained in Mevalone) in various plants were determined in multiple literature studies due to the common use of essential oils in alternative human and animal medicine. Taking this into account, the zRMS is of the opinion that high natural concentrations of eugenol, methyl-eugenol, geraniol and thymol in plants may be confirmed.

All considered compounds have high vapour pressure which will result with rapid volatilisation already directly after application. This was confirmed in multiple residue trials performed in grapes and apples in conditions representative for the Central and Southern Zone, where concentrations at 0 DAA (days after last application) were <LOD or <LOQ for eugenol and methyl-eugenol and from <LOD to 0.06 ppm for geraniol and thymol following application at 4x4.0 L Mevalone/ha. It has to be noted that in studies performed on grapes geraniol was also found at 0 DAT in controls at concentrations ranging from <LOD to 0.04 ppm, while in one trial thymol was found at <LOQ

at 7 DAA (although it was at <LOD at 0 DAA), which indicates that these compounds are naturally occurring in grapes. According to conclusions of the zRMS residue expert, performed trials demonstrated that application of Mevalone does not lead to increase of concentrations of eugenol, methyl-eugenol, geraniol and thymol over the natural background concentrations.

Taking into account physico-chemical properties of each compound it may be expected that lack of increase in concentrations of eugenol, methyl-eugenol, geraniol and thymol in grapes and apples after application of Mevalone is a result of rapid volatilisation of these compounds after treatment. This is confirmed in the EU agreed study by Kant (2008) in which release of eugenol, geraniol and thymol from the encapsulated formulation under conditions mimicking the various environmental conditions was investigated. Under dry conditions 60% of eugenol, 81% of geraniol and 79% of thymol was released into the atmosphere within 3 days. Under conditions with periodic wetting, moisture added every 24 hours over 4 days, 98% of eugenol and 100% of thymol and geraniol had been released into the atmosphere after four days (for details of the study, see e.g. Geraniol Vol. 3, B.8 of May 2011). It should be noted that such release was observed from the capsules, so it may be expected that volatilisation will be more rapid when the capsules will be dissolved during preparation of the spraying solutions in the tank.

Although the residue trials were performed on fruits, the rapid volatilisation is equally expected from other plant surfaces, such as leaves, leading to significant reduction of exposure after application of Mevalone.

Taking into account the high volatilisation rate as well as rapid degradation in the atmosphere (with DT_{50} ranging from 0.059 to 0.165 days) and soil ($DT_{50} < 1$ day for all compounds), long-term exposure of birds to eugenol, methyl-eugenol, geraniol and thymol is not expected. Furthermore, due to natural presence of eugenol, methyl-eugenol, geraniol and thymol at high concentrations in various plant species combined with results of residue trials and rapid volatilisation from plant surfaces it is not expected that application of Mevalone would lead to increase of residues of eugenol, methyl-eugenol, geraniol and thymol over the background concentrations.

This issue is expected to be discussed during the ongoing EU renewal process of all three compounds and further evaluation will be performed once final and firm conclusion is taken at the EU level. Until that time, informative long-term risk assessment based on $LD_{50}/10$ values is considered acceptable by the zRMS.

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).

The avian risk assessment has been carried out considering the critical GAP of four applications (7 day interval) which corresponds to 0.132 kg eugenol/ha, 0.264 kg geraniol/ha and 0.264 kg thymol/ha. For the risk assessment the risk envelope approach has been used. The application rate is the same for the intended uses vineyards and orchards, taking into account that the shortcut values for vineyards are higher than orchards, the risk envelope approach has been applied, and therefore the calculations with vineyards also cover the application in orchards (see 9.1.2).

A combined effects assessment according to Appendix B of the Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438) has not been performed since there are no available avian toxicity data for the individual active substances. However, as the avian risk assessment is based on an endpoint derived from a formulation study it can be considered that the presence of all three active substances has already been taken into account and no further combination toxicity assessment is therefore required.

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

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

As discussed above under Section 9.2.1.1, taking into account the short persistence of the compounds (DT_{50} in soil is 1 days) and their natural occurrence and volatility, long-term exposure to birds is not

expected. No avian reproductive toxicity data for Mevalone are available for the long-term risk assessment and further vertebrate testing is not justified. Therefore, as a conservative worst-case a long-term risk assessment has been conducted using the acute toxicity values LD₅₀/10 as a surrogate.

Table 9.2.2.1-1: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of Mevalone in vineyards and orchards (risk envelope)

Intended use		Vineyards (covering orchards due to higher SV)				
Active substance/product		Mevalone				
Application rate (g/ha)		4 x 4116 ^b g product/ha (7 day interval)				
Acute toxicity (mg/kg bw)		> 10000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Vineyards	Small omnivorous bird	95.3	1.8	707	>14	
Reprod. toxicity (mg/kg bw/d)		>1000 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Vineyards	Small omnivorous bird	38.9	2.2 x 0.53	187	>5.3	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

^b based on Mevalone nominal density of 1.029 g/mL

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

Table 9.2.2.1-2: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of eugenol in vineyards and orchards (risk envelope)

Intended use		Vineyards (covering orchards due to higher SV)				
Active substance/product		eugenol				
Application rate (g/ha)		4 x 132 g eugenol/ha (7 day interval)				
Acute toxicity (mg/kg bw)		> 320				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Vineyards	Small omnivorous bird	95.3	1.8	22.6	>14	
Reprod. toxicity (mg/kg bw/d)		>32 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Vineyards	Small omnivorous bird	38.9	2.2 x 0.53	5.98	>5.3	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

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

Table 9.2.2.1-3 Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of geraniol in vineyards and orchards (risk envelope)

Intended use		Vineyards (covering orchards due to higher SV)				
Active substance/product		geraniol				
Application rate (g/ha)		4 x 264 g geraniol/ha (7 day interval)				
Acute toxicity (mg/kg bw)		> 640				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Vineyards	Small omnivorous bird	95.3	1.8	45.3	>14	
Reprod. toxicity (mg/kg bw/d)		>64 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m ×	DDD_m (mg/kg bw/d)	TER_t	
Vineyards	Small omnivorous bird	38.9	2.2 x 0.53	12.0	>5.3	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

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

Table 9.2.2.1-4: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of thymol in vineyards and orchards (risk envelope)

Intended use		Vineyards (covering orchards due to higher SV)				
Active substance/product		thymol				
Application rate (g/ha)		4 x 264 g thymol/ha (7 day interval)				
Acute toxicity (mg/kg bw)		> 640				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Vineyards	Small omnivorous bird	95.3	1.8	45.3	>14	
Reprod. toxicity (mg/kg bw/d)		>64 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m ×	DDD_m (mg/kg bw/d)	TER_t	
Vineyards	Small omnivorous bird	38.9	2.2 x 0.53	12.0	>5.3	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

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 screening step indicated an acceptable acute and long-term/reproductive risk to birds for the proposed uses of Mevalone in vineyards and pome fruit, even when considering conservative worst-case surrogate reproductive endpoints. No further assessment is required.

zRMS comments:

The risk assessment for birds presented in Tables 9.2.2.1-1 to 9.2.2.1-4 is agreed by the zRMS. Calculations performed for vineyards are also protective for orchards due to higher SV values defined in EFSA (2009) for vineyards.

Separate calculations for particular active compounds were not necessary, since they were based on formulation endpoints expressed in terms of particular active compounds and application rate for each compound calculated proportionally from the formulation rate. Hence it could be expected that the TER values will be the same as these calculated for the formulated product.

It is noted that due to significant volatilisation of eugenol, geraniol and thymol already within first hours after application, the predicted acute and long-term dietary exposure of birds is considered to be highly conservative and exaggerated.

It should be pointed out that the reproductive risk assessment is only illustrative, since in absence of the

reproductive toxicity data it was based on LD₅₀/10 value, which was agreed by the zRMS (for discussion regarding this issue, please refer to zRMS comments in points 9.2.1 and 9.2.1.1 above).

No separate risk assessment was performed for methyl-eugenol, however according to Part C this compound is considered to be a relevant impurity, naturally present in eugenol and not formed during the formulation processes or product storage. Taking this into account, this compound was also present in the formulated product used in the acute toxicity study. Furthermore, according to the available literature data, methyl-eugenol is present in plant tissues at concentrations ranging from 0 to 14 650 ppm. Taking this into account, the zRMS is of the opinion that the risk assessment is also protective for this impurity. Nevertheless, further assessment may be deemed necessary once relevant data become available after the EU renewal process of eugenol.

Since evaluation was based on formulation toxicity data, the combined risk is considered to be covered.

Overall, based on results of the above calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of birds, no unacceptable risk to birds is anticipated from uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on some theoretical assumptions, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.2.2.2 Higher-tier risk assessment

The risk assessments presented above concluded acceptable risk to birds at the screening step. Therefore, no further studies or assessments are considered to be necessary.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since Mevalone 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 is not relevant.

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} ≥ 500 L/kg).

The application rate of 4.12 kg product/ha and MAF have been used in the calculations supposing no degradation between applications and K_{oc} < 500 L/kg.

Table 9.2.2.3-1: Screening step for the drinking water assessment to birds due to the use of Mevalone

Effective application rate (g/ha)	for acute risk ^a =	7437.6		
Acute toxicity (mg/kg bw)	=	>10000	quotient =	0.7
Effective application rate (g/ha)	for long-term risk ^a =	9064		
Reprod. toxicity (mg/kg bw/d)	=	>1000 ^b	quotient =	9

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.2.2.3-2: Screening step for the drinking water assessment to birds due to the use of eugenol

Effective application rate (g/ha)	for acute risk ^a =	237.6		
Acute toxicity (mg/kg bw)	=	>320	quotient =	0.7
Effective application rate (g/ha)	for long-term risk ^a =	290		
Reprod. toxicity (mg/kg bw/d)	=	>32 ^b	quotient =	9

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.2.2.3-3: Screening step for the drinking water assessment to birds due to the use of geraniol

Effective application rate (g/ha)	for acute risk ^a =	475.2		
Acute toxicity (mg/kg bw)	=	>640	quotient =	0.7
Effective application rate (g/ha)	for long-term risk ^a =	581		
Reprod. toxicity (mg/kg bw/d)	=	>64 ^b	quotient =	9

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.2.2.3-4: Screening step for the drinking water assessment to birds due to the use of thymol

Effective application rate (g/ha)	for acute risk ^a =	475.2		
Acute toxicity (mg/kg bw)	=	>640	quotient =	0.7
Effective application rate (g/ha)	for long-term risk ^a =	581		
Reprod. toxicity (mg/kg bw/d)	=	>64 ^b	quotient =	9

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

The acute and long-term risks to birds from drinking water exposure is considered acceptable as the ratios of effective application rate to the relevant endpoint are well below the trigger of 50 for less sorptive substances. No further assessment is required.

zRMS comments:

The drinking water risk assessment for birds presented in Tables 9.2.2.3-1 to 9.2.2.3-4 is agreed by the zRMS.

For discussion on considered endpoints, please refer to zRMS comments in points 9.2.1 and 9.2.2.1.

It is noted that with the single application rate of 4116 g product/ha, the effective application rates would be 7409 and 9055 g product/ha when MAF of 1.8 and 2.2 is considered for the acute and long-term risk assessment, respectively. Nevertheless, as the rates considered by the Applicant are higher, no corrections were made by the zRMS in tables above.

Separate calculations for particular active compounds were not necessary, since they were based on formulation endpoints expressed in terms of particular active compounds and application rate for each compound calculated proportionally from the formulation rate. Hence it could be expected that the TER values will be the same as these calculated for the formulated product.

Overall, based on results of the above calculations, no unacceptable risk to birds from exposure via drinking water is anticipated following uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on some theoretical assumptions, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.2.2.4 Effects of secondary poisoning

According to the EFSA Guidance Document on Risk assessment for Birds and Mammals (2009), substances with a log P_{ow} value greater than 3 have a potential for bioaccumulation. The risk assessment for bioaccumulation is not required for eugenol because the log P_{ow} values for eugenol are 2.30-2.45 (EFSA Journal 2012; 10(11):2914) and 2.49 (pH = 7) obtained from the new study (see Part B5 of this

dRR, CA 4.1.2/25 2020a).

In the case of geraniol the log P_{ow} value is 3.8 ((EFSA Journal 2012; 10(11):2915) and 3.11 – 3.13 between pH 4-9 obtained from the new study (see Part B5 of this dRR, CA 4.1.2/47 2020b) and thus exceed the trigger value of 3, requiring consideration of the risk to earthworm-eating and fish-eating birds due to secondary poisoning. However, due to its rapid volatilisation properties and ready biodegradation it is considered unlikely that geraniol will be persistent and accumulate in soil or natural water systems. Since geraniol is of very low persistence in soil and water, accumulation in worms and fish and exposure of birds from consumption of these is considered unlikely. According to the EFSA Conclusion Report for geraniol (EFSA Journal 2012; 10(11):2915), for bioaccumulation “...it was considered that geraniol is unlikely to bioaccumulate in fish, and therefore no studies are required”.

In the case of thymol the log P_{ow} values are 3.97 (EFSA Journal 2012; 10(11):2916) and 3.41 - 3.44 between pH 4-9 obtained from the new study (see Part B5 of this dRR, CA 4.1.2/25 2020) and thus exceed the trigger value of 3, requiring consideration of the risk to earthworm-eating and fish-eating birds due to secondary poisoning. However, due to its rapid volatilisation properties and ready biodegradation it is considered unlikely that thymol will be persistent and accumulate in soil or natural water systems. Since thymol is of very low persistence in soil and water, accumulation in worms and fish and exposure of birds from consumption of these is considered unlikely. According to the EFSA Conclusion Report for thymol (EFSA Journal 2012; 10(11):2916), for bioaccumulation “...it was considered that bioaccumulation in fish is unlikely”.

Whilst bioconcentration of eugenol, geraniol and thymol in prey of birds and mammals is considered unlikely, for completeness conservative secondary poisoning assessments are presented below for geraniol and thymol (as log P_{ow} values are >3 for these two active substances). These risk assessments have used worst-case initial PEC values, worst-case surrogate reproductive endpoints of LD₅₀/10 and QSAR-estimated BCF_{fish} values. The adsorption values (Koc) used below are consistent with those used in document B8, point 8.5, tables 8.5.2 and 8.5.3.

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water. A risk envelope approach has been performed considering the worst case PEC_{soil} values for grapes and PEC_{sw} values for apples. The most recent P_{ow} values have been used for the risk assessment performed below.

Table 9.2.2.4-1: Assessment of the risk for earthworm-eating birds due to exposure to geraniol via bioaccumulation in earthworms (secondary poisoning) for the intended use of Mevalone in grapes

Parameter	Geraniol	comments
PEC _{soil} (mg/kg soil) 21-d	0.01 0.142	21-d TWA PEC _{soil} Worst case initial PEC _{soil} for grapes (multiple applications); Table 8.7.2-5 of Section B8.
log P_{ow} / P_{ow}	3.13 / 1349	Study CA 4.1.2/47 2020b
Koc	10 27	In absence of reliable sorption data, worst case default was considered Section B8, point 8.5, Table 8.5.2
foc	0.02	Default
BCF _{worm}	85.14 31.53	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.85 4.48	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.89 4.70	DDD = $PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	>64	conservative worst-case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _{tt}	71.9 >13.6	i.e. above the trigger of 5

Table 9.2.2.4-2: Assessment of the risk for fish-eating birds due to exposure to geraniol via bioaccumulation in fish (secondary poisoning) for the intended use of Mevalone in apples

Parameter	Geraniol	comments
PEC _{sw} (mg/L)	0.40272	Worst case initial PEC _{sw} for apples (FOCUS STEP 1); Section B8, point 8.9, Table 8.9-15.
BCF _{fish} (L/kg wet wt)	53.97	Estimated using log P _{ow} of 3.13 and the BCFBAF (v3.01) programme of US EPA EPI Suite (Estimation Programs Interface Suite™ for Microsoft® Windows).
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	21.73	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	3.46	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	≥64	conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _{it}	≥18.5	i.e. above the trigger of 5

Table 9.2.2.4-3: Assessment of the risk for earthworm-eating birds due to exposure to thymol via bioaccumulation in earthworms (secondary poisoning) for the intended use of Mevalone in grapes

Parameter	Thymol	comments
PEC _{soil} (mg/kg soil)	0.01 0.142	21-d TWA PEC _{soil} Worst case initial PEC _{soil} for grapes (multiple applications); Table 8.7.2-7 of Section B8.
log P _{ow} / P _{ow}	3.44 / 2754	Study CA 4.1.2/25 2020
Koc	10 190	In absence of reliable sorption data, worst case default was considered Section B8, point 8.5, Table 8.5-3
foc	0.02	Default
BCF _{worm}	169.44 8.92	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) = (0.84 + 0.012 × P _{ow}) / foc × Koc
PEC _{worm}	1.69 1.27	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	1.78 1.33	DDD = PEC _{worm} × 1.05
NOEL (mg/kg bw/d)	≥64	conservative worst-case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _{it}	36.0 >48.1	i.e. above the trigger of 5

Table 9.2.2.4-4: Assessment of the risk for fish-eating birds due to exposure to thymol via bioaccumulation in fish (secondary poisoning) for the intended use of Mevalone in apples

Parameter	Thymol	comments
PEC _{sw} (mg/L)	0.40272	Worst case max PEC _{sw} for apples (FOCUS STEP 1); Section B8, point 8.9, Table 8.9-26
BCF _{fish} (L/kg wet wt)	86.44	Estimated using log P _{ow} of 3.44 and the BCFBAF (v3.01) programme of US EPA EPI Suite (Estimation Programs Interface Suite™ for Microsoft® Windows).
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	34.8	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	5.53	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	≥64	conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _{it}	≥11.6	i.e. above the trigger of 5

Even when considering the worst-case parameters (initial/max PEC values, worst-case surrogate reproductive endpoints of LD₅₀/10, QSAR-estimated BCF_{fish} values) in the conservative risk assessments above, all the TER_{it} values are well above the trigger of 5, concluding acceptable risks to earthworm-eating and fish-eating birds due to secondary poisoning following the proposed uses of Mevalone. No further data or assessments are considered necessary.

zRMS comments:

Since no reliable Kfoc values were available for geraniol and thymol following evaluation of the confirmatory data in August 2016, the evaluation of the risk of secondary poisoning for earthworms-eating birds was amended by the zRMS with consideration of the worst case default Kfoc of 10 mL/g, as agreed in area of Section 8 for groundwater modelling. However, the 21-d TWA PEC_{SOIL} was considered more relevant than initial PEC_{SOIL} to calculate the PEC_{WORM}.

Evaluation of the risk of secondary poisoning to fish-eating birds could not be validated since the BCF values estimated using QSAR were not EU agreed and no details of performed calculations were provided by the Applicant. Calculations presented in Tables 9.2.2.4-2 and 9.2.2.4-4 were thus struck through. Nevertheless, according to conclusions taken in the course of the EU review, neither geraniol nor thymol are expected to accumulate in fish tissues and for this reason secondary exposure of birds via fish is unlikely.

Overall, no unacceptable risk of secondary poisoning is anticipated following uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on some theoretical assumptions, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

Acceptable acute and long-term risk to birds is concluded at the screening step from the proposed uses of Mevalone in vineyards and pome fruit, ~~even when considering conservative worst case surrogate reproductive endpoints~~. The risk from secondary poisoning and drinking water is also considered to be acceptable.

It should be, however, noted that in absence of the EU agreed avian reproductive toxicity studies, the long-term risk assessment was performed with consideration of the surrogate LD₅₀/10 value and should be rather considered as illustrative. Nevertheless, in opinion of the zRMS, based on results of the performed calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of birds, no unacceptable risk to birds is anticipated from uses of Mevalone in line with the Central Zone GAP. Further evaluation will be performed once final and firm conclusions are taken at the EU level following the ongoing renewal process of all three active compounds.

Concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

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 the active substances eugenol, geraniol and thymol. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of Mevalone were also evaluated as part of the EU assessment of eugenol, geraniol and thymol. Full details of these studies are provided in the respective EU DAR and related documents.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. For completeness, as a conservative worst-case a long-term risk assessment for geraniol and thymol has also been conducted using the acute toxicity values LD₅₀/10 as a surrogate. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Rat	Mevalone	Oral Acute	LD ₅₀ > 2000 mg product/kg bw	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
Rat	Eugenol	Oral Acute	LD ₅₀ = 1930 mg eugenol/kg bw	EFSA Journal 2012;10(11):2914
Rat	Geraniol	Oral Acute	LD ₅₀ = 3600 mg geraniol/kg bw	EFSA Journal 2012;10(11):2915
Rat	Thymol	Oral Acute	LD ₅₀ = 980 mg thymol/kg bw	EFSA Journal 2012;10(11):2916
Rat	Eugenol	Long-term Developmental toxicity	NOAEL = 250 mg eugenol/kg bw	EFSA Journal 2012;10(11):2914
Rat	Geraniol	Long-term Carcinogenicity study	NOAEL = 558 mg a.s./kg bw/d	National Toxicology program (1987), NIH Publication No 88-2508 Summarised in Geraniol Vol. 3, B.6 of May 2011
Rat	Thymol	Long-term Reproductive screening study (comparable with OECD 422); dosing via gavage	Offspring NOAEL = 40 mg thymol/kg bw/d ¹⁾ Reproduction NOAEL = 200 mg thymol/kg bw/d Parental NOAEL: 200 mg thymol/kg bw/d	Matsuura et al. (no date given) Summarised in Thymol Vol. 3, B.8 of May 2011

1) Based on slightly reduced pup weights (<10% in both sexes) and weight gain (15% for males and 10% for females) during lactation, statistically not significant
Endpoints in bold are used in the risk assessment

zRMS comments:

Mammalian toxicity data provided in Table 9.3-1 above are in line with EU agreed endpoint reported in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

According to EFSA conclusions, for geraniol and thymol no reproductive toxicity study with mammals were performed and for this reason no endpoints could be established. However, both, geraniol and thymol, were authorised for uses in plant protection products within the EU despite the fact that the long-term risk assessment could not be finalised and confirmatory data were not sufficient to demonstrate that application of Mevalone will not result with concentrations of eugenol, geraniol and thymol higher than the background exposure. In opinion of the zRMS, responsibility for requesting submission of additional vertebrate studies should not be shifted to the zonal level, but should be dealt with during the ongoing renewal process, especially in the course of the peer-review it

may be concluded that performance of the mammalian reproductive studies is not required based on WoE approach. However, the zRMS reviewed the monographs for both compounds and some data that could be used for derivation of the provisional long-term endpoints were found.

For geraniol, no reproductive or developmental toxicity studies were performed and it is not clear if any will be performed for purposes of the renewal process, since according to conclusions of the RMS toxicology expert, the ADI could be derived based on the information available in the data bases. However, purposes of the first EU review of geraniol 2 carcinogenicity studies were submitted. Although reproductive performance is not covered by these studies, the investigations include assessment of effects on reproductive organs and in opinion of the zRMS in case no data are available from the reproductive toxicity studies, the NOAEL from the carcinogenicity studies where animals were dosed with the active substance via gavage 5 days a week for 103 weeks may be used as surrogate until relevant endpoints are established in the course of the EU renewal process.

Two carcinogenicity studies were performed for geraniol – one with rats and one with mice. The study with mice was considered by the RMS as not reliable due to possible infections and inadvertent overdose, which might have significant impact on the test results. The study with rats was considered fully acceptable by the RMS with NOAEL of 558 mg a.s./kg bw/d based on reduced survival and reduced bodyweight at 1116 mg a.s./kg bw/d. The endpoint from rat carcinogenicity study will be used by the zRMS as surrogate long-term endpoint in the risk assessment for geraniol applied as Mevalone, noting that effects on reproductive performance were not investigated in this study. Further assessment will be performed in case new endpoints will be concluded in the course of the ongoing EU renewal process.

For thymol the screening reproduction study was performed (Matsuura et al., no date available) and is summarised and evaluated by the RMS in Thymol Vol. 3, B.6 of May 2011. Although the information in the test report was not complete and the study did not follow all recommendations of the respective guideline for 2-generation study, it was considered by the RMS as sufficiently robust to be considered as supportive information. In the study thymol was administered to rats by oral gavage at doses of 0, 8, 40 and 200 mg a.s./kg bw/d. The test item had no effects on bodyweight, food consumption or reproductive parameters up to the highest dose tested of 200 mg a.s./kg bw/d. The NOAEL for general toxicity was set to 8.0 mg a.s./kg bw/d based on effects on forestomach at 40 and 200 mg a.s./kg bw/d, especially in males. Based on the necropsy findings these effects were related to the irritant effect of the high concentration of thymol in the upper GI tract. It should be noted that this effect would be not observed in case thymol was offered in the diet, which is the recommended route of administration of the test item in generational studies. Effects on stomach could be expected after repeated gavage dosing of males with high concentration of irritant essential oil over 43 days (females dosing was shorter and also effects on stomach were less pronounced). Taking this into account the zRMS is of the opinion that the NOAEL of 8.0 mg a.s./kg bw/d for general toxicity is ecotoxicologically not relevant, since wild mammals will be exposed to rather low concentrations in the diet.

The pup weight and weight gain were slightly reduced at 200 mg a.s./kg bw/d and the NOAEL was set to 40 mg a.s./kg bw/d. However, effects were statistically not significant. It should be noted that in several expert meetings in area of ecotoxicology effect on bodyweight gain were considered as not relevant for derivation of the ecotoxicologically relevant endpoint. Reduction of bodyweight at 200 mg a.s./kg bw/d was <10% and is thus considered ecotoxicologically not relevant. Overall, the zRMS is of the opinion that the reproductive NOAEL of 200 mg a.s./kg bw/d is relevant for purposes of the risk assessment until new endpoints are established in the course of the ongoing EU renewal process. It is not clear why results of this study were not considered in the course of the EU review of thymol, since the study was accepted by the RMS toxicology expert and could be thus considered in area of ecotoxicology.

9.3.1.1 Justification for new endpoints

It is noted that a data gap for further information to address the long-term risk to mammals was identified during the first EU review of eugenol, geraniol and thymol (EFSA Journal 2012; 10(11):2914; 2915; 2916). Further information on natural background levels of eugenol, geraniol and thymol was provided as confirmatory data, but this was not considered sufficient by EFSA to enable a comparison between the natural background exposure and the exposure due to the use of the plant protection product (EFSA Supporting publication 2017:EN-1165 ; EN-1163; EN-1162).

One developmental toxicity study in the rat study was previously evaluated as part of the EU review for the EU inclusion of eugenol (DAR, Volume 3, Annex B.6, 2011, B.6.6.2). The NOAEL value for rat of 250 mg eugenol/kg bw is considered valid for use in the ecotoxicological risk assessment (EFSA Journal 2012;10(11):2914). No further studies are considered necessary for eugenol.

Geraniol and thymol are naturally occurring terpene oils that can be found in a wide variety of plant species, fruit, foods and herbs.

Geraniol is found in a wide variety of fruits, vegetables, herbs and spices and can be found in concentrations varying between 30000 ppm to 1.26 ppm. Geraniol is also found in bergamot orange, carrot, coriander, lavender, lemon, lime, nutmeg, orange, rose, blueberry, blackberry and geranium (please, see geraniol Addendum – Confirmatory Data Table B.7.1).

Thymol is found in a variety of herbs and foods, particularly citrus fruit. Thymol is present in a variety of herbs including bergamot, thyme and crops such as blackberry, grapefruit, liquorice and celery seed oil. A comprehensive list of the concentrations of thymol in various edible plant species is presented in the thymol Addendum – Confirmatory Data Table B.7.1, with concentrations ranging from 1 mg/kg in the leaves of bitter orange to 24100 mg/kg in common thyme and 111000 mg/kg in lemon).

In accordance with Regulation (EU) No 283/2013, a test for the effects on reproduction in mammals is currently requested if adult mammals are likely to be exposed during the breeding season. Following field application of the representative formulated product, Mevalone, initial environmental exposure of geraniol and thymol will decline rapidly in relation to the applied dose due to volatilisation and degradation. It is observed that the DT₅₀ in soil for geraniol and thymol are less than one day. The DT₅₀ in air obtained from the Atkinson model is 0.713 hours for geraniol and the DT₅₀ in air obtained from the Atkinson model is 1.197 hours for thymol (please see document B8, section 8.10 and EFSA Journal 2012;10(11):2914; 2915 ; 2916 for details).

Consequently, the duration of exposure under typical conditions will be very limited, particularly in relation to background levels of eugenol, geraniol and thymol in the environment. This is also confirmed by the results of the residue trials conducted with Mevalone on grapevines and apples (please see section B7, Appendix 2, studies KCP 8.3/01 and KCP 8.3/02 for further details). A total of 11 trials in grapes were conducted in Northern EU countries (Austria, Germany and Northern France) in 2006 and 2020. All 2020 trials were conducted according to the critical GAP to support the use of Mevalone in the CEU. In the 2020 season trials in grapes, residues of eugenol were not detected in the untreated control samples and not detected or detected up to 0.02 mg/kg in the treated samples. All residues of eugenol in grapes had declined to not detectable by 1 day after the last application.

In the 2020 season trials in grapes, residues of thymol were not detected or detected up to 0.01 mg/kg in the untreated control samples and not detected or detected up to 0.06 mg/kg in the treated samples. All residues of thymol in grapes had declined to the background levels found in the control samples by 7 days after the last application.

In the 2020 season trials in grapes, the mean residues of geraniol were below the limit of quantification (<0.01 mg/kg) to 0.04 mg/kg in the untreated control samples and <0.01 mg/kg to 0.07 mg/kg in the treated samples. All residues of geraniol in grapes had declined to the background levels found in the control samples by 7 days after the last application.

Furthermore, a total of 6 trials in apples were conducted in Northern EU countries (Austria, Germany and Northern France) in 2020 with an LOQ of 0.01 mg/kg. All trials were conducted according to the critical GAP to support the use of Mevalone in the CEU. No residues of eugenol and geraniol were detected at or above the LOQ of 0.01 mg/kg in any of the treated samples even on the day of the last application of Mevalone according to the critical GAP. Mean residues of thymol in the treated samples were <0.01 to 0.02 mg/kg on the day of application and not detected or below the LOQ of <0.01 mg/kg by 7 days after the last application of Mevalone according to the critical GAP.

This indicates that even the acute exposure will be significantly less than that estimated by the shortcut value (SV). There is thus a clear pattern of exposure with very low acute levels and very short duration so that the long-term residue burden resulting from dietary exposure after application of Mevalone will be very limited.

The active substances geraniol and thymol are of low acute toxicity to mammals. Two acute oral toxicity studies were previously evaluated as part of the EU review for the EU inclusion of geraniol and thymol (DAR, Volume 3, Annex B.9, 2011, B.9.3.1). The acute oral LD₅₀ was 3600 mg geraniol/kg bw and the acute oral LD₅₀ was 980 mg thymol/kg b.w (please see Table 9.3-2).

Given the characteristics of the active substance, it is highly unlikely that such a short-lived substance would result in any effects on reproduction. In the interests of minimising vertebrate testing, it is not justified to conduct a new reproductive mammalian toxicity study for an active substance that is ubiquitous in the environment and degrades rapidly following application as a plant protection product, and is of known low acute oral mammalian toxicity. As stated in Section 4.3 of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7 (12): 1438), the lowest of the acute LD₅₀/10 and the lowest NOAEL from mammalian reproduction studies should be used for the long-term screening assessment. Therefore, in the absence of mammalian reproductive toxicity data the surrogate endpoint of LD₅₀/10 is considered an acceptable endpoint for the long-term screening risk assessment for geraniol and thymol. The NOAEL value for rat of 250 mg eugenol/kg bw is also considered valid for use in the ecotoxicological risk assessment. Therefore, no further studies on vertebrates are considered necessary.

zRMS comments:

As already indicated in point 9.3.1 above, the zRMS is not in the position to request additional studies on reproductive toxicity of the active compounds to any of the vertebrate species, since this issue – being a basic data requirement – should be dealt with at the EU level. However, despite obvious data gaps not resolved in the confirmatory data package, all three active compounds were authorised for uses in plant protection products within the EU with no restrictions resulting from not finalised long-term risk assessment for birds, mammals and aquatic species. Taking this into account, only provisional long-term risk assessment may be performed at the zonal level, at least until this issue is resolved in the course of the ongoing renewal process of all three active compounds.

In general, the zRMS agrees with discussion provided by the Applicant above. It should be noted that it was also agreed in the course of evaluation of Mevalone in the Southern Zone.

Eugenol, geraniol and thymol are natural essential oils found in various parts of multiple plant species which are constituents of the herbivorous and omnivorous mammals diet. Although no specific trials investigating level of residues of particular compounds in plants stem and leaves were performed, the available public literature data clearly indicate that the natural concentrations of eugenol, methyl-eugenol, geraniol and thymol in plant tissues are in range of 0.02-180 000 ppm, 0-14 650 ppm, 1.26-30 000 ppm and 1.0-111 000 ppm, respectively. Although some concerns regarding the data base used to retrieve the information on the natural occurrence of the active substances in plant tissues were expressed by the RMS in the course of the confirmatory data evaluation, it has to be noted that the literature data rarely contain information sufficient for the proper validation. However, similar information on the background concentrations of eugenol, methyl-eugenol, geraniol and thymol may be found in publications of various authors and concentration of the essential oils (also other than compounds contained in Mevalone) in various plants were determined in multiple literature studies due to the common use of essential oils in alternative human and animal medicine. Taking this into account, the zRMS is of the opinion that high natural concentrations of eugenol, methyl-eugenol, geraniol and thymol in plants may be confirmed.

All considered compounds have high vapour pressure which will result with rapid volatilisation already directly after application. This was confirmed in multiple residue trials performed in grapes and apples in conditions representative for the Central and Southern Zone, where concentrations at 0 DAA (days after last application) were <LOD or <LOQ for eugenol and methyl-eugenol and from <LOD to 0.06 ppm for geraniol and thymol following application at 4x4.0 L Mevalone/ha. It has to be noted that in studies performed on grapes geraniol was also found at 0 DAT in controls at concentrations ranging from <LOD to 0.04 ppm, while in one trial thymol was found at <LOQ at 7 DAA (although it was at <LOD at 0 DAA), which indicates that these compounds are naturally occurring in grapes. According to conclusions of the zRMS residue expert, performed trials demonstrated that application of Mevalone does not lead to increase of concentrations of eugenol, methyl-eugenol, geraniol and thymol over the natural background concentrations.

Taking into account physico-chemical properties of each compound it may be expected that lack of increase in concentrations of eugenol, methyl-eugenol, geraniol and thymol in grapes and apples after application of Mevalone is a result of rapid volatilisation of these compounds after treatment. This is confirmed in the EU agreed study by Kant (2008) in which release of eugenol, geraniol and thymol from the encapsulated formulation under conditions

mimicking the various environmental conditions was investigated. Under dry conditions 60% of eugenol, 81% of geraniol and 79% of thymol was released into the atmosphere within 3 days. Under conditions with periodic wetting, moisture added every 24 hours over 4 days, 98% of eugenol and 100% of thymol and geraniol had been released into the atmosphere after four days (for details of the study, see e.g. Geraniol Vol. 3, B.8 of May 2011). It should be noted that such release was observed from the capsules, so it may be expected that volatilisation will be more rapid when the capsules will be dissolved during preparation of the spraying solutions in the tank.

Although the residue trials were performed on fruits, the rapid volatilisation is equally expected from other plant surfaces, such as leaves, leading to significant reduction of exposure after application of Mevalone.

Taking into account the high volatilisation rate as well as rapid degradation in the atmosphere (with DT_{50} ranging from 0.059 to 0.165 days) and soil ($DT_{50} < 1$ day for all compounds), long-term exposure of mammals to eugenol, methyl-eugenol, geraniol and thymol is not expected. Furthermore, due to natural presence of eugenol, methyl-eugenol, geraniol and thymol at high concentrations in various plant species combined with results of residue trials and rapid volatilisation from plant surfaces it is not expected that application of Mevalone would lead to increase of residues of eugenol, methyl-eugenol, geraniol and thymol over the background concentrations.

This issue is expected to be discussed during the ongoing EU renewal process of all three compounds and further evaluation will be performed once final and firm conclusion is taken at the EU level. Until that time, the long-term risk assessment for geraniol and thymol will be performed with consideration of the provisional endpoints as discussed in the zRMS comment in point 9.3-1. For eugenol the EU agreed NOAEL from the developmental toxicity with rats is available.

The Applicants' proposal to use the $LD_{50}/10$ value is not agreed by the zRMS since in EFSA (2009) this approach is indicated as specific for birds only.

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).

The mammalian risk assessment has been carried out considering the critical GAP of four applications of Mevalone 4.12 kg/ha (7 day interval) in vineyards BBCH 60-89 and pome fruit BBCH 75-89, which corresponds to 0.132 kg eugenol/ha, 0.264 kg geraniol/ha and 0.264 kg thymol/ha (please, see table 9.1.2 for details).

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 9.3.2.1-1: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Mevalone in Orchard/vineyards

Intended use		Orchard/vineyards				
Active substance/product		Mevalone				
Application rate (g/ha)		4 × 4116 ^b g Mevalone/ha				
Acute toxicity (mg/kg bw)		>2000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening Step – Orchard and vineyards	Small herbivorous mammal	136.4	1.8	1012	>2.0	
Reprod. toxicity (mg/kg bw/d)		>200				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Screening Step – Orchard and vineyards	Small herbivorous mammal	72.3	2.2 × 0.53	187	>1.1	

^bBased on Mevalone nominal density of 1.029 g/mL

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

Table 9.3.2.1-2: Screening step for the acute and long-term/reproductive risk for mammals due to the use of eugenol in Orchard/vineyards

Intended use		Orchard/vineyards				
Active substance/product		eugenol				
Application rate (g/ha)		4 × 132 g eugenol/ha				
Acute toxicity (mg/kg bw)		1930				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening Step – Orchard and vineyards	Small herbivorous mammal	136.4	1.8	32.4	60	
Reprod. toxicity (mg/kg bw/d)		250				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Screening Step – Orchard and vineyards	Small herbivorous mammal	72.3	2.2 × 0.53	11.12	22	

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

Table 9.3.2.1-3: Screening step for the acute and long-term/reproductive risk for mammals due to the use of geraniol in vineyards

Intended use		Vineyards				
Active substance/product		geraniol				
Application rate (g/ha)		4 x 264 g thymol/ha (7 day interval)				
Acute toxicity (mg/kg bw)		3600				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening Step – Orchard and vineyards	Small herbivorous mammal	136.4	1.8	64.8	56	
Reprod. toxicity (mg/kg bw/d)		558 360 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Screening Step – Orchard and vineyards	Small herbivorous mammal	72.3	2.2 x 0.53	22.25	25.1 16	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

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

Table 9.3.2.1-4: Screening step for the acute and long-term/reproductive risk for mammals due to the use of thymol in vineyards

Intended use		Vineyards				
Active substance/product		thymol				
Application rate (g/ha)		4 x 264 g thymol/ha (7 day interval)				
Acute toxicity (mg/kg bw)		980				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening Step – Orchard and vineyards	Small herbivorous mammal	136.4	1.8	64.8	15	
Reprod. toxicity (mg/kg bw/d)		200 98 ^a				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t	
Screening Step – Orchard and vineyards	Small herbivorous mammal	72.3	2.2 x 0.53	22.25	9.0 4.4	

^a conservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

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 screening step indicated acceptable risk to mammals except for acute risk to the product Mevalone and the active substance thymol, and long term risk to the active substance thymol.

A Tier 1 acute risk assessment for Mevalone and thymol is provided below for the intended uses in vineyards and orchards.

Table 9.3.2.1-5: First tier assessment for the acute oral risk to mammals due to the use of Mevalone in vineyards

Intended use		Vineyards			
Product		eugenol+thymol+geraniol / Mevalone			
Application rate (g/ha)		4 × 4116 ^b (g prod/ha) (132 g eugenol/ha, 264 g geraniol/ha and 264 g thymol/ha)			
Acute toxicity (mg/kg bw)		>2000 mg Mevalone/kg bw			
TER criterion		10			
Crop scenario Growth stage	Generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg prod/kg bw)	TER _a
Application crop directed BBCH ≥ 40	Large herbivorous mammal “lagomorph”	8.1	1.8	60.1	>33
Application crop directed BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.8	40.1	>50
Application crop directed BBCH ≥ 40	Small herbivorous mammal “vole”	40.9	1.8	303.3	>6.6 ^a
Application crop directed BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.8	38.6	>52

^a Combined toxicity risk assessment, using Finney equation (Finney, 1942) indicates acute TER is 33 and an acute risk to mammals exposed to the active substance components of the formulation is unlikely.

^b Based on Mevalone nominal density of 1.029 g/mL

Table 9.36: First tier assessment for the acute oral risk to mammals due to the use of Mevalone in orchards

Intended use		Orchards			
Product		eugenol+thymol+geraniol / Mevalone			
Application rate (g/ha)		4 × 4116 ^c (g prod/ha) (132 g eugenol/ha, 264 g geraniol/ha and 264 g thymol/ha)			
Acute toxicity (mg/kg bw)		>2000 mg Mevalone/kg bw			
TER criterion		10			
Crop scenario Growth stage	Generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg prod/kg bw)	TER _a
Application crop directed BBCH ≥ 40	Small herbivorous mammal “vole”	40.9	1.8	303.3	>6.6 ^a
Fruit stage BBCH 71-79	Frugivorous mammal “dormouse”	47.9	1.8	355.2	>5.6 ^b
Application crop directed BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1.8	77.9	>26
Application crop directed BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.8	38.6	>52

^a Combined toxicity risk assessment, using Finney equation (Finney, 1942) indicates acute TER is 33 and an acute risk to mammals exposed to the active substance components of the formulation is unlikely.

^b Combined toxicity risk assessment, using Finney equation (Finney, 1942) indicates acute TER is 28 and an acute risk to mammals exposed to the active substance components of the formulation is unlikely.

^c Based on Mevalone nominal density of 1.029 g/mL

All the TER_A values at the screening step and the Tier 1 risk assessment are above the relevant trigger of 10 except for the small herbivorous mammal “vole” and frugivorous mammal “dormouse”. Therefore the risk assessments indicated a potential acute risk for the formulation Mevalone. It is observed that the risk is acceptable for the active substance data. Taking into account that the acute endpoint for Mevalone is a “greater than” value, with no mortalities observed at 2000 mg Mevalone/kg bw, an acute risk to herbivorous mammals to the formulation is unlikely. In addition, it is considered that mammals are unlikely to be exposed to Mevalone in their diet because following application the formulation will rapidly breakdown into its component active substances, thymol, geraniol and eugenol, which are all highly volatile. As discussed in the Final Addendum to the eugenol, geraniol and thymol DAR (2012), it should be noted that the active substances thymol, geraniol and eugenol are present in the formulation in low amounts of 6.4%, 6.4% and 3.2%, respectively. All active substances have low acute toxicity to mammals at 980, 3600 and 1930 mg a.s./kg bw, respectively. It is therefore considered that the true

LD₅₀ value for the formulation, based on the low active substance content, will be considerably greater than 2000 mg Mevalone/kg bw.

Since the acute formulation toxicity study did not derive an actual value for use as an endpoint (i.e. LD₅₀ >2000 mg Mevalone/kg bw), the combined toxicity of the three active substance components can be calculated using the Finney (1942) equation, as summarised in the table below.

Considering that the formulation is 3.2% eugenol, 6.40% geraniol and 6.4% thymol the LD₅₀ mixture is calculated with the Finney's equation.

$$LD_{50} \text{ mixture} = \left(\frac{1}{\left(\frac{0.032}{1930} \right) + \left(\frac{0.064}{3600} \right) + \left(\frac{0.064}{980} \right)} \right) = 10033.7 \text{ mg Mevalone/kg bw.}$$

As discussed above, the predicted toxicity to mammals exposed to the active substances in combination is lower than that indicated by the acute mammalian formulation study, where the measured toxicity was >2000 mg Mevalone/kg bw. If the predicted endpoint (10033.7 mg Mevalone/kg bw) is compared with the daily dietary dose for the formulation (DDD₉₀ 355.2 mg Mevalone/kg bw and DDD₉₀ 303.3 mg Mevalone/kg bw for “dormouse” and “vole” respectively), the resulting TER value is 28 for “dormouse” and the resulting TER value is 33 for vole, clearly indicating a low acute risk to mammals.

A Tier 1 long term risk assessment for thymol is provided below for the intended uses in vineyards and orchards:

Table 9.3.2.1-7: First tier assessment for the long term risk to mammals due to the use of thymol in vineyards

Intended use		Vineyards			
Active substance		thymol			
Application rate (g/ha)		4 × 264 g thymol/ha			
Acute toxicity (mg/kg bw)		98 ^a			
TER criterion		5			
Crop scenario Growth stage	Generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Application crop directed BBCH≥40	Large herbivorous mammal “lagomorph”	3.3	2.2 × 0.53	1.016	97
BBCH≥20	Small insectivorous mammal “shrew”	1.9	2.2 × 0.53	0.585	167
Application crop directed BBCH≥40	Small herbivorous mammal “vole”	21.7	2.2 × 0.53	6.680	15
Application crop directed BBCH≥40	Small omnivorous mammal “mouse”	2.3	2.2 × 0.53	0.708	138

^aconservative worst case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.3.2.1-8: First tier assessment for the long-term risk to mammals due to the use of thymol in orchards

Intended-use		Orchards			
Active substance		thymol			
Application rate (g/ha)		4 × 264 g thymol/ha			
Acute toxicity (mg/kg bw)		98 ^a			
TER criterion		5			
Crop scenario	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Application crop directed BBCH≥40	Large herbivorous mammal “lagomorph”	4.3	2.2 × 0.53	1.324	74
Fruit stage BBCH 71-79	Frugivorous mammal “dormouse”	22.7	2.2 × 0.53	6.987	14
Application crop directed BBCH≥40	Small herbivorous mammal “vole”	21.7	2.2 × 0.53	6.680	15
Application crop directed BBCH≥40	Small omnivorous mammal “mouse”	2.3	2.2 × 0.53	0.708	138

^aconservative worst-case surrogate endpoint using the acute toxicity value LD₅₀/10

As all TER_{LT} values were above the trigger of 5 at the screening or first tier step considering a conservative surrogate long-term endpoint based on the acute LD₅₀/10 for geraniol and thymol, and developmental NOAEL for eugenol, an acceptable long-term risk to mammals is concluded when Mevalone is applied according to the proposed uses. No further assessment is required.

zRMS comments:

The acute risk assessment for particular active compounds and the formulated product provided by the Applicant in Tables 9.3.2.1- to 9.3.2.1-4 is agreed by the zRMS. The long-term risk assessment for eugenol is also agreed. The long-term risk assessment for geraniol and thymol was amended by the zRMS with consideration of the provisional endpoints discussed by the zRMS in point 9.3.1 above.

Based on performed calculations, acceptable acute and long-term risk from particular active compounds may be concluded,. Taking this into account, additional long-term evaluation for thymol presented in Tables 9.3.2.1-7 and 9.3.2.1-8 was not necessary and is struck through.

Performed evaluation demonstrated unacceptable acute risk from the formulated product at the screening step and Tier 1 calculations were performed. At Tier 1 the risk from uses in vineyards was acceptable for all relevant generic focal species with exception of small herbivorous mammals. In orchards acceptable risk could be concluded for large herbivores and small omnivores, but unacceptable risk was indicated for small herbivores and frugivores.

No specific refinement options were available, but the Applicant correctly indicated that the acute toxicity endpoint considered in evaluation performed for the formulated product is greater than value, meaning that the real endpoint would be higher. No mortality was observed in the acute study performed with Mevalone, but unlike for birds, consideration of extrapolation factor for the limit dose in case of no mortality is not possible, since data for calculation of an average probit slope for variety of substances was not available for mammals.

The Applicant decided to refine the risk using the surrogate LD₅₀mix derived using the Finney equation. This approach is agreed by the zRMS since for the active compounds definite endpoints were available, while for the formulation LD₅₀ was greater than the maximum dose tested, being only indicative and giving no information on the actual endpoint. It is, however, noted that in calculations the Applicant used concentrations of the active compounds, while in line with indications of Appendix B of EFSA (2009), fractions of the active substances in the product should be used and these must sum up to 1. Fractions of eugenol, geraniol and thymol in Mevalone are 0.2, 0.4 and 0.4, respectively and when these fractions are considered together with the toxicity endpoints for particular active compounds, the surrogate LD₅₀mix if 1605.4 mg/kg bw may be calculated and will be used in calculations presented below.

Intended use		Vineyards			
Product		eugenol+thymol+geraniol / Mevalone			
Application rate (g/ha)		660 g sum of a.s./ha (132 g eugenol/ha, 264 g geraniol/ha and 264 g thymol/ha)			
Acute toxicity (mg/kg bw)		1605.4 mg sum of a.s./kg bw			
TER criterion		10			
Crop scenario Growth stage	Generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg prod/kg bw)	TER_a
Application crop directed BBCH ≥ 40	Large herbivorous mammal “lagomorph”	8.1	1.8	9.6	166.8
Application crop directed BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.8	6.4	250.2
Application crop directed BBCH ≥ 40	Small herbivorous mammal “vole”	40.9	1.8	48.6	33.0
Application crop directed BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.8	6.2	259.9

Table 9.36: First tier assessment for the acute oral risk to mammals due to the use of Mevalone in orchards

Intended use		Orchards			
Product		eugenol+thymol+geraniol / Mevalone			
Application rate (g/ha)		660 g sum of a.s./ha (132 g eugenol/ha, 264 g geraniol/ha and 264 g thymol/ha)			
Acute toxicity (mg/kg bw)		1605.4 mg sum of a.s./kg bw			
TER criterion		10			
Crop scenario Growth stage	Generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg prod/kg bw)	TER_a
Application crop directed BBCH ≥ 40	Small herbivorous mammal “vole”	40.9	1.8	48.6	33.0
Fruit stage BBCH 71-79	Frugivorous mammal “dormouse”	47.9	1.8	56.9	28.2
Application crop directed BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1.8	12.5	128.7
Application crop directed BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.8	6.2	259.9

TER values derived with consideration of the surrogate LD₅₀mix estimated using the exact active substance toxicity data are all above the trigger of 10 demonstrating acceptable acute risk to mammals exposed to the mixture following intended Central Zone uses of Mevalone. In opinion of the zRMS no further assessment in this area is deemed necessary and performance of additional formulation study testing higher doses of the product is not justified.

It is noted that the chronic combined risk assessment was not addressed by the Applicant, although it is mandatory in the Central Zone. Its performance was possible, since the long-term risk assessment was based on the specific endpoints for particular active substances. As a first step of evaluation the TERmix approach has been considered by the zRMS. Results are presented in table below. All calculations were performed with unbound values.

Compound						Σ1/TER	Σ1/TER ⁻¹	Trigger
Eugenol		Geraniol		Thymol				
TER ¹⁾	1/TER	TER ¹⁾	1/TER	TER ¹⁾	1/TER			
Vineyards and orchards (the same TER values for both crops)								
22.0	0.0455	22.5	0.0398	9.0	0.1111	0.1964	5.1	5

¹⁾ Screening step TER values

The TERmix calculated with consideration of the screening step TER values for particular active compounds is greater than the trigger of 5 indicating acceptable long-term combined risk to mammals following intended Central Zone uses of Mevalone.

No separate risk assessment was performed for methyl-eugenol, however according to Part C this compound is considered to be a relevant impurity, naturally present in eugenol and not formed during the formulation processes or product storage. Taking this into account, this compound was also present in the acute and long-term toxicity studies performed with eugenol. Furthermore, according to the available literature data, methyl-eugenol is present in plant tissues at concentrations ranging from 0 to 14 650 ppm. Taking this into account, the zRMS is of the opinion that the risk assessment is also protective for this impurity. Nevertheless, further assessment may be deemed necessary once relevant data become available after the EU renewal process of eugenol.

Overall, based on results of the above calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of mammals, no unacceptable risk to mammals is anticipated from uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on the provisional long-term toxicity endpoints for geraniol and thymol, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.3.2.2 Higher-tier risk assessment

The risk assessments presented above concluded acceptable risk to mammals at the screening or first-tier step. Therefore, no further studies or assessments are considered to be necessary.

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).

Leaf scenario

Since Mevalone 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 is not relevant.

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).

The application rate of 4.12 kg product/ha and MAF have been used in the calculations supposing no degradation between applications and $K_{oc} < 500$ L/kg.

Table 9.3.2.3-1: Screening step for the drinking water assessment to mammals due to the use of Mevalone

Effective application rate (g/ha) for acute risk ^a =	7416		
Acute toxicity (mg/kg bw) =	2000	quotient =	3.7
Effective application rate (g/ha) for long-term risk ^a =	9064		
Reprod. toxicity (mg/kg bw/d) =	$\geq 200^b$	quotient =	45

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.3.2.3-2: Screening step for the drinking water assessment acute risk to mammals due to the use of eugenol

Effective application rate (g/ha) for acute risk ^a =	237.6		
Acute toxicity (mg/kg bw) =	1930	quotient =	0.12
Effective application rate (g/ha) for long-term risk ^a =	290		
Reprod. toxicity (mg/kg bw/d) =	250	quotient =	1.2

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

Table 9.3.2.3-3: Screening step for the drinking water assessment acute risk to mammals due to the use of geraniol

Effective application rate (g/ha) for acute risk ^a =	475.2		
Acute toxicity (mg/kg bw) =	3600	quotient =	0.132
Effective application rate (g/ha) for long-term risk ^a =	581		
Reprod. toxicity (mg/kg bw/d) =	558 360 ^b	quotient =	1.04 1.6

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst case surrogate endpoint using the acute toxicity value LD₅₀/10

Table 9.3.2.3-4: Screening step for the drinking water assessment to mammals due to the use of thymol

Effective application rate (g/ha) for acute risk ^a =	475.2		
Acute toxicity (mg/kg bw) =	980	quotient =	0.48
Effective application rate (g/ha) for long-term risk ^a =	581		
Reprod. toxicity (mg/kg bw/d) =	200 98 ^b	quotient =	2.9 6

^a MAF₉₀ for acute risk: 1.8 (4 applications, 7 day interval); MAF_m for long-term risk: 2.2 (4 applications, 7 day interval)

^b conservative worst case surrogate endpoint using the acute toxicity value LD₅₀/10

The acute and long-term risks to mammals from drinking water exposure is considered acceptable as the ratios of effective application rate to the relevant endpoint are well below the trigger of 50 for less sorptive substances. No further assessment is required.

zRMS comments:

The drinking water risk assessment for birds presented in Tables 9.3.2.3-1 to 9.3.2.3-4 is in general agreed by the zRMS.

The calculation of the long-term ratio for formulation has been struck through as being not relevant (long-term exposure to the formulation is not expected).

The long-term risk assessment for geraniol and thymol was amended by the zRMS with consideration of the provisional endpoints discussed by the zRMS in point 9.3.1 above.

It is noted that with the single application rate of 4116 g product/ha, the effective application rates would be 7409 and 9055 g product/ha when MAF of 1.8 and 2.2 is considered for the acute and long-term risk assessment, respectively. Nevertheless, as the rates considered by the Applicant are higher, no corrections were made by the zRMS in tables above.

Overall, based on results of the above calculations, no unacceptable risk to birds from exposure via drinking water is anticipated following uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on the provisional long-term toxicity endpoints for geraniol and thymol, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.3.2.4 Effects of secondary poisoning

According to the EFSA Guidance Document on Risk assessment for Birds and Mammals (2009), substances with a log P_{ow} value greater than 3 have a potential for bioaccumulation. The risk assessment for bioaccumulation is not required for eugenol because the log P_{ow} value for eugenol is 2.30-2.45 (EFSA Journal 2012; 10(11):2914) and 2.49 (pH = 7) obtained from the new study (see Part B5 of this dRR, CA 4.1.2/25 2020a).

In the case of geraniol the log P_{ow} values is 3.8 ((EFSA Journal 2012; 10(11):2915) and 3.11 – 3.13 between pH 4-9 obtained from the new study (see Part B5 of this dRR, CA 4.1.2/47 2020b) and thus exceed the trigger value of 3, requiring consideration of the risk to earthworm-eating and fish-eating mammals due to secondary poisoning. However, due to its rapid volatilisation properties and ready biodegradation it is considered unlikely that geraniol will be persistent and accumulate in soil or natural water systems. Since geraniol is of very low persistence in soil and water, accumulation in worms and fish and exposure of mammals from consumption of these is considered unlikely. According to the EFSA Conclusion Report for geraniol (EFSA Journal 2012; 10(11):2915), for bioaccumulation “...it was considered that geraniol is unlikely to bioaccumulate in fish, and therefore no studies are required”.

In the case of thymol the log P_{ow} values are 3.97 (EFSA Journal 2012; 10(11):2916) and 3.41 - 3.44 between pH 4-9 obtained from the new study (see Part B5 of this dRR, CA 4.1.2/25 2020) and thus exceed the trigger value of 3, requiring consideration of the risk to earthworm-eating and fish-eating mammals due to secondary poisoning. However, due to its rapid volatilisation properties and ready biodegradation it is considered unlikely that thymol will be persistent and accumulate in soil or natural water systems. Since geraniol is of very low persistence in soil and water, accumulation in worms and fish and exposure of mammals from consumption of these is considered unlikely. According to the EFSA Conclusion Report for thymol (EFSA Journal 2012; 10(11):2916), for bioaccumulation “...it was considered that bioaccumulation in fish is unlikely”.

Whilst bioconcentration of eugenol, geraniol and thymol in prey of birds and mammals is considered unlikely, for completeness conservative secondary poisoning assessments are presented below for geraniol and thymol (as log P_{ow} values are >3 for these two active substances). These risk assessments have used worst-case initial PEC values, worst-case surrogate reproductive endpoints of $LD_{50}/10$ and QSAR-estimated BCF_{fish} values. The adsorption values (K_{oc}) used below are consistent with those used in document B8, point 8.5, tables 8.5.2 and 8.5.3.

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

To achieve a concise risk assessment, the risk envelope approach is applied. A risk envelope approach has been performed considering the worst case PEC_{soil} values for grapes and PEC_{sw} values for apples. The most recent P_{ow} values have been used for the risk assessment performed below.

Table 9.3.2.4-1: Assessment of the risk for earthworm-eating mammals due to exposure to geraniol via bioaccumulation in earthworms (secondary poisoning) for the intended use of Mevalone in grapes

Parameter	Geraniol	comments
PEC _{soil} (mg/kg soil)	0.01 0.142	21-d TWA PEC _{soil} Worst-case initial PEC _{soil} for grapes (multiple applications); Table 8.7.2-5 of Section B8.
log P _{ow} / P _{ow}	3.13 / 1349	Study CA 4.1.2/47 2020b
Koc	10 27	In absence of reliable sorption data, worst case default was considered Section B8, point 8.5, Table 8.5-2
foc	0.02	Default
BCF _{worm}	85.14 31.53	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.85 4.48	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	1.09 5.73	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	558 360	Provisional long-term endpoint, see discussion in point 9.3.1 above conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _t	512 62.8	i.e. above the trigger of 5

~~Table 9.3.2.4-2: Assessment of the risk for fish-eating mammals due to exposure to geraniol via bioaccumulation in fish (secondary poisoning) for the intended use of Mevalone in apples~~

Parameter	Geraniol	comments
PEC _{sw} (mg/L)	0.40272	Worst-case initial PEC _{sw} for apples (FOCUS STEP 1); Section B8, point 8.9, Table 8.9-15.
BCF _{fish} (L/kg wet wt)	53.97	Estimated using log P _{ow} of 3.13 and the BCFBAF (v3.01) programme of US EPA EPI Suite (Estimation Programs Interface Suite™ for Microsoft® Windows).
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	21.7	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	3.09	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	360	conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _t	117	i.e. above the trigger of 5

Table 9.3.2.4-3: Assessment of the risk for earthworm-eating mammals due to exposure to thymol via bioaccumulation in earthworms (secondary poisoning) for the intended use of Mevalone in grapes

Parameter	Thymol	comments
PEC _{soil} (mg/kg soil)	0.01 0.142	21-d TWA PEC _{soil} Worst-case initial PEC _{soil} for grapes (multiple applications); Table 8.7.2-7 of Section B8.
log P _{ow} / P _{ow}	3.44 / 2754	Study CA 4.1.2/25 2020
Koc	10 190	In absence of reliable sorption data, worst case default was considered Section B8, point 8.5, Table 8.5-3
foc	0.02	Default
BCF _{worm}	169.44 8.92	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	1.69 1.27	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	2.16 1.62	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	200 98	Provisional long-term endpoint, see discussion in point 9.3.1 above conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _t	92.6 60.5	i.e. above the trigger of 5

Table 9.3.2.4-4: Assessment of the risk for fish-eating mammals due to exposure to thymol via bioaccumulation in fish (secondary poisoning) for the intended use of Mevalone in apples

Parameter	Thymol	Comments
PEC _{sw} (mg/L)	0.40272	Worst case max PEC _{sw} for apples (FOCUS STEP 1); Section B8, point 8.9, Table 8.9-26
BCF _{fish} (L/kg wet wt)	86.44	Estimated using log P _{ow} of 3.44 and the BCFBAF (v3.01) programme of US EPA EPI Suite (Estimation Programs Interface Suite™ for Microsoft® Windows).
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	34.8	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	4.94	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	98	conservative worst case surrogate endpoint using the acute toxicity value LD ₅₀ /10
TER _t	19.8	i.e. above the trigger of 5

Even when considering the worst-case parameters (initial/max PEC values, worst-case surrogate reproductive endpoints of LD₅₀/10, QSAR-estimated BCF_{fish} values) in the conservative risk assessments above, all the TER_t values are well above the trigger of 5, concluding acceptable risks to earthworm-eating due to secondary poisoning following the proposed uses of Mevalone. No further data or assessments are considered necessary.

zRMS comments:

Since no reliable K_{foc} values were available for geraniol and thymol following evaluation of the confirmatory data in August 2016, the evaluation of the risk of secondary poisoning for earthworms-eating mammals was amended by the zRMS with consideration of the worst case default K_{foc} of 10 mL/g, as agreed in area of Section 8 for groundwater modelling. However, the 21-d TWA PEC_{SOIL} was considered more relevant than initial PEC_{SOIL} to calculate the PEC_{WORM}.

In addition to that, the TER values were amended with consideration of the provisional endpoints discussed by the zRMS in point 9.3.1 above.

Evaluation of the risk of secondary poisoning to fish-eating mammals could not be validated since the BCF values estimated using QSAR were not EU agreed and no details of performed calculations were provided by the Applicant. Calculations presented in Tables 9.3.2.4-2 and 9.3.2.4-4 were thus struck through. Nevertheless, according to conclusions taken in the course of the EU review, neither geraniol nor thymol are expected to accumulate in fish tissues and for this reason secondary exposure of mammals via fish is unlikely.

Overall, no unacceptable risk of secondary poisoning is anticipated following uses of Mevalone in line with the Central Zone GAP.

Nevertheless, as evaluation was based on the provisional long-term toxicity endpoints for geraniol and thymol, concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

Acceptable acute and long-term risk to mammals is concluded at the screening step or first-tier for the proposed uses of Mevalone in vineyards and pome fruit, ~~even when considering conservative worst case surrogate reproductive endpoints~~. The risk from secondary poisoning and drinking water is also considered to be acceptable.

It should be, however, noted that in absence of the EU agreed mammalian reproductive toxicity studies for geraniol and thymol, the long-term risk assessment was performed with consideration of the provisional long-term toxicity endpoints derived by the zRMS with consideration of information available in the DAR (May 2011) for both active compounds. Nevertheless, in opinion of the zRMS, based on results of the performed calculations, rapid dissipation of active compounds due to volatilisation and degradation as well as natural occurrence of eugenol, methyl-eugenol, geraniol and thymol in various food items of mammals, no unacceptable risk to mammals is anticipated from uses of Mevalone in line with the Central Zone GAP. Further evaluation will be performed once final and firm conclusions are taken at the EU level following the ongoing renewal process of all three active compounds.

Concerned Member States may wish to reconsider the approach of the zRMS at the product authorisation in their countries.

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

The above risk assessments for birds and mammals are expected to cover the effects in other terrestrial vertebrates. No further assessments are considered necessary.

zRMS comments:

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

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 the active substances eugenol, geraniol and thymol. Full details of these studies are provided in the respective EU DAR and related documents. New chronic *Daphnia* data for all three active substances, as well as a new algae study with geraniol, are submitted with this application; listed in Appendix 1 and summarised in Appendix 2.

Effects on aquatic organisms of Mevalone were evaluated as part of the EU assessment of eugenol, geraniol and thymol.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, with the exception of algal data for geraniol, in which a new study is submitted for completeness. Justifications are provided below.

It is noted that a data gap for further information to address the chronic risk to aquatic organisms was identified during the first EU review of eugenol (EFSA Journal 2012; 10(11): 2914; 2915; 2916). Further information on natural background levels of eugenol, geraniol and thymol were provided as confirmatory data, but this was not considered sufficient by EFSA to enable a comparison between the natural background exposure and the exposure due to the use of the plant protection product (EFSA Supporting publication 2017:EN-1165 ; EN-1163; EN-1162)).

A waiver is requested for long-term toxicity data to fish as further vertebrate testing is not justified. Additional weight of evidence to support this waiver is presented below. Significant long-term exposure of eugenol, geraniol and thymol in surface waters are not expected due to its rapid volatilisation properties and ready biodegradation. Following field application of the formulated product, Mevalone, initial environmental exposure of eugenol, geraniol and thymol will decline rapidly in relation to the applied dose. For eugenol, according to the acute toxicity endpoints obtained from EFSA Journal 2012; 10(11):2914, it is observed that *Daphnia magna* is more sensitive (up to 10 times more acutely toxic) than fish. For geraniol and thymol, according to the acute toxicity endpoints obtained from EFSA Journal 2012; 10(11):2915 and EFSA Journal 2012; 10(11):2916 respectively, it is observed that fish are no more sensitive to geraniol, thymol or the representative product, Mevalone, than *Daphnia magna* (acute LC₅₀ and EC₅₀ values for fish and *Daphnia* are in same order of magnitude). Therefore, no new long-term toxicity studies have been carried out for fish to waive further vertebrate testing. The new long-term studies with *Daphnia magna* (please see studies CP 10.2.1/01-03) are expected to be sufficient to address the long-term toxicity for aquatic organism.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – eugenol, geraniol and thymol

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	eugenol	Acute toxicity 96 h, ss	LC ₅₀ > 10 mg eugenol/L (nom)	EFSA Journal 2012;10(11):2914
<i>Oncorhynchus mykiss</i>	geraniol	Acute toxicity 96 h, ss	LC ₅₀ = 11.6 mg geraniol/L (nom)	EFSA Journal 2012;10(11):2915
<i>Oncorhynchus mykiss</i>	thymol	Acute toxicity 96 h, ss	LC ₅₀ = 3.0 mg thymol/L (nom)	EFSA Journal 2012;10(11):2916
<i>Danio rerio</i>	eugenol	Acute toxicity 96 h, ss	LC ₅₀ =11.9 mg eugenol/L (nominal)	EFSA Journal 2012;10(11):2914
<i>Danio rerio</i>	geraniol	Acute toxicity 96 h, ss	LC ₅₀ =23.6 mg geraniol/L (nom)	EFSA Journal 2012;10(11):2915
<i>Danio rerio</i>	thymol	Acute toxicity 96 h, ss	LC ₅₀ =7.1 mg thymol/L (nom)	EFSA Journal 2012;10(11):2916
<i>Daphnia magna</i>	eugenol	Acute toxicity 48 h, s	EC ₅₀ = 1.11 mg eugenol/L (nominal)	EFSA Journal 2012;10(11):2914
<i>Daphnia magna</i>	geraniol	Acute toxicity 48 h, s	EC ₅₀ = 16.1 mg geraniol/L (nom)	EFSA Journal 2012;10(11):2915
<i>Daphnia magna</i>	thymol	Acute toxicity 48 h, s	EC ₅₀ = 4.9 mg thymol/L (nom)	EFSA Journal 2012;10(11):2916
<i>Daphnia magna</i>	eugenol	Chronic toxicity 21 d, ss	NOEC = 0.0959 mg eugenol/L (mm) (highest concentration tested) EC ₁₀ could not be determined	Study CP 10.2.1/01
<i>Daphnia magna</i>	geraniol	Chronic toxicity 21 d, ss	NOEC = 0.0392 mg geraniol/L (mm) ¹⁾ EC ₁₀ = 0.0520 mg geraniol/L (mm) ¹⁾ NOEC = 0.191 mg geraniol/L (nom) EC ₁₀ = 0.278 mg geraniol/L (nom)	Study CP 10.2.1/02 ¹⁾
<i>Daphnia magna</i>	thymol	Chronic toxicity 21 d, ss	NOEC = 0.137 mg thymol/L (mm) EC ₁₀ = 0.292 mg thymol/L (mm)	Study CP 10.2.1/03
<i>Pseudokirchneriella subcapitata</i>	eugenol	96 h, s Results reported for 72 hours	E _r C ₅₀ = 15.4 mg eugenol/L E _y C ₅₀ = 10.8 mg eugenol/L E _b C ₅₀ = 10.0 mg eugenol/L (mm)	EFSA Journal 2012;10(11):2914
<i>Pseudokirchneriella subcapitata</i>	geraniol	96 h, s Results reported for 72 hours	E _r C ₅₀ = 48.0 mg geraniol/L E _y C ₅₀ = 10.3 mg geraniol/L E _b C ₅₀ = 12.7 mg geraniol/L (nom)	EFSA Journal 2012;10(11):2915

Species	Substance	Exposure System	Results	Reference
<i>Pseudokirchneriella subcapitata</i>	geraniol	72 h, s	E _r C ₅₀ = 9.51 mg geraniol/L E _y C ₅₀ = 5.41 mg geraniol/L E _b C ₅₀ = 5.84 mg geraniol/L (mm)	Study CP 10.2.1/04
<i>Pseudokirchneriella subcapitata</i>	thymol	96 h, s Results reported for 72 hours	E _r C ₅₀ = 11.1 mg thymol/L E _y C ₅₀ = 4.89 mg thymol/L E _b C ₅₀ = 5.14 mg thymol/L (mm)	EFSA Journal 2012;10(11):2916

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

Endpoints in bold are used in the risk assessment

¹⁾ Endpoints not fully reliable, since the measured concentrations of the test item in the aged solutions were <LOD/LOQ and ½ of LOD/LOQ was used to calculate the time weighted mean measured concentrations. Therefore the endpoints are kept for illustrative risk assessment, while final decision should be taken at the EU level during the substance renewal, especially during the first EU review of geraniol endpoints from study with algae were kept in the LoEP although the measured concentration of the test item at test termination was <LOD.

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

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Mevalone	Acute toxicity 96 h, ss	LC ₅₀ = 31.1 mg product /L (nom)	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
<i>Daphnia magna</i>	Mevalone	Acute toxicity 48 h, s	EC ₅₀ = 35.4 mg product/L (nom)	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
<i>Pseudokirchneriella subcapitata</i>	Mevalone	96 h, s Results reported for 72 hours	E _r C ₅₀ = 100.8 mg product/L E _y C ₅₀ = 69.0 mg product/L E _b C ₅₀ = 65.2 mg product/L (nom)	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916

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

Endpoints in bold are used in the risk assessment

zRMS comments:

The acute aquatic toxicity data for particular active compounds and the formulated product provided in Table 9.5-1 and 9.5-2 are in line with endpoints reported in in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

In support of evaluation of Mevalone at the Central Zone level, the Applicant submitted studies on chronic toxicity of particular active compounds to *Daphnia magna*. Also algae study with geraniol was submitted due to issues with the measured concentrations identified in the course of the first EU review of this active compound. All submitted studies were evaluated and agreed by the zRMS, however it was noted that in the study on chronic toxicity of geraniol to *Daphnia magna* the measured concentrations of test item dropped to <LOD/LOQ in the aged solutions (renewed every 3 days) and for this reason calculations of the reliable geometric mean measured concentrations in the test solutions was not possible. The study authors considered ½ LOD or LOQ to derive the mean measured concentrations, which may be in general accepted in case when the measured concentrations are between LOD and

LOQ, but is not fully relevant in case when concentrations are <LOD. Nevertheless, the zRMS decided to provisionally accept the approach of the study authors in order to avoid situation when the study is rejected at the zonal level, but then accepted during the EU renewal process, which is already ongoing. Furthermore, in opinion of the zRMS authorisation of the products based on natural active compounds should not be restrained in case the provisional risk assessment may be performed. Detailed discussion regarding this issue may be found in Appendix 2 in the zRMS comments to the study (KCP 10.2.1/02) and is not repeated here. All summaries of the studies together with zRMS evaluation may be found in Appendix 2.

The zRMS agrees with the Applicant that based on the acute toxicity studies *Daphnia magna* is clearly (by a factor of 9) seems to be more sensitive to eugenol than fish. Sensitivity of fish and *Daphnia magna* to other active compounds and the formulated product is at the similar level with differences resulting rather from natural and inter-laboratory variation. and for the Therefore, in opinion of the zRMS, the long-term studies with fish may be waived, especially for the animal welfare reasons testing of fish should be avoided and especially available data clearly indicate that all active compounds are rapidly (within hours from application) volatilised and degraded, so long-term exposure of fish (and other aquatic species) is unlikely. Decision on further vertebrate testing should be taken at the EU level and responsibility for such decision should not be shifted to the zonal level.

9.5.1.1 Justification for new endpoints

Three chronic toxicity studies with *Daphnia magna* have been provided to address the EFSA data gaps for chronic risk to aquatic organisms for the active substances eugenol, geraniol and thymol. Moreover, one green algal study with geraniol was previously evaluated as part of the EU review for the EU inclusion of geraniol (DAR, Volume 3, Annex B.9, 2011, B.9.2.1.3). In this study geraniol could not be detected by the end of the 96-hour test and endpoints were therefore reported based on initial nominal concentrations. Given the rapid degradation of geraniol in this first study, for completeness a new study has also been conducted for geraniol. In this new study (summarised below in Appendix 2), intermediate analytical samples were taken at 4 and 24 hours, as well as at test end (72 hours) to allow calculation of mean measured concentrations throughout the exposure period. Since the E_rC_{50} value of 9.51 mg geraniol/L (mean measured) from this new algal study is worst-case compared to the EU-agreed E_rC_{50} value of 48.0 mg geraniol/L (nominal), the lower endpoint is considered the most robust endpoint for the risk assessment of algae for geraniol.

zRMS comments:

The chronic *Daphnia magna* studies were deemed necessary to address data gaps identified in EFSA reports for all three active compounds. As testing have not included vertebrates, submission of the studies for purposes of the zonal evaluation of Mevalone was justified.

Although new algae study with geraniol was not identified to be a data gap, the new study was evaluated by the zRMS since it provided more robust endpoints due to the measured concentrations maintained at the level enabling calculation of the geometric mean measured concentrations.

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. The PEC_{sw} were calculated taking into account deposition after volatilisation as a worst case. For the risk assessment the risk envelope approach has been used. The risk assessment conducted below has been performed using apple (covers all proposed uses in pome fruit) and vineyards.

In the following table, the ratios between predicted environmental concentrations in surface water bodies

(PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5.2-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on instantaneous formulation PEC_{sw} calculations (spray drift only) for the use of Mevalone –Vineyards and pome fruit 4 applications at 4116 g product/ha

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC50	EC50	ErC50
AF		31100	35400	100800
RAC (µg/L)		100	100	10
Crop	PEC gl-max (µg/L)	PEC/RAC	PEC/RAC	PEC/RAC
		311	354	10080
PEC _{sw} formulation, Document B8, point 8.9.2.4, Table 8.9-30				
Vines (late)	110.034	0.354	0.311	0.011
Pome fruit (late)	215.816	0.694	0.610	0.021

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

For the intended uses of Mevalone in vines and pome fruit, calculated PEC/RAC ratios for the formulated product indicate an acceptable risk for the most sensitive group of aquatic organisms (acute risk for fish) based on instantaneous formulation PEC_{sw} calculations (spray drift only). Therefore, no further assessment is necessary for Mevalone.

zRMS comments:

The aquatic risk assessment based on the formulation toxicity data compared with surface water exposure to the product resulting from spray drift is agreed by the zRMS. Acceptable risk may be concluded.

It should be, however, noted that approach taken by the Applicant is not foreseen in EFSA (2013) which clearly states that the risk assessment for the formulated product should be based on PEC_{mix} (being the sum of PEC_{SW} for particular active compounds) compared with endpoints expressed in terms of the sum of active substances.

The combined risk assessment if considered further below.

Eugenol

Table 9.5.2-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS STEP 1, 2 and 3 calculations for the use of eugenol – Vineyards and pome fruit 4 applications at 132 g eugenol/ha

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC ₅₀	EC ₅₀	NOEC	ErC ₅₀
AF		>10000	1110	95.9	15400
RAC (µg/L)		100	100	10	10
FOCUS Scenario	PEC gl-max (µg/L)	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC
		>100	11.1	9.59	1540
STEP 1 - Document B8, point 8.9.2.1, Table 8.9-5 and Table 8.9-6					
Vines	187.81	1.87	16.9	19.6	0.121
Apples	201.36	2.01	18.14	21.0	0.131
STEP 2 - Document B8, point 8.9.2.1, Table 8.9-5 and Table 8.9-6					
Vines	7.64	<0.076	0.688	0.797	0.005
Apples	11.67	<0.117	1.05	1.22	0.008
STEP 3 - Document B8, point 8.9.2.1, Table 8.9-12					
Apples D3/ditch	4.854	-	0.437	0.506	-
Apples D4/pond	0.2173	-	0.020	0.023	-
Apples D4/stream	4.755	-	0.428	0.496	-

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Algae
Apples D5/pond	0.2174	-	0.020	0.023	-
Apples D5/stream	5.254	-	0.473	0.548	-
Apples R1/pond	0.2172	-	0.020	0.023	-
Apples R1/stream	3.725	-	0.336	0.388	-
Apples R2/stream	4.993	-	0.450	0.521	-
Apples R3/stream	5.25	-	0.473	0.547	-
Apples R4/stream	3.724	-	0.335	0.388	-

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

For the intended uses of Mevalone in vines and pome fruit, calculated PEC/RAC ratios for the active substance eugenol indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for *Daphnia*) at FOCUS STEP 2 for vines, and in all FOCUS STEP 3 scenarios for pome fruit. Therefore, no further assessment is necessary for eugenol.

Geraniol

Table 9.5.2-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS STEP 1, 2 and 3 calculations for the use of geraniol – Vineyards and pome fruit 4 applications at 264 g geraniol/ha

Group		Fish acute	Inverteb. acute	Algae	Inverteb. chronic		
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>D. magna</i>		
Endpoint (µg/L)		LC ₅₀	EC ₅₀	E _r C ₅₀	NOEC	EC ₁₀	NOEC
AF		11600	16100	9510	39.2 (mean measured)	52.0 (mean measured)	191 (nominal)
RAC (µg/L)		100	100	10	10	10	10
FOCUS Scenario	PEC _{gl-max} (µg/L)	116	161	951	3.92	5.20	19.1
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC
STEP 1 - Document B8, point 8.9.2.1, Table 8.9-14 and Table 8.9-15							
Vines	375.63	3.24	2.33	0.395	95.8	72.2	19.7
Apples	402.72	3.47	2.50	0.423	102.7	77.4	21.1
STEP 2 - Document B8, point 8.9.2.1, Table 8.9-14 and Table 8.9-15							
Vines	15.28	0.132	0.095	-	3.90	2.94	0.800
Apples	23.34	0.201	0.145	-	5.95	4.49	1.22
STEP 3 - Document B8, point 8.9.2.1, Table 8.9-20 and Table 8.9-21							
Vines D3 ditch	4.658*	-	-	-	1.19	0.896	-
Vines D4 pond	0.3603*	-	-	-	0.092	0.069	-
Vines D4 stream	3.897*	-	-	-	0.994	0.749	-
Vines D5 pond	0.3645*	-	-	-	0.093	0.070	-
Vines D5 stream	4.109*	-	-	-	1.05	0.790	-
Vines D6 ditch	4.729*	-	-	-	1.21	0.909	-
Vines R1 pond	0.4854	-	-	-	0.124	0.093	-
Vines R1 stream	3.703*	-	-	-	0.945	0.712	-
Vines R2 stream	4.882*	-	-	-	1.25	0.939	-
Vines R3 stream	4.967*	-	-	-	1.27	0.955	-
Vines R4 stream	3.704*	-	-	-	0.945	0.712	-
Apples D3 ditch	9.707*	-	-	-	2.48	1.87	0.508
Apples D4 pond	0.9714	-	-	-	0.248	0.187	0.051
Apples D4 stream	9.815*	-	-	-	2.50	1.87	0.514
Apples D5 pond	0.7855	-	-	-	0.200	0.151	0.041

Group		Fish acute	Inverteb. acute	Algae	Inverteb. chronic		
Apples D5 stream	10.54*	-	-	-	2.69	2.03	0.552
Apples R1 pond	0.6407	-	-	-	0.163	0.123	0.034
Apples R1 stream	7.769*	-	-	-	1.98	1.49	0.407
Apples R2 stream	10.44*	-	-	-	2.66	2.01	0.547
Apples R3 stream	10.57*	-	-	-	2.70	2.03	0.553
Apples R4 stream	7.694*	-	-	-	1.96	1.48	0.403

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

*Single application gives higher PEC_{sw} than multiple application at Step 3 therefore single application values reported at Step 3.

As it was explained in document B8, point 8.9 vines scenario only covers one D scenario (D6) and a surrogate crop was chosen (pome fruits) to cover D3, D4 and D5 scenarios. Therefore, additional calculations were provided to cover the additional scenarios. For the intended uses of Mevalone in vines and pome fruit, calculated PEC/RAC ratios for the active substance geraniol indicate an acceptable acute risk for fish, aquatic invertebrates and algae at FOCUS STEP 2. For the most sensitive group of aquatic organisms (chronic risk for *Daphnia*), in the first instance the worst-case NOEC value of 39.2 µg geraniol/L (based on mean measured concentrations) has been considered in the risk assessment in the table above. For some (but not all) scenarios at FOCUS STEP 3, the PEC/RAC ratio is slightly above 1 (1.05 – 1.27 for vines; 1.96 – 2.70 for apples) using this worst-case NOEC value of 39.2 µg geraniol/L (RAC of 3.92 µg geraniol/L). However, as detailed in the study summary (CP 10.2.1/02), in this 21-day semi-static chronic *Daphnia* study, by the end of each 2-3 day renewal period the measured concentrations of geraniol in the aged test solutions were below the LOD/LOQ in the majority of cases (including all analyses at the nominal NOEC concentration of 191 µg geraniol/L). The mean measured concentrations were conservatively calculated using half of the LOD or LOQ for the aged test solutions and therefore the NOEC value of 39.2 µg geraniol/L based on these mean measured concentrations is considered extremely worst-case. Therefore, the table above also presents additional assessments using the EC₁₀ value based on mean measured concentrations, as well as the NOEC based on nominal concentrations. ~~PEC/RAC values are greater than 1 for all FOCUS STEP 3 scenarios for vines using the EC₁₀ (mean measured) value of 52.0 µg geraniol/L; and additionally for all FOCUS STEP 3 scenarios for apples using the NOEC (nominal) value of 191 µg geraniol/L. Given the high volatility of geraniol (vapour pressure 4.6 Pa at 20 °C (please see dRR Section 2: Physical and chemical properties) it is likely that the rapid loss of test item in the *Daphnia* test media resulted largely from volatilisation. Since similar rapid dissipation of geraniol from the environment is expected following the intended application of Mevalone, long term chronic exposure in natural water systems is considered unlikely.~~ Additional FOCUS Step 4 calculations were performed in document B8, point 8.9 to address the potential risk of geraniol considering the RAC values of 3.92 µg geraniol/L in apples. As it was stated in section B8, please see point 8.9.2 for details, additional scenarios were provided for vines to cover D3, D4 and D5 FOCUS scenarios using orchards as surrogate. Therefore, the risk assessment with FOCUS Step 4 values for geraniol in apples and vines has been provided below.

Thymol

Table 9.5.2-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS STEP 1, 2 and 3 calculations for the use of thymol – Vineyards and pome fruit 4 applications at 264 g thymol/ha

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC ₅₀ 3000	EC ₅₀ 4900	NOEC 137	E _r C ₅₀ 11100
AF		100	100	10	10
RAC (µg/L)		30	49	13.7	1110
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC
STEP 1 - Document B8, point 8.9.2.1, Table 8.9-25 and Table 8.9-26					
Vines	375.63	12.5	7.67	27.4	0.338
Apples	402.72	13.4	8.22	29.4	0.363
STEP 2 - Document B8, point 8.9.2.1, Table 8.9-25 and Table 8.9-26					
Vines (S-Europe)	15.28	0.509	0.312	1.12	-
Apples (S-Europe)	22.34	0.745	0.456	1.63	-
STEP 3 - Document B8, point 8.9.2.1, Table 8.9-31 and Table 8.9-32					
Vines D3 ditch	4.755	-	-	0.347	-
Vines D4 pond	0.6944	-	-	0.051	-
Vines D4 stream	3.946	-	-	0.288	-
Vines D5 pond	0.5412	-	-	0.040	-
Vines D5 stream	4.172*	-	-	0.305	-
Vines D6 ditch	4.801*	-	-	0.350	-
Vines R1 pond	0.5514	-	-	0.040	-
Vines R1 stream	3.725*	-	-	0.272	-
Vines R2 stream	4.888*	-	-	0.357	-
Vines R3 stream	5.055*	-	-	0.369	-
Vines R4 stream	3.733*	-	-	0.272	-
Apples D3 ditch	9.707*	-	-	0.709	-
Apples D4 pond	1.106	-	-	0.081	-
Apples D4 stream	9.852*	-	-	0.719	-
Apples D5 pond	0.9065	-	-	0.066	-
Apples D5 stream	10.54*	-	-	0.769	-
Apples R1 pond	0.725	-	-	0.053	-
Apples R1 stream	7.816*	-	-	0.571	-
Apples R2 stream	10.45*	-	-	0.763	-
Apples R3 stream	10.57*	-	-	0.772	-
Apples R4 stream	7.737*	-	-	0.565	-

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

*Single application gives higher PEC_{sw} than multiple applications at Step 3 therefore single application values reported at Step 3.

For the intended uses of Mevalone in vines and pome fruit, calculated PEC/RAC ratios for the active substance thymol indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for *Daphnia*) in all FOCUS STEP 3 scenarios for vines and apples. Therefore, no further assessment is necessary for thymol.

Step 4 risk assessment for geraniol

For the intended uses of Mevalone in vines and apples, the calculated PEC/RAC ratios for the active substance geraniol indicated potential risk for the most sensitive group of aquatic organisms (chronic risk for *Daphnia*) at FOCUS STEP 3. The risk assessment considering FOCUS Step 4 values for the intended use on apples and vines has been provided below.

Table 9.5.2-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for geraniol based on FOCUS Step 4 calculations and toxicity data for aquatic invertebrates with mitigation of spray drift and run-off for the use of geraniol in apples

Intended use		Apples	
Active substance		geraniol	
Application rate (g/ha)		4 × 264 g a.s./ha	
Nozzle reduction	No-spray buffer (m)	10	20
	Vegetated filter strip (m)	10	20
None	D3 ditch	3.047*	-
None	D4 pond	0.6372	-
None	D4 stream	3.576*	-
None	D5 pond	0.5153	-
None	D5stream	3.705*	-
None	R1 pond	0.4203	-
None	R1 stream	2.882*	-
None	R2 stream	3.793*	-
None	R3 stream	4.557	2.363
None	R4 stream	2.828*	-
RAC (µg/L)		PEC/RAC ratio	
3.92			
None	D3 ditch	0.777	-
None	D4 pond	0.163	-
None	D4 stream	0.912	-
None	D5 pond	0.131	-
None	D5stream	0.945	-
None	R1 pond	0.107	-
None	R1 stream	0.735	-
None	R2 stream	0.968	-
None	R3 stream	1.163	0.603
None	R4 stream	0.721	-

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

*Single application gives higher PEC_{sw} than multiple application at Step 3 therefore single application values reported at Step 3.

For the intended uses of Mevalone, calculated PEC/RAC ratios for the active substance geraniol indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for *Daphnia*) in all FOCUS STEP 4 scenarios for apples with a vegetated filter strip of 20 m.

Table 9.5.2-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for geraniol based on FOCUS Step 4 calculations and toxicity data for aquatic invertebrates with mitigation of spray drift and run-off for the use of geraniol in vines

Intended use		Vines	
Active substance		geraniol	
Application rate (g/ha)		4 × 264 g a.s./ha	
Nozzle reduction	No-spray buffer (m)	10	20
	Vegetated filter strip (m)	10	20
None	D3 ditch	3.047 ^{a*}	-
None	D5 stream	3.705 ^{a*}	-
None	D6 ditch	1.488	-
None	R2 stream	1.468*	-
None	R3 stream	1.575*	-
RAC (µg/L)		PEC/RAC ratio	
3.92			
None	D3 ditch	0.777	-
None	D5 stream	0.945	-
None	D6 ditch	0.380	-
None	R2 stream	0.374	-
None	R3 stream	0.402	-

^a D3 ditch and D5 stream scenarios for apples were used as surrogate for vines.

*Single application gives higher PEC_{sw} than multiple application at Step 3 therefore single application values reported at Step 3. PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the intended uses in vines, calculated PEC/RAC ratios for the active substance geraniol indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for *Daphnia*) in all FOCUS STEP 4 scenarios for apples with a vegetated filter strip of 10 m.

zRMS comments:

The risk assessment for aquatic organisms from particular active substances is in general agreed by the zRMS.

The EU agreed data as well as endpoints derived from the newly submitted studies were considered and compared with Surface water exposure agreed in area of Section 8 (lower PEC_{sw} of single and multiple applications was used). Surface water exposure calculated with consideration of the deposition from volatilisations was used. For Central Zone scenarios not defined for vines, pome fruits were used as surrogate.

It should be noted that the chronic risk assessment for *Daphnia magna* from geraniol is not fully reliable due to issues discussed in point 9.5.1 above. Also for this reason in the study evaluation the zRMS concluded that the NOEC values, being lower than the EC₁₀, should be used for PEC/RAC calculations, even though according to EFSA (2013), EC₁₀ values should be used. Taking this into account, Applicants' calculations performed with consideration of the EC₁₀ and NOEC based on nominal concentrations of geraniol was struck through in Table 9.5.2-3.

For eugenol and thymol acceptable risk to aquatic organisms could be concluded for Step 1-3 PEC_{sw} values. For geraniol, acceptable risk with no need for risk mitigation measures could be concluded for Step 1-3 PEC_{sw} in scenarios D4, R1 and R4 and for uses in vines. For uses in apples the chronic risk to *Daphnia magna* based on Step 3 PEC_{sw} was unacceptable in all scenarios. The risk was refined with Step 4 PEC_{sw} values and demonstrated that following risk mitigation measures are deemed necessary:

1. Vines:

- 10 m vegetated filter strip in scenarios D3, D5, D6, R2 and R3,
- no mitigation in scenarios D4, R1 and R4.

2. Apples:

- 10 m vegetated filter strip in scenarios D3, D4, D5, R1, R2 and R4,
- 20 m vegetated filter strip in scenario R3.

Additional calculations may be required by the Member States that do not accept surface water exposure calculated according to FOCUS recommendations.

Mixture toxicity risk assessment

Since the proposed use of Mevalone (3AEY) may result in exposure of aquatic organisms to eugenol, geraniol and thymol simultaneously, it is necessary to check that there is no evidence of synergistic effects, whereby the toxicity of one active substance is enhanced by the presence of the other.

A theoretical endpoint for the formulation can be calculated from the appropriate LC₅₀ and EC₅₀ values for each of the active substances by applying the concentration addition model (CA model) as described in 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” (EFSA Journal 2013; 11(7): 3290), implemented by the Commission Services (SANTE-2015-00080, 15 January 2015):

$$ECx_{mix-CA} = \left(\sum_{i=1}^n \frac{p_i}{ECx_i} \right)^{-1}$$

Where:

- n: number of mixture components
- i: index from 1...n mixture components
- p_i: the ith component as a relative fraction of the mixture composition (note: Σ p_i must be 1)
- EC_{x_i}: concentration of component i provoking x % effect

Subsequently, the measured formulation toxicity is compared with the calculated mixture toxicity EC_{x_{mix-CA}} by means of the model deviation ratio (MDR), which indicates whether relevant toxicity contributions of co-formulants not included in the calculation do occur:

$$MDR = \frac{ECx_{mix-CA} \text{ (calculated mixture toxicity)}}{ECx_{PPP} \text{ (measured mixture toxicity)}}$$

Where:

- EC_{x_{mix}} calculated mixture toxicity (assuming concentration addition (CA))
- EC_{x_{PPP}} measured formulation toxicity

The observed and calculated mixture toxicities are considered in agreement if the MDR is between 0.2 and 5. More than additive (i.e. synergistic) mixture toxicity is indicated if the MDR is > 5. A MDR < 0.2 indicates a less-than additive (i.e. antagonistic) mixture toxicity.

Table 9.5.2-7 Summary of the mixture toxicity assessment.

Organisms	Measured toxicity of Mevalone EC _{x_{PPP}} [mg product/L]	Toxicity of Mevalone (a.s. based) (EC _{x_{PPP}}) [mg a.s./L]	Calculated mixture toxicity ((EC _{x_{mix-CA}}) [mg a.s./L]	Model deviation ratio (MDR = EC _{x_{mix-CA}} /EC _{x_{PPP}})
Fish	31.1	4.987	5.324	1.068
Daphnia	35.4	5.676	3.488	0.615
Algae	100.8	16.163	10.979	0.679

* worst case Step 3 values for apple (pome fruit).

The MDR values for fish, daphnia and algae are 1.068, 0.615 and 0.679 respectively. Therefore, the MRD

values are between 0.2-5 for each relevant aquatic organism group. Thus, no additive/synergistic effect is expected. Therefore the risk assessment can be based on the active substances. For completeness, a risk assessment with the formulated product is also presented above in Table 9.5.2-1.

zRMS comments:

The MDR values are in range of 0.2-5 indicating that the measured mixture toxicity is not more toxic than predicted based on the individual active substance data. However, in line with EFSA (2013) this does not mean that the risk assessment for the active substance covers the risk resulting from exposure to the mixture, but indicates that the experimentally derived endpoints for the formulation may be used in the risk assessment and compared with PECmix.

Respective calculation was performed by the zRMS using the lowest mixture endpoint (estimated EC₅₀ of 3488 µg sum of a.s./L resulting with RAC of 34.88 µg sum of a.s./L) for *Daphnia magna* and sum of Step 3 PEC_{sw} for particular active compounds obtained for uses in apples as giving higher exposure than this predicted for uses in vines. Since the lowest endpoint was taken into account, performed evaluation covers other aquatic species.

FOCUS scenario	Step 3 PEC _{sw} [µg a.s./L]			PECMix [µg sum of a.s./L]	Lowest RAC [µg sum of a.s./L]	PEC/RAC	Trigger
	Eugenol	Geraniol	Thymol				
D3/ditch	4.854	9.707	9.707	24.268	34.88	0.696	1
D4/pond	0.2173	0.9714	1.106	2.2947		0.066	
D4/stream	4.755	9.815	9.852	24.422		0.700	
D5/pond	0.2174	0.7855	0.9065	1.9094		0.055	
D5/stream	5.254	10.54	10.54	26.334		0.755	
R1/pond	0.2172	0.6407	0.725	1.5829		0.045	
R1/stream	3.725	7.769	7.816	19.31		0.554	
R2/stream	4.993	10.44	10.45	25.883		0.742	
R3/stream	5.25	10.57	10.57	26.39		0.757	
R4/stream	3.724	7.694	7.737	19.155		0.549	

All PEC/RAC ratios are below the trigger of 1 indicating acceptable acute risk to aquatic species from the mixture.

Based on the rapid dissipation of active compounds due to volatilisation and degradation, the long-term exposure to the mixture is not expected and the combined chronic risk assessment is thus deemed not necessary.

9.5.3 Overall conclusions

An acceptable risk to aquatic organisms following the proposed uses of Mevalone (including the three active substances eugenol, geraniol and thymol) is concluded based on the available data when considering mitigation measures are considered. For the intended use orchards, the risk is acceptable with a vegetative filters strip of 10 m in scenarios D3, D4, D5, R1, R2 and R4 and 20 m in scenario R3. For the intended uses on vines, the risk is acceptable considering a vegetative filter strip of 10 m in scenarios D3, D5, D6, R2 and R3. No risk mitigation measures are deemed necessary for uses in vines in scenarios D4, R1 and R4. Concerned Member States must decide on applicability of proposed mitigation measures in their countries.

It should be noted that due to measured concentrations of geraniol in aged test solutions being <LOD/LOQ, the evaluation performed for geraniol is provisional and further evaluation will be performed once decision on acceptability of the study is taken at the EU level following the ongoing renewal process.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation Mevalone, which was performed in line with the EU agreed methodology.

“The endpoint E_rC₅₀ is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic

Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”

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 the formulation Mevalone. Full details of the acute toxicity studies are provided in the respective EU DAR and related documents.

Chronic effects on adult and larval bees of Mevalone are now available since the first EU assessment of the active substances and these new data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of acute toxicity studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below for the new chronic toxicity data.

Table 9.6.1-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Honey bee (<i>Apis mellifera</i>)	Mevalone	Acute oral toxicity	LD₅₀ > 224.6 µg product/bee	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
Honey bee (<i>Apis mellifera</i>)	Mevalone	Acute contact toxicity	LD₅₀ > 200 µg product/bee	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
Honey bee (<i>Apis mellifera</i>)	Mevalone (referred to in report under Tradename ARAW)	10 day chronic adult feeding study	LDD ₁₀ = 64.62 µg product/bee /day LDD ₂₀ = 83.65 µgproduct/bee/day LDD₅₀ = 123.53 µg product/bee/day NOEDD = 66.96 µg product/bee/day	Study CP 10.3.1.2/01
Honey bee (<i>Apis mellifera</i>)	Mevalone (referred to in report under Tradename ARAW)	22 day, repeated exposure larval toxicity test	NOED = 1300 µg product/larva/developmental period	Study CP 10.3.1.3/01

Endpoints in bold are used in the risk assessment

zRMS comments:

Mevalone was the representative formulation for all three active compounds and acute bee toxicity data presented in Table 9.6.1-1 are in line with EU agreed endpoints reported in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

Studies on chronic toxicity Mevalone to adult bees and larvae were evaluated and agreed by the zRMS. Summaries of the studies together with zRMS evaluation are presented in Appendix 2. Endpoints reported in Table 9.6.1-1 are confirmed to be correct.

9.6.1.1 Justification for new endpoints

Two new studies have been provided with Mevalone, a new chronic oral honey bee toxicity study (KCP 10.3.1.2/01) and a new repeated exposure honey bee larval toxicity study (KCP 10.3.1.3/01), to meet new data requirements under Regulation (EU) No 284/2013. A full summary is provided in Appendix 2, point 2.3.1.2 and 2.3.1.3 (respectively).

zRMS comments:

Studies on chronic toxicity of Mevalone to adult bees and larvae were necessary to fulfil the data requirements set by the Commission Regulation (EU) No 284/2013.

9.6.2 Risk assessment

The evaluation of the acute risk for bees was first 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 EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA Journal 2013;11(7):3295) has not yet been noted at the EU level. Nevertheless, the current SANCO/10329/2002 guidance document does not cover the risk assessment for honey bee larvae and chronic adults, while endpoints for these are available according to the current data requirements. In the absence of alternative approaches, it was agreed in a general ecotoxicology meeting (EFSA Supporting publication 2015:EN-924) that the first-tier risk assessment to honey bees should be performed according to the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA Journal 2013;11(7):3295, hereafter referred to as EFSA/2013/3295). On the basis of all the available information, a conclusion should be drawn with regards to the risk to honey bees. For bumblebees and solitary bees, it was agreed that if any data are submitted, they should be evaluated. However, currently it cannot be recommended to routinely perform a risk assessment for these organisms. No data for bumble bees or solitary bees have been submitted, and based on the recommendation, an assessment for bumble bees and solitary bees has not been conducted.

The risk assessment presented below according to SANCO/10329/2002 rev.2 was conducted using the worst case single application rate which covers the intended uses vines and pome fruit. Separate Tier 1 chronic oral risk assessments according to EFSA/2013/3295 are presented for each crop group using the worst case application rate.

9.6.2.1 Hazard quotients / Exposure Toxicity Ratios for bees

Table 9.6.2.1-1: First-tier assessment of the acute risk for bees due to the use of Mevalone in vineyards and orchards in accordance with SANCO/10329/2002 rev.2 (final)

Intended use	vineyards and pome fruit		
Active substance	Mevalone		
Application rate (g/ha)	4 × 4116 ¹⁾		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 224.6 µg product/bee	4116	<18.3
Contact toxicity	> 200 µg product /bee		<20.6

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

¹⁾ Based on the nominal relative density of 1.029 g/mL, as agreed in Part C

The hazard quotients (HQ) are well below the trigger of 50, indicating an acceptable acute oral and contact risk to bees following the proposed use of Mevalone in vineyards and orchards. The potential for inhalation exposure is at least partly covered by the available acute oral and contact toxicity tests. In any case it is noted that as a consequence of the high volatility of all three active substances, a low residence time in the treated field is expected after each application. Taking into account the HQ values for acute oral and contact toxicity, there is a wide margin of safety which is expected to also cover the potential risk to bees *via* the inhalation route of exposure.

Risk assessment according to EFSA/2013/3295

Contact exposure

Table 9.6.2.1-2: First tier acute contact risk assessment to bees for the intended uses of Mevalone in vineyards and orchards in accordance with EFSA/2013/3295 SANCO/10329/2002 rev.2 (final)

Intended use	vineyards and pome fruit				
Product	Mevalone				
Application rate (g product /ha)	4 × 4116 (7-day interval)				
Exposure scenario	Acute contact (screening assessment)				
Test group	LD₅₀ (lab.) (µg product /bee)	Single application rate (g product/ha)	HQ_{contact}	Trigger	Acceptable risk?
Honey bee (adult)	>200	4116	<20.6	85	Yes

Oral exposure

The acute and chronic oral risk assessment for adult honey bees and honey bee larva is carried out according to EFSA/2013/3295.

The acute oral and chronic oral risk to honey bees from the use of Mevalone was assessed using the maximum single application rate and the respective LD₅₀, LDD₅₀ and NOED values to calculate the exposure toxicity ratio (ETR).

$$ETR_{\text{acute adult oral}} = \frac{AR(\text{application rate}) * SV(\text{shortcut value})}{LD_{50 \text{ oral}}}$$

Where: AR = in kg product/ha
 SV = shortcut value
 LD₅₀ oral is expressed as µg product/bee

$$ETR_{\text{chronic adult oral}} = \frac{AR * SV(\text{application rate}) * SV(\text{shortcut value})}{LDD_{50}}$$

Where: AR = in kg Mevalone/ha
 SV = shortcut value
 LDD₅₀ oral is expressed as µg product/bee/day

$$ETR_{\text{larvae}} = \frac{AR(\text{application rate}) * SV(\text{shortcut value})}{NOEClarvae}$$

Where: AR = in kg product/ha
 SV = shortcut value
 NOEC is expressed as µg product/larva/development period

Table 9.6.2.1-3: Screening assessment of the risk for honey bees from oral exposure due to the use of Mevalone in vineyards and orchards; according to EFSA/2013/3295

Intended use	Vineyards and pome fruit				
Product	Mevalone				
Application rate (g product /ha)	4 × 4116 (7-day interval)				
Exposure scenario	Oral (screening assessment)				
Test group	LD₅₀ (lab.) (µg product/bee)	SV (sideward spray)	ETR_{oral}	Trigger	Acceptable risk¹?
Honey bee (adult) - acute	224.6	10.6	0.194	0.2	Yes
Honey bee (adult) - chronic	123.53	10.6	0.354	0.03	No
Honey bee (larvae) - chronic	1300	6.1	0.019	0.2	Yes

¹ETR calculation below the trigger value indicates acceptable risk

¹ETR calculation shown in bold fall below the relevant trigger.

The ETR values for acute oral exposure to adult bees and for the chronic oral exposure to larvae are

below the respective trigger, indicating an acceptable acute oral risk for honey bee adults and chronic oral risk for honey bee larvae. In the case of the chronic oral exposure to adult bees, the risk is not acceptable at the screening step and a first-tier assessment is presented in Tables 9.6.2.1-4 to 9.6.2.1-6 below in accordance with EFSA/2013/3295:

Table 9.6.2.1-4: Tier 1 chronic oral risk assessment for honey bee adults according to EFSA/2013/3295 following the use of Mevalone in orchards (pome fruit) BBCH 75-89

Intended use	Pome fruit						
Product	Mevalone						
Application rate (g product /ha)	4 × 4116 (7-day interval)						
Exposure scenario	Tier 1 chronic oral risk assessment						
Test group	LDD₅₀ (lab.) (µg product/bee)	Exposure factor (EF)	SV (sideward / upward spray)	TWA	ETR_{oral}	Trigger	Acceptable risk¹?
Adjacent crop							
Honey bee (adult) - chronic	123.53	0.031	5.8	0.72	0.004	0.03	Yes
Weeds in treated field							
Honey bee (adult) - chronic	123.53	0.3	2.9	0.72	0.021	0.03	Yes
Field margin							
Honey bee (adult) - chronic	123.53	0.052	2.9	0.72	0.004	0.03	Yes
Next crop							
Honey bee (adult) - chronic	123.53	1	0.54	0.72	0.013	0.03	Yes

Treated crop is not relevant for this BBCH stage (fruit stage)

¹ETR calculation below the trigger value indicates acceptable risk

Table 9.6.2.1-5: Tier 1 chronic oral risk assessment for honey bee adults according to EFSA/2013/3295 following the use of Mevalone in vineyards BBCH 60-69

Intended use	vineyards						
Product	Mevalone						
Application rate (g product /ha)	4 × 4116 (7 day interval)						
Exposure scenario	Tier 1 chronic oral risk assessment						
Test group	LDD₅₀ (lab.) (µg Mevalone /bee)	Exposure factor (EF)	SV (sideward / upward spray)	TWA	ETR_{oral}	Trigger	Acceptable risk¹?
Treated crop							
Honey bee (adult) - chronic	123.53	1	8.2	0.72	0.197	0.03	No
Adjacent crop							
Honey bee (adult) - chronic	123.53	0.0143	5.8	0.72	0.002	0.03	Yes
Weeds in treated field							
Honey bee (adult) - chronic	123.53	0.3	2.9	0.72	0.021	0.03	Yes
Field margin							
Honey bee (adult) - chronic	123.53	0.027	2.9	0.72	0.002	0.03	Yes
Next crop							
Honey bee (adult) - chronic	123.53	1	0.54	0.72	0.013	0.03	Yes

¹ETR calculation below the trigger value indicates acceptable risk

¹ETR calculation shown in bold fall below the relevant trigger.

Table 9.6.2.1-6: Tier 1 chronic oral risk assessment for honey bee adults according to EFSA/2013/3295 following the use of Mevalone in vineyards (BBCH ≥ 70)

Intended use		vineyards					
Product		Mevalone					
Application rate (g product /ha)		4 × 4116 (7day interval)					
Exposure scenario		Tier 1 chronic oral risk assessment					
Test group	LDD₅₀ (lab.) (µg Mevalone/bee)	Exposure factor (EF)	SV (sideward / upward spray)	TWA	ETR_{oral}	Trigger	Acceptable risk¹?
Adjacent crop							
Honey bee (adult) - chronic	123.53	0.0143	5.8	0.72	0.002	0.03	Yes
Weeds in treated field							
Honey bee (adult) - chronic	123.53	0.3	2.9	0.72	0.021	0.03	Yes
Field margin							
Honey bee (adult) - chronic	123.53	0.027	2.9	0.72	0.002	0.03	Yes
Next crop							
Honey bee (adult) - chronic	123.53	1	0.54	0.72	0.013	0.03	Yes

Treated crop is not relevant for this BBCH stage (fruit stage)

¹ETR calculation below the trigger value indicates acceptable risk

The Tier 1 chronic oral adult assessment has been performed according to EFSA Journal 2013; 11(7):3295; Mevalone is intended to be applied in orchards (pome fruit) at BBCH 75-89 and vines at BBCH 60-89. The results above demonstrate acceptable chronic oral risk to adult bees for all relevant scenarios in orchards (BBCH 75-89). For the intended uses in vineyard, the risk is acceptable for all scenarios at BBCH ≥ 70. The Tier 1 chronic oral adult risk assessment is above the trigger value of 0.03 only for the treated crop scenario in the intended uses on vineyards at BBCH 60-69. According to the Appendix D of EFSA Journal 2013;11(7):3295, grapevines are of low attractiveness to bees for collection of nectar. It is known that grapevines are wind-pollinated so although they produce nectar they are rarely visited by bees for collection of pollen (Attractiveness of Agriculture Crops to Pollinating Bees- USDA Report 2017¹).

Due to the extremely short half-lives and high volatility of the active substances, the duration of exposure under typical conditions will be very limited, particularly in relation to background levels of thymol, geraniol and eugenol in the environment. Eugenol, geraniol and thymol are naturally occurring terpene oils found in a wide variety of fruits, vegetables, herbs and spices.

Furthermore, thymol is routinely used by beekeepers as an acaricide treatment, applied directly into honey bee hives to control *Varroa* mites without adverse effects on the honey bee colony.

Taking into account that the EFSA Journal 2013;11(7):3295 chronic oral trigger value is still highly conservative and given the rapid degradation, volatility and the natural occurrence of the three active substances, and the low attractiveness of grapevines to bees for the collection of nectar, the chronic oral exposure to adult honey bees in treated vineyards at BBCH 60-69 is unlikely. Therefore, the intended uses in vineyards at BBCH 60-69 are also considered to be acceptable and no further assessment is considered necessary.

Risk assessment from exposure to contaminated water

Honey bees can potentially be exposed to pesticide residues in guttation water, surface water bodies and puddles present in the field. Exposure of bees to thymol, geraniol or eugenol residues in guttation water following the proposed uses of Mevalone will be negligible as the active substances are not systemic. No quantitative risk assessment is therefore considered necessary and a low risk to honey bees *via* exposure to guttation water is concluded. The acute oral risk to adults, the chronic oral risk to adults and the

¹https://www.ars.usda.gov/ARSUserFiles/OPMP/Attractiveness%20of%20Agriculture%20Crops%20to%20Pollinating%20Bees%20Report-FINAL_Web%20Version_Jan%202018_2018.pdf

chronic risk to larvae from exposure to contaminated surface water and puddles is assessed based on the following equation:

$$ETR_{acute} = \frac{W(\text{water uptake of adult bee}) * PEC(\text{the concentration of a.s.in water in } \mu\text{g}/\mu\text{L})}{LD50}$$

Where: W = 11.4 µL/bee per day
 PEC = 0.000216 µg/µL (based on worst-case PEC_{sw} formulation value of 215.816 mg/L for apples; Part B8, Table 8.9-30)
 LD₅₀ = 48-h oral LD₅₀ in µg/bee

$$ETR_{chronic\ adult} = \frac{W(\text{water uptake of adult bee}) * PEC(\text{the concentration of a.s.in water in } \mu\text{g}/\mu\text{L})}{LDD50}$$

Where: W = 11.4 µL/bee per day
 PEC = 0.000216 µg/µL (based on worst-case PEC_{sw} formulation value of 215.816 mg/L for apples; Part B8, Table 8.9-30)
 LDD₅₀ = 10-d oral LD₅₀ in µg/bee/d

$$ETR_{chronic\ larvae} = \frac{W(\text{water uptake of adult bee}) * PEC(\text{the concentration of a.s.in water in } \mu\text{g}/\mu\text{L})}{NOEL}$$

Where: W = 111 µL/bee per day
 PEC = 0.000216 µg/µL (based on worst-case PEC_{sw} formulation value of 215.816 mg/L for apples; Part B8, Table 8.9-30)
 NOEC = 5-d NOEC in µg/larvae (covered with the 22-d NOEC)

Table 9.6.2.1-7: Assessment of the risk for adult and larval honey bees via surface water and puddle water exposure due to the use of Mevalone in orchards

Intended use	apples (worst-case PEC value also covers uses in vineyards)					
Product	Mevalone					
Application rate (g product /ha)	4 × 4116 (7day interval)					
Exposure scenario	Drinking water					
Test group	Endpoint (lab.) (µg product/bee)	Daily water consumption (µL)	PEC (µg/µL)	ETR_{oral}	Trigger	Acceptable risk¹?
Surface water and puddle water						
Honey bee (adult) - acute	>224.6	11.4	0.000216	<0.00001	0.2	Yes
Honey bee (adult) - chronic	123.53	11.4		0.00002	0.03	Yes
Honey bee (larvae) - chronic	1300	111		0.00002	0.2	Yes

¹ETR calculation below the trigger value indicates acceptable risk

The oral ETR values are well below the relevant triggers for all scenarios, demonstrating an acceptable risk to honey bees *via* surface water and puddle water exposure at the screening step with a large margin of safety.

Combination toxicity

A combined effects assessment has not been performed since there are no available bee toxicity data for the individual active substances. However, as the bee risk assessment is based on endpoints derived from formulation studies it can be considered that the presence of all three active substances has already been taken into account and no further combination toxicity assessment is therefore required.

zRMS comments:

The risk assessment for bees based on indications of SANCO/10329 rev 2 final and EFSA (2013) is agreed by the zRMS.

It should be noted that according to conclusions of the Central Zone Steering Committee (CZSC), recommendations

of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Nevertheless, as some cMS follow risk assessment scheme provided in EFSA (2013) at the national level, the CZSC indicated that the evaluation performed in accordance with EFSA (2013) should be presented in the Core Assessment, even if its results are not the basis for conclusion on the risk to bees at the zonal level.

The screening step evaluation demonstrated acceptable acute oral and contact risk to bees. The risk to larvae was also acceptable, but the ETR for chronic risk was above the respective trigger and Tier 1 evaluation was deemed necessary for the chronic oral exposure.

Tier 1 calculations demonstrated acceptable chronic risk to bees in all relevant scenarios following uses of Mevalone in orchards and vineyards at BBCH ≥ 70 . The treated crop scenario is not relevant for BBCH ≥ 70 . For uses in vineyards at BBCH 60-69 the chronic risk was acceptable in scenarios weeds in treated crop. Field margin, adjacent crop and next crop. For treated crop scenario the ETR was above the respective trigger.

Since the risk assessment based on indications of EFSA (2013) is not the basis to take decision on acceptability of the risk at the zonal level, the chronic ETR above the trigger is considered to be indicative and further assessment will have to be performed at the product authorisation by cMS that do consider EFSA (2013) at the national level.

It should be, however, pointed out that the long-term exposure of bees to the active compounds is unlikely due to rapid dissipation resulting from high volatilisation. Furthermore, thymol is indeed used by beekeepers in anti-varroa treatments. In addition to that, each active compound is present in plants from which bees collect nectar and pollen, such as thyme, lemon, roses, carnation etc., so bees are naturally exposed to all active compounds, however available data are insufficient to compare the background exposure with exposure resulting from application of Mevalone. All these aspects have to be considered by cMS at the national level.

The combined risk is covered by the performed evaluation since the formulation data were used to calculate HQ / ETR values.

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

Not relevant.

9.6.3 Effects on bumble bees

No data available.

9.6.4 Effects on solitary bees

No data available.

9.6.5 Overall conclusions

The risk assessment conducted according to “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) indicated acceptable acute oral and contact risk to bees. The risk assessment conducted according to EFSA/2013/3295 indicated acceptable acute oral and contact risk to bees and acceptable risk *via* exposure of contaminated water. The Tier 1 chronic oral adult risk assessment indicated acceptable risk to bees for orchards (all intended BBCH stages) and vineyards at BBCH ≥ 70 . The Tier 1 chronic oral adult risk assessment is above the conservative trigger value of 0.03 only for the treated crop scenario in the intended uses on vineyards at BBCH 60-69. Due to the characteristics of the active substances (extremely short half-lives, high volatility and natural occurrence) and the low attractiveness of grapevines to bees for the collection of nectar, the chronic oral exposure to adult honey bees in treated vineyards at BBCH 60-69 is unlikely. Therefore, the intended uses in vineyards at BBCH 60-69 are also considered to be acceptable. However, this will have to be dealt with at the product authorisation by the cMS that performed bee risk assessment in line with EFSA (2013) at the national level, since at the zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level. ~~and no further assessment is considered necessary.~~

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 the formulation Mevalone and were evaluated as part of the EU assessment of thymol, geraniol and eugenol. Full details of these studies are provided in the respective EU DAR and related documents.

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

Table 9.7.1-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
<i>T. pyri</i>	Mevalone (3AEY)	Mortality Glass Plate 2D	LR ₅₀ > 12420 g product/ha	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
<i>A. rhopalosiphi</i>	Mevalone (3AEY)	Mortality Glass Plate 2D	LR ₅₀ > 12420 g product/ha	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916

Endpoints in bold are used in the risk assessment

zRMS comments:

Mevalone was a representative formulation in the course of the EU review of eugenol, geraniol and thymol. Endpoints reported in Table 9.7.1-1 are confirmed to be in line with EU agreed toxicity data reported in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

It is noted that in EFSA conclusions for active compounds it is indicated that the endpoints for non-target arthropods are uncertain due to expected high volatilisation from the glass plates during the studies. However, ESCORT 2 does not provide indications how to perform tests with substances prone to volatilisation. Taking this into account together with the fact that similar volatilisation is expected from soil and plant surfaces after application of Mevalone, results of the studies are considered relevant for the risk assessment, similarly as they were considered in the course of the EU review.

9.7.1.1 Justification for new endpoints

Not relevant.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 2 (orchards) also covers the risk for non-target arthropods from all other intended uses in group 1 (vineyards) (see 9.1.2).

The in-field exposure (predicted environmental rate, PER_{in-field}) is calculated according to the ESCORT 2 guidance document using the following equation:

$$PER_{in-field} = \text{Application rate} \times \text{MAF}$$

The potential risk to non-target arthropods exposed in-field to Mevalone was assessed calculating the hazard quotient (HQ = exposure in-field / toxicity) and comparing with the trigger value of 2.

Table 9.7.2.1-1: Tier 1 in-field risk assessment to non-target arthropods for the use of Mevalone in orchards

Intended use	Orchards (Pome fruit)		
Product	Mevalone		
Application rate (g product/ha)	4 x 4116 g product/ha (7-day interval)		
MAF	2.7 (foliar); 3.4 (soil) Appendix V of ESCORT 2		
Foliar exposure			
Test species Tier I	L/ER₅₀ (lab.) (g product/ha)	PER_{in-field} (foliar) (g product/ha)	HQ_{in-field} (foliar) criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	> 12420	11113	<0.9
<i>Aphidius rhopalosiphi</i>	> 12420	11124	<0.9
Soil exposure			
<i>Typhlodromus pyri</i>	> 12420	13994	<1.1
<i>Aphidius rhopalosiphi</i>	> 12420	14008	<1.1

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

As the HQ values are below the trigger of 2 for both indicator species, the in-field risk to non-target arthropods for the intended uses of Mevalone in vineyards and orchards is considered to be acceptable.

zRMS comments:

The in-field risk assessment presented above is in general agreed by the zRMS with minor correction of the in-field exposure.

Based on the above calculations, acceptable in-field risk to non-target arthropods may be concluded.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 2 (orchards) also covers the risk for non-target arthropods from all other intended uses in groups 1 (vineyards) (see 9.1.2) because according to the drift values obtained from ESCORT 2, orchards represent the worst-case drift values.

The off-field exposure (predicted environmental rate, PER_{off-field}) is calculated according to the ESCORT 2 guidance document using the following equation:

$$PER_{off-field} = \frac{\text{maximum } PER_{in-field} \times \left(\frac{\%drift}{100}\right)}{\text{vegetation distribution factor}} \times \text{correction factor}$$

The potential off-field risk to non-target arthropods exposed to Mevalone was assessed calculating the hazard quotient (HQ = exposure off-field / toxicity) and comparing with the trigger value of 2.

Table 9.7.2.2-1: Tier 1 off-field risk assessment to non-target arthropods for orchards early application (as a worst-case)

Intended use	Orchards (Pome fruit)				
Product	Mevalone				
Application rate (g product/ha)	4 × 4116 g product/ha (7-day interval)				
MAF	2.7 (foliar); 3.4 (soil) (Appendix V of ESCORT2)				
vdf	10 (Tier 1) / 5 (in line with current discussion at the EU level)				
Test species Tier I	LR₅₀ (lab.) (g product/ha)	%Drift rate	CF	PER_{off-field} (g product/ha)	HQ_{off-field} criterion: HQ ≤ 2
Foliar exposure (VDF 10)					
<i>Typhlodromus pyri</i>	>12420	23.61 ^a	10	2626.3	<0.21
<i>Aphidius rhopalosiphi</i>	>12420				<0.21
Foliar exposure (VDF 5)					
<i>Typhlodromus pyri</i>	>12420	23.61 ^a	10	5248	<0.42
<i>Aphidius rhopalosiphi</i>	>12420				<0.42
Soil exposure					
<i>Typhlodromus pyri</i>	>12420	23.61 ^a		3307.3	<0.27
<i>Aphidius rhopalosiphi</i>	>12420				<0.27

^a Appendix IV of ESCORT 2. Early application in orchards is considered as a worst-case (3 m), covering also uses in vines. MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

As the HQ values are below the trigger of 2 for both indicator species, the results of the off-field risk assessment indicate an acceptable off-field risk for non-target arthropods following the intended uses of Mevalone in vineyards and orchards without the need for mitigation measures.

zRMS comments:

The off-field risk assessment presented above is in general agreed by the zRMS with some corrections.

According to ESCORT 2, for the off-field exposure only MAF for leaf substrates (2.3:1) is applicable and for this reason calculation for soil off-field exposure was struck through in Table 9.7.2.2-1 above.

With regard to the vegetation distribution factor, some Member States prefer to consider VDF of 5 in the off-crop risk assessment. However, in line with implementation schedule indicated in the Bullet points in area of ecotoxicology agreed by the CZSC in November 2021, VDF of 5 should be considered since 1st of July 2022. Furthermore, Bullet point 4 presented in this document indicates that:

The majority of MSs agreed to be in line with the EFSA Technical Report (2019) and use a VDF of 5

It should be pointed out that EFSA Technical Report (EFSA Supporting publication 2019:EN-1673) does not indicate that currently VDF of 5 must be used in evaluations, but that VDF of 5 should be considered as an interim solution that will be reflected in the SANCO/10329/2002-rev.2 guidance document with its implementation considered further. However, the SANCO guidance document was not amended yet and this is acknowledged in the most recent version of the Working document on Risk Assessment of Plant Protection Products in the Central Zone (May 2021):

The CZSC will make an urgent request to the Commission to adjust this issue in the guidance document as soon as possible.

Therefore, from the formal point of view, VDF of 10 is still applicable and may be used for purposes of calculation of the off-field exposure. It is also uncertain if consideration of VDF of 5 will be possible after 1st of July 2022 in case it will not be reflected in the terrestrial GD as an interim solution.

Nevertheless, additional calculations based on VDF of 5 have been included in Table 9.7.2.2-1 above for convenience of the cMS preferring this option.

Calculations were based on the worst case drift of 23.61% relevant for four early application in orchards, covering both late uses in apples and vines.

Based on the above calculations, acceptable off-field risk to non-target arthropods may be concluded for the intended uses of Mevalone with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

The risk assessments presented above concluded acceptable risk at the first tier. Therefore, no further studies or assessments are considered to be necessary.

9.7.2.4 Risk mitigation measures

The risk assessments presented above concluded acceptable risk at the first tier. No risk mitigation needed.

9.7.3 Overall conclusions

The results of the risk assessment indicate an acceptable **in- and off-field** risk for non-target arthropods following the intended uses of product Mevalone in vineyards and orchards without the need for mitigation measures.

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

9.8.1 Toxicity data

The acute toxicity of Mevalone to earthworms was evaluated as part of the first EU assessments of thymol, geraniol and eugenol. Full details of this study are provided in the respective EU DAR and related documents.

To meet new product data requirements, new studies assessing the chronic effects on earthworms and *Folsomia* due to the use of Mevalone are also submitted with this application; listed in Appendix 1 and summarised in Appendix 2.

Justifications to support the new endpoints are provided below.

Table 9.8.1-1: 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>	Mevalone	Acute toxicity (mixed into substrate 14 days, 10% peat)	LC ₅₀ > 1000 mg product/kg soil LC _{50,corr} > 500 mg product/kg soil	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
<i>Eisenia andrei</i>	Mevalone	Mixed into substrate 28 days, Chronic 10% peat content	NOEC (reproduction) = 52.9 mg product/kg dry soil NOEC_{corr} (reproduction) = 26.5 mg product/kg dry soil Corresponding to: NOEC _{corr} (reproduction) = 0.85 mg eugenol/kg dry soil NOEC _{corr} (reproduction) = 1.85 mg geraniol/kg dry soil NOEC _{corr} (reproduction) = 1.65 mg thymol/kg dry soil NOEC (mortality) = 556 mg product/kg dry soil EC ₁₀ (reproduction) = 86.8 mg product/kg dry soil EC _{10,corr} (reproduction) = 43.4 mg product/kg dry soil	CP 10.4.1.1/01
<i>Folsomia candida</i>	Mevalone	Mixed into substrate 28 days, Chronic 5% peat content	NOEC = 45.0 mg product/kg dry soil NOEC _{corr} = 22.5 mg product/kg dry soil EC ₁₀ = 37.3 mg product /kg dry soil EC_{10,corr} = 18.65 mg product/kg dry soil Corresponding to: EC _{10,corr} = 0.6 mg eugenol/kg dry soil EC _{10,corr} = 1.3 mg geraniol/kg dry soil EC _{10,corr} = 1.15 mg thymol/kg dry soil NOEC (mortality) = 45.0 mg product/kg dry soil	CP 10.4.2/01

*corr: corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. Endpoints in bold are used in the risk assessment

zRMS comments:

Studies on effects of Mevalone on earthworms and *Folsomia candida* were evaluated and agreed by the zRMS. Summaries of the studies together with details of evaluation are presented in Appendix 2. Endpoints reported in Table 9.8.1-1 are confirmed with some corrections resulting from the zRMS evaluation of the studies (i.e. EC₁₀ values for earthworm reproduction are struck through as being not reliable).

No study with *Hypoaspis aculeifer* was performed, but in line with data requirements set by the Commission Regulation (EU) No 284/2013:

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 (see point 10.4.2.1).

As acceptable in- and off-field risk to *Typhlodromus pyri* and *Aphidius rhopalosiphi* could be concluded based on Tier I data with no concerns and Mevalone is not applied directly to soil, a study performed with *Folsomia candida* only is deemed sufficient, as in general testing with any of the species is in this case not mandatory.

9.8.1.1 Justification for new endpoints

A new chronic earthworm toxicity study has been provided with formulation Mevalone to meet new data requirements under Regulation (EU) No 284/2013; references are listed in Appendix 1 and the summaries are included in Appendix 2 of this document. It is noted that *Hypoaspis aculeifer* and *Folsomia candida* studies are not formally required as there is no direct application to soil and a low risk is concluded at Tier 1 with *T. pyri* and *A. rhopalosiphi*, but a new *Folsomia candida* study is provided for completeness.

zRMS comments:

Please, refer to the commenting box in point 9.8.1 above for zRMS comments on the data requirements for Mevalone.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 to 8.7-9. According to the assessment of environmental-fate data, multi-annual accumulation in soil is not needed to be considered for eugenol, geraniol and thymol.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 vineyards also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in groups 2 orchards (see 9.1.2).

Table 9.8.2.1-1: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Mevalone in vineyards

Intended use	Vineyards		
Chronic effects on earthworms			
Product/active substance	NOEC_{corr} (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
Mevalone	26.5	2.195	12.1
Chronic effects on other soil macro- and mesofauna (<i>Folsomia</i>)			
Product/active substance	EC_{10,corr} (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
Mevalone	18.65	2.195	8.5

Table 9.8.2.1-2: 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 eugenol, geraniol and thymol in vineyards

Intended use	Vineyards		
Chronic effects on earthworms			
Product/active substance	NOEC_{corr} (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
eugenol	0.85	0.071	12.0
geraniol	1.85	0.142	13.0
thymol	1.65	0.142	11.6
Chronic effects on other soil macro- and mesofauna (<i>Folsomia</i>)			
Product/active substance	EC_{10,corr} (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
eugenol	0.6	0.071	8.5
geraniol	1.30	0.142	9.2
thymol	1.15	0.142	8.1

As all TER_{LT} values are above the trigger of 5, the risk to earthworms and *Folsomia* following the proposed uses of Mevalone in vineyards and orchards is considered to be acceptable.

Combination toxicity

A combined effects assessment has not been performed since there are no available soil organism toxicity data for the individual active substances. However, as the above risk assessment is based on endpoints derived from formulation studies it can be considered that the presence of all three active substances has already been taken into account and no further combination toxicity assessment is therefore required.

zRMS comments:

The risk assessment provided above is agreed by the zRMS. Since neither of active compounds is expected to accumulate in soil and initial PEC_{SOIL} are relevant for TER calculations, the risk assessment based on formulation toxicity data and exposure is deemed sufficient and no separate calculations for particular active compounds are deemed necessary, especially the toxicity endpoints for active compounds were formulation endpoints (already used to calculate TER for formulated product) expressed in terms of particular active substances. Nevertheless, calculations presented in Table 9.9.2-2 were retained as additional information.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded for the intended uses of Mevalone in orchards and vineyards.

The combined risk assessment is not required since evaluation performed above was based on endpoints derived from the formulation studies, so combined effects of all three active compounds were already accounted for. It should be also noted that current guidance document on the risk assessment for soil organisms (SANCO/10329/2002 rev2 final) does not foresee combined risk assessment based on the endpoints derived for particular active substances and the risk assessment performed with consideration of the formulation toxicity data is considered sufficient since to combined effects of the active compounds are already covered in the formulations studies. It should be also noted that in line with the Commission Regulation (EU) No 283/2013, in case of soil organisms testing with the formulated product is more feasible and generation of endpoints for particular active compounds is not mandatory.

9.8.2.2 Higher-tier risk assessment

The risk assessments presented above concluded acceptable risk at the first tier. Therefore, no further studies or assessments are considered to be necessary.

9.8.3 Overall conclusions

As all TER_{LT} values are above the trigger of 5, the risk to earthworms and other soil macro- and mesofauna following the proposed use of Mevalone in vineyards and orchards is considered to be acceptable.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

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

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

Table 9.9.1-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
Soil nitrogen transformation	Mevalone	56 days	Deviations from the control of -9.23% and -3.19% at 5.44 and 54.4 mg product/kg soil dw respectively (54.4 mg product/kg dry soil corresponds to 1.7 mg eugenol/kg dry soil; 3.5 mg geraniol/kg dry soil; and 3.5 mg thymol/kg dry soil).	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916
Carbon mineralisation	Mevalone	28 days	Deviations from the control of -9.3% and -7.1% at 5.44 and 54.4 mg product/kg soil dw respectively (54.4 mg product/kg dry soil corresponds to 1.7 mg eugenol/kg dry soil; 3.5 mg geraniol/kg dry soil; and 3.5 mg thymol/kg dry soil).	EFSA Journal 2012;10(11):2914 EFSA Journal 2012;10(11):2915 EFSA Journal 2012;10(11):2916

Endpoints in bold are used in the risk assessment

zRMS comments:

Mevalone was the representative formulation for all three active compounds and toxicity data for soil microorganisms presented in Table 9.9.1-1 are in line with EU agreed endpoints reported in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively.

Results of studies on effects on carbon mineralisation were struck through in table above as being no longer a data requirement.

9.9.1.1 Justification for new endpoints

Not relevant.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 to 8.7-9 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9.2-1: Assessment of the risk for effects on soil micro-organisms due to the use of Mevalone in vineyards

Intended use	vineyards		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC_{soil} (mg/kg dw)	Risk acceptable?
Mevalone	54.4	2.195	Yes

Table 9.9.2-2: Assessment of the risk for effects on soil micro-organisms due to the use of eugenol, geraniol and thymol in vineyards

Intended use	vineyards		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC_{soil} (mg/kg dw)	Risk acceptable?
eugenol	1.74	0.071	Yes
geraniol	3.50	0.142	Yes
thymol	3.50	0.142	Yes

zRMS comments:

The risk assessment provided above is agreed by the zRMS.

Since neither of active compounds is expected to accumulate in soil and initial PEC_{SOIL} are relevant for the risk assessment purposes, evaluation based on formulation toxicity data and exposure is deemed sufficient and no separate calculations for particular active compounds are deemed necessary, especially the toxicity endpoints for active compounds were formulation endpoints (already used to calculate TER for formulated product) expressed in terms of particular active substances. Nevertheless, calculations presented in Table 9.9.2-2 were retained as additional information.

Overall, no unacceptable effects on soil microbial activity are expected following the intended uses of Mevalone in orchards and vineyards.

9.9.3 Overall conclusions

No significant effects (<25%) on soil micro-organisms were shown for Mevalone (including eugenol, geraniol and thymol) at concentrations greater than the maximum predicted environmental concentrations in soil. The risk to soil micro-organisms from the proposed uses of Mevalone is therefore considered to be acceptable.

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

9.10.1 Toxicity data

Effects on non-target terrestrial plants of Mevalone were evaluated as part of the EU assessments of thymol, geraniol and eugenol. No specific studies were undertaken to evaluate effects on non-target plants, but preliminary screening data found no effects on a range of dicotyledonous and monocotyledonous non-target plants exposed to application rates including 4 x 4 L product/ha and higher (EFSA Journal 2012;10(11):2914; EFSA Journal 2012;10(11):2915; EFSA Journal 2012;10(11):2916). No adverse effects were observed on any crops adjacent to those on which any of the efficacy or taint trials were located. The efficacy of Mevalone against diseases on other crop types has been investigated in glasshouse and field trial studies and these have not reported any observed phytotoxic effects at rates equivalent or higher than that proposed for Mevalone in vineyards or orchards.

Table 9.10.1-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Project / Trial No.	Year	Crop	Location/ Country	Organisation	GEP (Y/N)	Trial Type (glasshouse / field / efficacy / other)	Application rate L product/ha / No of applic. to crop	Phytotoxicity Observed (Y/N)
N/A	2006	Glasshouse mini potted Rose	Cornell University, USA	Long Island Horticultural Extension Centre	N	Glasshouse efficacy	4 L/ha x3	N
EDZI002	2006	Potato	As, Norway	Bioforsk Plantehelse	Y	Field efficacy	2 L/ha x8	N
N/A	2006	Cucumber	Malaysia	Zagro	N	Field efficacy	3 L/ha x4	N
N/A	2006	Water Melon	Malaysia	Zagro	N	Field efficacy	3 L/ha x4	N
EDB011	2007	Cucumber	Cornell University, USA	Long Island Horticultural Extension Centre	N	Glasshouse efficacy	2 L/ha x3	N
EDB012	2007	Lettuce	Cornell University, USA	Long Island Horticultural Extension Centre	N	Glasshouse efficacy	4 L/ha x4	N
EDB020	2006	Lettuce	Cornell University, USA	Long Island Horticultural Extension Centre	N	Glasshouse efficacy	4 L/ha x4	N
Eden-1	2006	Capsicum	Virginia, South Australia	University of Adelaide	N	Glasshouse efficacy	2 L/ha x6	N
EDN-1-1	2006	Walnut	Forth, Tasmania	Agromico Research	N	Field efficacy	4.5 L/ha x3	N
EDR-605	2006	Capsicum	Adelaide, Australia	SARDI	N	Glasshouse efficacy	2 L/ha x3	N
EDZE002	2006	Lucumo	Peru	Agrarian National University	N	Field efficacy	3 L/ha x3	N
N/A	2006	Papaya	Balm, Florida	I.F.A.S., University of Florida	N	Glasshouse efficacy	3 L/ha x1	N
1110055	2005	Strawberry	Valldal, Norway	Bioforsk Plantehelse	Y	Field efficacy	4 L/ha x4	N
N/A	2006	Cacao	Nkometou, Cameroon	IRAD Cameroon	N	Field efficacy	4 L/ha x8	N
N/A	2007	Turf	Yorkshire, UK	STRI	Y	Field efficacy	N /A	N/A
BX1161	2007	Oilseed Rape	ADAS Rosemaund,	Rothamsted Research	Y	Field efficacy	2 L/ha x2	N
WW814-01	2007	Winter Wheat	Harpenden, Hertfordshire	Rothamsted Research	Y	Field efficacy	4 L/ha x2	N
S08-01991	2008	Pasture	Derbyshire, UK	Eurofins	Y	Field efficacy	12 L/ha x1	N
AF/10729/ED/A	2006	Courgette / Cucumber	3x Spain 4x Greece	Eurofins	Y	Field efficacy	4 L/ha x4-6	N
AF/10729/ED/B	2006	Courgette / Cucumber	1x Spain 2x Greece	Eurofins	Y	Field efficacy	4 L/ha x4-6	N

zRMS comments:

Information presented in Table 9.10.1-1 was taken from the DARs for particular active compounds (see e.g. Geraniol, Vol. 3, B.9 of May 2011) and at the EU level it was considered sufficient to address any potential concerns regarding phytotoxic activity of Mevalone to non-target plants.

following conclusion regarding the risk to non-target plants is provided in EFSA Journal 2012;10(11):2914, EFSA Journal 2012;10(11):2915 and EFSA Journal 2012;10(11):2916 for eugenol, geraniol and thymol, respectively:

No effects seen on a range of dicotyledonous and monocotyledonous non-target plants exposed to application rates including 4 x 4 L „Mevalone 3AEY“/ha and higher.

9.10.1.1 Justification for new endpoints

Not relevant.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Due to the relative crop safety of Mevalone it is considered unlikely that Mevalone, applied as per the proposed label recommendations, would have any impact on other non-target terrestrial plants *via* off-field exposure. The available information is sufficient to address any potential concerns about the phytotoxic activity of Mevalone to off-field non-target terrestrial plants. The risk is therefore concluded as acceptable and no further consideration is required.

zRMS comments:

Since Mevalone is not a herbicide, the screening data taken from the Efficacy section are deemed sufficient to address the risk to non-target terrestrial plants.

No phytotoxic effects were observed in any of the EU agreed efficacy trials performed on a range of monocotyledonous and dicotyledonous crops at applications rates including 4x4.0 L product/ha and higher.

In addition to that, no phytotoxic effects were observed in efficacy studies performed in vines and apples up to 4x4.0 L product/ha, evaluated and agreed by the zRMS efficacy expert (see Core Assessment, Part B, Section 3 for details).

Based on lack of phytotoxic effects in any of the efficacy trials, acceptable risk to non-target terrestrial plants may be concluded for the intended uses of Mevalone in orchards and vineyard.

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

Not relevant.

9.10.3 Overall conclusions

The risk to non-target terrestrial plant due to the use of the product Mevalone is considered to be acceptable based on preliminary screening data **with no need for risk mitigation measures.**

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

No further data are considered to be necessary. The risk to standard organisms has been shown to be acceptable.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

The proposed classification and labelling of Mevalone (3AEY) for environmental hazards has been determined according to the ECHA guidance on the application of the CLP criteria, version 5.0 (July 2017). Available aquatic data on the formulation are summarized below.

Available formulation data for Mevalone (3AEY) for classification to the aquatic environment

96 hour LC ₅₀ , fish	Mevalone (3AEY) LC ₅₀ = 31.1 mg/L
48 hour EC ₅₀ , <i>Daphnia</i>	Mevalone (3AEY) EC ₅₀ = 35.4 mg/L
72 hour EC ₅₀ , algae	Mevalone (3AEY) E _r C ₅₀ = 100.8 mg/L
72 hour NOEC EC ₅₀ , algae	Mevalone (3AEY) NOE _r C = 32.0 mg/L

Mevalone (3AEY) contains three active substances, eugenol, geraniol and thymol. Only thymol has a harmonised classification under Annex VI of Regulation (EC) No 1272/2008 as follows:

Thymol

Aquatic Chronic 2; H411

Conclusion short-term (acute) aquatic hazard

The available acute toxicity of Mevalone (3AEY) as a whole formulation has been tested and can be used for classification of the mixture. Mevalone (3AEY) is not classified for short-term (acute) hazard as acute toxicity data are available for three trophic levels (fish, crustacean and algae), demonstrating L/EC₅₀ values >1 mg/L.

Conclusion long-term (chronic) aquatic hazard

Chronic toxicity of Mevalone (3AEY) as a whole formulation has not been tested. Classification of Mevalone (3AEY) for the long-term (chronic) aquatic hazard is therefore based on the available data for its components. With the exception of thymol all other components of the formulation (see confidential section) are not classified for environmental hazards under Annex VI of Regulation (EC) No 1272/2008. Furthermore, due to its rapid volatilisation properties and ready biodegradation it is considered unlikely that thymol will be persistent and accumulate in natural water systems. The long-term exposure from thymol is regarded as minimal, hence the need for classification based on thymol (with a log Kow <4) should not be required.

Mevalone (3AEY) is not classified for long-term (chronic) hazard as toxicity data are available for three trophic levels (fish, crustacea and algae), demonstrating L/EC₅₀ values >1 mg/L. Based on the active substance thymol being present at 6.4% and the sum of the other components triggered for chronic risk is less than 25%. The overall conclusion is that the formulation is also not classified as chronic aquatic hazard category.

The proposed environmental classification and labelling of Mevalone (3AEY) according to the CLP Regulation (EC) No 1272/2008 is presented below.

Table 9.13-1: Proposed environmental classification and labelling of Mevalone (3AEY) according to the CLP Regulation (EC) No 1272/2008

Classification categories for hazard to the aquatic environment	-	
Hazard Pictograms	-	
Signal words	-	
Hazard Statements	-	
Proposed precautionary statements	Refer to the extract legislation*	

* Applicant proposed precautionary statements ~~P273~~ and P501

zRMS comments:

CLP classification proposed by the Applicant is agreed by the zRMS. Mevalone is no classified for acute and chronic hazard.

It is noted that precautionary statement P273 was proposed by the Applicant. However, this precautionary statement (avoid release to the environment) is not applicable in case release to the environment is the intended use of the product. Hence, precautionary statement P273 is not relevant for Mevalone, which is intended to be used in the environment.

Although precautionary statement is not mandatory in case the mixture in not classified for aquatic hazard, it may be displayed on the label in case proposed by the Applicant.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

These studies have also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

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	Egeler, P.	2021a	Eugenol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> Company Report No 20GC3DB IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc
KCP 10.2.1/02	Egeler, P.	2021b	Geraniol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> Company Report No 20GC1DB IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc
KCP 10.2.1/03	Egeler, P.	2021c	Thymol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> Company Report No 20GC2DB IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc
KCP 10.2.1/04	Siedel, U., Emnet, P.	2021	Geraniol: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth inhibition Test. Company Report No 155771210 IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc
KCP 10.3.1.2/01	Pecorari, F.	2019a	Chronic oral effects of ARAW on adult worker honeybees <i>Apis mellifera</i> L., 10-day feeding laboratory test Report No. BT059/19 BioTecnologie BT S.r.l., Italy GLP Unpublished	N	Eden Research plc
KCP 10.3.1.3/01	Pecorari, F.	2019b	Effects of ARAW on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure Report No. BT060/19 BioTecnologie BT S.r.l., Italy GLP Unpublished	N	Eden Research plc

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 10.4.1.1/01	Straube, D.	2021	Mevalone: Effects on Reproduction and Growth of Earthworms <i>Eisenia andrei</i> in Artificial Soil Company Report No 155781022 IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc
KCP 10.4.2.1/01	Straube, D.	2020	Mevalone: Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil Company Report No 155781016 IBACON Gmbh, Rossdorf, Germany GLP Unpublished	N	Eden Research plc

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

These studies have been submitted within the first approval dossier of active substances (RMS: UK) and/or for registration of product in SEU (see part B0 for Regulatory history of active substances and product).

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1.1/01	XXXXX	2007	3AEY (thymol/geraniol/eugenol mixture): An acute oral toxicity study with the Northern Bobwhite Report No. 648-101 Wildlife International, Ltd, USA GLP Unpublished	Y	Eden Research plc
KCP 10.2.1/01	XXXX	2008a	Acute toxicity of 3AEY to rainbow trout (<i>Oncorhynchus mykiss</i>) in a 96-hour semi-static test Report No. 34301230 IBACON GmbH, Germany GLP Unpublished	Y	Eden Research plc
KCP 10.2.1/02	Grade, R., Wydra, V.	2008a	Acute toxicity of 3AEY to <i>Daphnia magna</i> in a static 48-hour immobilization test Report No. 34302220 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc
KCP 10.2.1/03	Grade, R., Wydra, V.	2008c	Toxicity of 3AEY to <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test Report No. 34303210 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc
KCP 10.3.1.1.1/01	Schmitzer, S.	2007	Effects of 3AEY (acute contact and oral) on honey bees (<i>Apis mellifera L.</i>) in the laboratory Report No. 34304035 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.1/01	Moll, M.	2007a	Effects of 3AEY on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory - Dose Response Test Report No. 34306063 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc
KCP 10.3.2.1/02	Moll, M.	2007b	Effects of 3AEY on the Parasitoid <i>Aphidius rhopalosiphi</i> in the Laboratory - Dose Response Test Report No. 34305001 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc
KCP 10.4.1.1/01	Lührs, U.	2007	Acute toxicity (14 days) of 3AEY to the earthworm <i>Eisenia fetida</i> in artificial soil Report No. 34307021 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc
KCP 10.5/01	Reis, K.H.	2007	Effects of 3AEY on the activity of the soil microflora in the laboratory Report No. 34308080 IBACON GmbH, Germany GLP Unpublished	N	Eden Research plc

zRMS comments:

As most of endpoints for eugenol, geraniol and thymol were taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for particular active compounds.

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
There were no data submitted by the Applicant and not relied on.					

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
There were no data relied on and not submitted by the Applicant.					

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 and chronic toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Study 1

Comments of zRMS:	<p>The study was performed in line with OECD 211 with no deviations.</p> <p>The test item concentrations were measured in fresh and aged media, but not at each renewal, but once a week. This is considered acceptable, since from performed analyses sufficient data for determination of the mean measured concentrations are available.</p> <p>Correction of mortality in test item groups is not relevant in case of aquatic toxicity study and is thus not considered. Nevertheless, no increased mortality was observed in the treatment groups with exception of the second lowest concentration with 30% mortality, which is, however, considered to be incidental, since at higher test concentrations mortality was at level comparable with controls (i.e. 10%) and no dose-response was observed.</p> <p>All the validity criteria were met and the study is considered acceptable with following endpoints are relevant for the risk assessment:</p> <p>NOEC (reproduction) = 0.0959 mg a.s./L (based on time weighted mean measured concentrations) NOEC (immobilisation) = 0.0959 mg a.s./L (based on time weighted mean measured concentrations)</p> <p>ECx could not be determined due to lack of the dose-response, but it would be >0.0959 mg a.s./L, the maximum measured concentration tested.</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.2.1/01
Report	Eugenol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> XXXXXX, 2021, report No 20GC3DB
Guideline(s):	Yes. OECD Test Guideline 211 (2012)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	Eugenol
Chemical Name:	2-Methoxy-4-(2-propenyl)phenol
Formulation type:	Not applicable
Source and lot/batch no.:	40002011619
Active substance content:	99.74%
Appearance:	Pale yellow liquid
Expiry date of lot/batch:	31 July 2021
Storage conditions:	In original container at ambient temperature, in the dark

Test organism

Species:	<i>Daphnia magna</i> (water flea).
Strain/clone:	M10
Age at study initiation:	<24 hours old
Source:	Originally supplied by KU Leuven, Belgium, cultured at ECT Oekotoxikologie GmbH since December 22, 2011
Feeding during test:	Three times per week with fresh algae suspension
Acclimation:	Not applicable

Test conditions

Test concentrations:	0, 7.81, 15.6, 31.3, 62.5 and 125 µg test item/L
Exposure regime:	Semi-static
Replicates:	10 individually held Daphnids per control and test item groups (relevant for semi-static design)
Test medium:	Elendt medium M4
Hardness:	250 - 268 mg/L as CaCO ₃
Test temperature:	19.7 – 21.6 °C* (manual measurement)
pH:	7.7 – 8.9*
Dissolved oxygen:	9.2 – 11.2 mg/L
Photoperiod:	16 hours of light / 8 hours of dark
Light intensity:	16.45 – 17.36 µE m ⁻² s ⁻¹

* Measured (x 2) on days 0, 3, 10, 12, 17 and 19 of the test

Ten replicate vessels, each a glass beaker containing a single daphnid in 50-60 mL medium, were allocated to each test concentration and control. Test solutions were renewed three times per week (semi-static test system). The daphnids were fed three times per week after transfer to fresh test solutions. Daily observations were made of the parental daphnids in all test vessels; immobile parental daphnids were removed upon recording. From day 8, onwards, the live offspring (F1 generation) was counted daily and removed from the vessels. Deviations in behaviour compared to the control animals, presence of aborted eggs or dead offspring were recorded.

Samples of test media were taken once a week at the start and end of selected media renewal cycles, during the 21-day exposure period, and analysed for eugenol by GC-MS. Water quality parameters (dissolved oxygen concentration, pH, water hardness and temperature (manual measurement)) were determined once per test week in fresh and aged solutions of the control and the highest test item concentration.

The Cochran-Armitage test procedure was applied with immobility at 21 days to detect an increasing trend in responses (Alpha: 0.050; one-sided greater). Determination of EC_x values for parental immobility by Probit analysis was not possible due to the lacking concentration-response relationship. Parental immobility was additionally corrected for control immobility using Abbott's formula. Determination of EC_x values for reproduction, using non-linear regression analysis, was not possible due to the poor concentration-response relationship. Prior to threshold concentration testing, a qualitative trend analysis by contrasts was applied to check for monotonicity of the concentration-response relationship. Dunnett's multiple t-test procedure (p≤0.05) was used to determine the threshold concentrations for reproduction. Fisher's Exact Binomial Test with Bonferroni correction was used to determine the threshold concentration for parental immobility.

The statistical software package ToxRat Professional 3.3.0 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations using the nominal concentrations.

Results and discussions

The GC-MS analytical method for the determination of eugenol in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for eugenol in the control sample. The analytical calibration was shown to be linear ($r = 0.9951$) over the range of 1 - 200 μg eugenol/L. Accuracy was confirmed with recovery determined by fortification of eugenol at 3.5 and 150 μg eugenol/L; all recoveries were within the range of 81-110% and mean recoveries were within 82-107% (i.e. within the guideline range of 70-110%). Precision was confirmed with five determinations made at each fortification level; the relative standard deviation was between 0.9-4.9% (i.e. within the guideline limit of $\leq 20\%$). The limit of quantification (LOQ) was 3.50 μg eugenol/L (i.e. below the biological NOEC value). The limit of detection (LOD) was 1.05 μg eugenol/L in test medium. All samples were analysed within 24 h after extraction, therefore the stability of eugenol in the final extracts was not assessed.

The measured concentrations of eugenol during the 21-day exposure period of the *Daphnia magna* toxicity study are summarised in the tables below.

Table 10.2.1/01-1: Nominal and measured concentrations of eugenol in each replicate during the 21-day study

Nominal concentration (μg eugenol/L)	Measured concentration (μg eugenol/L)							Percentage of nominal (%)						
	Day						Mean ^a	Day						Mean ^a
	0F	3A	10F	12A	17F	19A		0F	3A	10F	12A	17F	19A	
Control	<LOD	-	-	-	-	<LOQ (1.36)*	-	-	-	-	-	-	-	-
7.81	6.82	3.83	7.58	<LOQ (1.93)	8.01	<LOQ (1.47)	4.50	87.3	49.0	97.1	n.a	103.0	n.a	57.6
15.6	14.0	10.5	16.5	4.41	15.2	6.66	10.8	89.7	67.3	106	28.3	97.4	n.a	69.2
31.3	27.3	19.5	28.6	9.78	31.6	13.4	21.0	87.2	62.3	91.4	31.2	101	42.7	67.1
62.5	63.2	40.7	64.0	29.1	65.7	27.5	47.1	101	65.1	102	46.6	105	42.8	75.4
125	127	81.2	130	65.1	135	53.9	95.9	102	65.0	104	52.1	108	44.0	76.7

^a time weighted mean

n.a. = not applicable

*both qualifier mass fragments yielded a residue < LOD

F: fresh media; A:aged media

The measured concentrations of 7.81 μg eugenol/L (nominal) were below LOQ but above LOD (measured values shown in brackets in table above) on days 12 and 19. The measured values were used for calculation of the time-weighted mean (TWM) as shown in the following table.

Table 10.2.1/01-2: Summary of nominal and time-weighted mean measured concentrations of eugenol during the 21-day study

Nominal concentration (μg eugenol/L)	Measured concentration (μg eugenol/L)					
	Control	7.81	15.6	31.3	62.5	125
Range (min - max)	-	4.41 – 16.5	9.78 – 31.6	13.4–28.6	27.5–65.7	53.9–135
Mean*	-	4.50	10.8	21.0	47.1	95.9
% of nominal	-	57.6	69.2	67.1	75.4	76.7

* time weighted mean

Since the analytical verification of the test item concentrations confirmed that measured concentrations were unstable and below 80% of nominal concentrations, the biological endpoints are therefore based on mean measured (time-weighted) concentrations.

Biological results

Summaries of the effects of eugenol on parental daphnid survival/immobility and the fecundity of the introduced and surviving parent daphnids are presented in the tables below.

Table 10.2.1/01-3: Total mobility/immobility of parental daphnids at the end of the 21-day study

Nominal concentration (µg eugenol/L)	Mean measured concentration (µg eugenol/L)	Number of daphnids			% Immobility
		Introduced	Mobile	Immobile	
Control	Control	10	9	1	10.0
7.81	4.50	9 ¹	9	0	0.0
15.6	10.8	10	7	3	30.0
31.3	21.0	9 ¹	8	1	11.1
62.5	47.1	10	9	1	10.0
125	95.9	10	9	1	10.0

¹ dead parental daphnid after unintended handling error: excluded from all further analysis

One parental daphnid at nominal test concentrations of 7.81 µg eugenol/L and 31.3 µg eugenol/L died following documented unintended handling errors, before first production of offspring. These replicates were excluded from all further analysis. For any other immobile parental daphnids, a concentration-response relationship could not be confirmed.

A few sublethal effects were observed in the living parental daphnids at all concentration levels, but no concentration-response relationship based on sublethal effects in living parental daphnids was determined.

Table 10.2.1/01-4: Summary of effects of eugenol on the total number of living offspring per surviving *Daphnia magna* parent after 21-days' exposure

Nominal concentration (µg eugenol/L)	Mean measured concentration (µg eugenol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
Control	-	1	132	129.4	-
		2	152		
		3	157		
		4	102		
		5	179		
		6	108		
		7	102		
		8	125		
		9	-		
		10	108		
7.81	4.50	1	74	128.1	1.0
		2	108		
		3	114		
		4	+		
		5	150		
		6	157		
		7	111		
		8	152		
		9	163		
		10	124		
15.6	10.8	1	-	133.7	-3.3
		2	-		
		3	92		
		4	123		
		5	177		
		6	146		

Nominal concentration (µg eugenol/L)	Mean measured concentration (µg eugenol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
		7	61		
		8	185		
		9	152		
		10	-		
31.3	21.0	1	173	156.1	-20.6
		2	-		
		3	+		
		4	97		
		5	195		
		6	145		
		7	159		
		8	191		
		9	124		
		10	165		
62.5	47.1	1	131	138.2	-6.8
		2	-		
		3	102		
		4	166		
		5	135		
		6	131		
		7	163		
		8	125		
		9	131		
		10	160		
125	95.9	1	117	135.4	-4.6
		2	126		
		3	127		
		4	107		
		5	-		
		6	161		
		7	163		
		8	93		
		9	149		
		10	176		

+ = documented handling accident: excluded from all evaluation

- = inadvertent mortality (unknown cause): offspring excluded from statistical analysis

* % offspring reduction compared to control (negative values = higher number than control)

The total number of living offspring was evaluated per surviving parent daphnid and per introduced parent daphnid, which did not die accidentally or inadvertently during the test. No concentration-response relationship was observed for reproduction.

Summary of biological results

Nominal concentration	[µg/L]	Control	7.81	15.6	31.3	62.5	125
TWM concentration	[µg/L]	Control	4.50	10.8	21.0	47.1	95.9
Number of replicates ¹	n	10	9	10	9	10	10
Mean living offspring per introduced	mean offspring [% of control]	127.2	128.1	112.3	139.6	137.1	125.1
	inhibition [% of control]	--	100.7	88.3	109.7	107.8	98.3
		--	-0.7	11.7	-9.7	-7.8	1.7
Adult immobility	[% of initial number]	10.0	0.0	30.0	11.1	10.0	10.0
	[% corrected for control immobility]	0.0	0.0	22.2	1.2	0.0	0.0
Mean living offspring per survivor	mean offspring [% of control]	129.4	128.1	133.7	156.1	138.2	135.4
	inhibition [% of control]	--	99.0	103.3	120.6	106.8	104.6
		--	1.0	-3.3	-20.6	-6.8	-4.6

¹: Replicates with parental mortality due to documented unintended handling error were excluded.

Since no concentration-response relationship was observed for reproduction, EC_x values could not be calculated, but are estimated to be greater than the highest concentration tested (i.e. >95.9 µg eugenol/L (mean measured)). The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 95.9 µg eugenol/L and the corresponding LOEC value was estimated to be >95.9 µg eugenol/L (mean measured).

Validity

All validity criteria were met in accordance with OECD test guideline 211 (2012):

- The mortality of the parent animals (female *Daphnia*) in the controls does not exceed 20% at the end of the test; (actual value: 10%)
- The mean number of living offspring produced per surviving parent animal in the controls at the end of the test is >60 (actual value: 129.4 ~~134.5%~~)
- Analytical measurement of test concentrations was included.

Conclusion

The 21-day chronic toxicity of eugenol to *Daphnia magna* was studied under static-renewal conditions in accordance with OECD test guideline 211 (2012). Since no concentration-response relationship was observed for reproduction, EC_x values could not be calculated.

The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 95.9 µg eugenol/L and the corresponding 21-day LOEC value was estimated to be >95.9 µg eugenol/L (mean measured).

Parameter	Endpoints based on measured concentrations [µg test item/L]				
	EC ₁₀	EC ₂₀	EC ₅₀	NOEC	LOEC
Reproduction based on surviving F0 daphnids	n.d.	n.d.	n.d.	95.9	>95.9
Reproduction based on introduced F0 daphnids	n.d.	n.d.	n.d.	95.9	>95.9
Mortality/immobility of parental daphnids	n.d.	n.d.	n.d.	95.9	>95.9

n.d.: not determined due to lacking concentration-response relationship.

Study 2

Comments of zRMS:	<p>The study was performed in line with OECD 211 with no deviations regarding the test design and conditions. All the validity criteria were met.</p> <p>Correction of mortality in test item groups is not relevant in case of aquatic toxicity study and is thus not considered.</p> <p>The measured concentrations in fresh media were at 80-120% of nominal. However, the measured concentrations in aged test media were <LOQ (at the highest test item concentration) or <LOD (in remaining groups), so calculation of the mean measured concentrations was in general not possible.</p> <p>The study authors decided to calculate the time-weighted mean measured concentrations assuming $\frac{1}{2}$ LOQ or $\frac{1}{2}$ LOD. This procedure is used in the kinetic evaluation of the degradation data, however it should be taken into account that in case of degradation studies there are at least 4-5 data points and in case samples at the last samplings give concentrations <LOQ or <LOD, there are still earlier data points that may be sufficient to obtain reliable fits. In case of this study there are only two “data points” – one on day 0 in fresh solution and second on day 3, in aged solution at renewal and it is not known when the concentration of the test item dropped below LOQ/LOD. In case more data points were available, it would be possible to model the degradation in test solutions and use this information for estimation of the exposure over time.</p> <p>It should be noted that in case of highly unstable substances (to which geraniol obviously belongs to) it is recommended to increase the sampling intervals to obtain more information on the degradation time. Alternatively, more frequent renewal of test solutions should be considered or the flow-through study should be performed (provided that it will not increase mortality of the test organisms).</p> <p>Taking this into account it may be concluded that the properties of the test item were not taken into account in selection of the sampling intervals, renewal intervals or the exposure regime and the study should be in general invalidated.</p> <p>However, there is currently increasing need to authorise products based on natural substances and rejection of this study would mean that the chronic risk to invertebrates would not be addressed and in consequence – authorisation of the product would be not possible. For this reason the zRMS decided to discuss in more detail the issue of insufficient data on measured concentrations in test solutions</p> <p>It is noted that in the aged solutions of the highest test concentrations geraniol was present at quantifiable levels (although <LOQ), which gives some indication that the dissipation have not occurred within one day and the test organisms were exposed to the test item for more than several hours. It is further noted that in the EU agreed acute toxicity studies for fish and <i>Daphnia magna</i> performed under static conditions, geraniol concentrations were maintained at 80-120% over the study period, so it is likely that the dissipation in the chronic study was rather gradual and the test organisms were exposure for longer period of time than only first hours. Reason for faster dissipation in the chronic study comparing to acute fish and <i>Daphnia</i> studies is unknown, but potentially it could be caused by presence of food for <i>Daphnia</i> (algal suspension), since in the EU agreed algae study concentrations of geraniol dropped <LOD after 96 hours at the test termination, although the initial concentrations were maintained at 80-120% of nominal. Despite this, endpoints based on nominal concentrations were reported in EFSA Journal 2012;10(11):2915. It is noted that EFSA in its comment indicated that the endpoints should not be based on nominal concentrations due to lack of chemical analyses after 48 and 72 hours and no detectable residues of geraniol at 96 hours, but no action in relation to the active substance study was requested by EFSA in the Reporting Table and an Open point was set for the formulation study only (to indicate that derived E_rC_{50} values is an extrapolated value just above the tested range). Issue of no detectable residues of geraniol in the algae study was not a subject of further discussion in the expert meeting and no data gap due to lack of relevant chemical analyses was set in the EFSA conclusions. It may be thus deduced that the</p>
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	<p>explanation of the Applicant provided in the Reporting Table was agreed by EFSA (“Notifier: Since no material was detected at the end of the test it is not possible to calculate geometric mean exposures. As a result it was decided to use nominal concentrations, since the degradation seen is representative of degradation that will be observed in the environment”).</p> <p>Although geraniol was not stable in the EU agreed algae study, its mean measured concentrations were maintained at 80-120% of nominal in the newly submitted study with <i>Pseudokirchneriella subcapitata</i> summarised below (Siedel & Emnet, 2021, KCP 10.2.1/04), despite presence of algal cells.</p> <p>It should be pointed out that geraniol is currently under the EU renewal process and the study on chronic toxicity of geraniol to <i>Daphnia magna</i> has been provided to the RMS and will be discussed during the peer-review once the DRAR will be finalised. Taking into account that during the first EU review residues of geraniol in test solutions being <LOD at test termination and lack of possibility for calculation of the geometric mean concentrations were not considered to be a problem, the zRMS for Mevalone is of the opinion that the same approach should be taken, while decision on rejection of the chronic <i>Daphnia magna</i> study should be taken at the EU level, since this is an active substance study. It should be also noted that consideration of ½ LOD/LOQ in calculation of the geometric mean measured concentration is still more conservative approach than this taken at the EU level, where endpoints based on nominal concentrations were considered sufficiently reliable despite shortcomings described above. Taking all this into account and given the importance of authorisation of natural products, the zRMS decided to keep the study for illustrative risk assessment.</p> <p>The Applicant should be, however, aware that the study likely will be invalidated at the EU level and new test will have to be performed with either more frequent test solutions renewal or under flow through conditions.</p> <p>Due to too rapid dissipation of the test item from the solutions the endpoints cannot be considered fully reliable, but will be used in the illustrative risk assessment:</p> <p>NOEC (reproduction) = 0.0392 mg a.s./L (based on time weighted mean measured concentrations) EC₁₀ = 0.0520 mg a.s./L (based on time weighted mean measured concentrations) NOEC (immobilisation) = 0.119 mg a.s./L (based on time weighted mean measured concentrations)</p> <p>Although according to EFSA (2013) EC₁₀ values are preferred for the risk assessment purposes, the zRMS is of the opinion that in case of geraniol the lower endpoint (i.e. NOEC) should be used in the illustrative risk assessment for reasons discussed above.</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.2.1/02
Report	Geraniol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> Egeler, P., 2021, report No 20GC1DB
Guideline(s):	Yes. OECD Test Guideline 211 (2012)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Accepted for illustrative risk assessment due to measured concentrations of geraniol in aged solutions being <LOD/LOQ and calculated geometric mean concentrations not fully reliable as based on ½ LOD/LOQ.
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Name: Geraniol

Chemical Name:	(2E)-3,7-Dimethylocta-2,6-diene-1-ol
Formulation type:	not applicable
Source and lot/batch no.:	L4363091
Active substance content:	98.91%
Appearance:	Colourless to pale yellow liquid
Expiry date of lot/batch:	31 December 2021
Storage conditions:	Store in cool, dry, well-ventilated and dark place away from direct sunlight, in tightly closed container; stored at test facility in closed container at ambient temperature in the dark

Test organism

Species:	<i>Daphnia magna</i> (Straus) (water flea).
Strain/clone:	M10
Age at study initiation:	<24 hours old
Source:	Originally supplied by KU Leuven, Belgium, cultured at ECT Oekotoxikologie GmbH since December 22, 2011
Feeding during test:	Three to four times per week with fresh algae suspension
Acclimation:	Not applicable

Test conditions

Test concentrations:	0, 0.0244, 0.0683, 0.191, 0.536 and 1.50 mg test item/L
Exposure regime:	Semi-static
Replicates:	10 individually held Daphnids per control and test item groups (relevant for semi-static design)
Test medium:	Elendt medium M4
Hardness:	246 - 254 mg/L as CaCO ₃
Test temperature:	19.5 – 22.0 °C*
pH:	7.8 – 9.7*
Dissolved oxygen:	9.0 – 13.6 mg/L*
Photoperiod:	16 hours of light / 8 hours of dark
Light intensity:	16.18 – 17.67 μE m ⁻² s ⁻¹

*Measured (x 2) on days 0, 2, 7, 9 and 16 of the test

Ten replicate vessels, each a glass beaker containing a single daphnid in 50-60 mL medium, were allocated to each test concentration and control. Test solutions were renewed three times per week (semi-static test system). The daphnids were fed three to four times per week after transfer to fresh test solutions. Daily observations were made of the parental daphnids in all test vessels; immobile parental daphnids were removed upon recording. From day 8, onwards, the live offspring (F1 generation) was counted daily and removed from the vessels. Observations of abnormal behaviour of the test animals were recorded.

Samples of test media were taken once a week at the start and end of selected media renewal cycles, during the 21-day exposure period, and analysed for geraniol by GC-MS. Taking into account the biological and analytical results, reserve samples of the intermediate concentration levels C2-C4 were also designated for analysis. Water quality parameters (dissolved oxygen concentration, pH, water hardness and temperature (manual measurement)) were determined once per test week in fresh and aged solutions of the control and the highest test item concentration.

The Cochran-Armitage test procedure was applied with immobility at 21 days to detect an increasing

trend in responses (Alpha: 0.050; one-sided greater). Determination of EC_x values for parental immobility was performed by Weibull analysis. Parental immobility was additionally corrected for control immobility using Abbott's formula. The Step-down Cochran-Armitage Test Procedure (Alpha: 0.050; one-sided greater) was used to determine the threshold concentration for parental immobility. Non-linear regression analysis (3-parameter normal Cumulative Distribution Function (CDF)) was used to determine EC_x values for reproduction. Prior to threshold concentration testing, a qualitative trend analysis by contrasts was applied to check for monotonicity of the concentration-response relationship. The Williams Multiple Sequential t-test Procedure (p≤0.05) was used to determine the threshold concentrations for reproduction.

The statistical software package ToxRat Professional 3.3.0 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations using the nominal concentrations.

The time-weighted mean (TWM) of the measured concentrations of the fresh and aged test solutions was calculated as described in the test guideline.

The biological endpoints (EC_x, NOEC and LOEC values) were expressed based on nominal and measured concentrations of the test item.

Results and discussions

Analytical results

The GC-MS analytical method for the determination of geraniol in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for geraniol in the control sample. The analytical calibration was shown to be linear (r² ≥0.9998) over the range of 0.0036 mg geraniol/L to 0.100 mg geraniol/L. Accuracy was confirmed with recovery determined by fortification of geraniol at 0.0120 and 1.50 mg geraniol/L; all recoveries were within the range of 94-105% and mean recoveries were within 96-99% (i.e. within the guideline range of 70-110%). Precision was confirmed with five determinations made at each fortification level; the relative standard deviation was between 1.6-4.0% (i.e. within the guideline limit of ≥20%). The limit of quantification (LOQ) was 0.0120 mg geraniol/L (i.e. below the biological NOEC value). The limit of detection (LOD) was 0.0036 mg geraniol/L in test medium. All samples were analysed within 24 h after extraction, therefore the stability of geraniol in the final extracts was not assessed.

The measured concentrations of geraniol during the 21-day exposure period of the *Daphnia magna* toxicity study are summarised in the tables below.

Table 10.2.1/02-1: Nominal and measured concentrations of geraniol in each replicate during the 21-day study

Nominal concentration (mg geraniol/L)	Measured concentration (mg geraniol/L)							Percentage of nominal (%)						
	Day						Mean ^a	Day						Mean ^a
	0F	2A	7F	9A	16F	19A		0F	2A	7F	9A	16F	19A	
Control	<LOD	-	-	-	-	<LOD	-	n.a.	-	-	-	-	-	-
0.0244	0.0237	<LOD	0.0241	<LOD	0.0207	<LOD	0.0228	97.1	n.a.	98.8	n.a.	84.8	n.a.	93.5
0.0683	0.0639	<LOD	0.0652	<LOD	0.0627	<LOD	0.0639	93.6	n.a.	95.5	n.a.	91.8	n.a.	93.6
0.191	0.187	<LOD	0.181	<LOD	0.181	<LOD	0.183	97.9	n.a.	94.8	n.a.	94.8	n.a.	95.8
0.536	0.540	<LOD	0.550	0.032	0.538	<LOD	0.415	101	n.a.	103	6.0	100	n.a.	77.5
1.50	1.53	<LOQ (0.0050)	1.51	0.559	1.48	<LOQ (0.0100)	1.269	102	n.a.	101	37.3	98.7	n.a.	84.7

^a time weighted mean

^b Measured concentrations <LOD. In order to calculate time-weighted mean measured concentrations (TWM) for the values below LOQ/LOD, the respective measured concentrations were replaced by 1/2 of the LOQ (0.0120 mg/L/2=0.00600 mg/L) or 1/2 of the LOD (0.0036 mg/L/2 = 0.0018 mg/L), respectively. This approach is considered justified, since it integrates the decreased concentrations in the aged test solutions, and since otherwise the calculation of TWM, and consequently the expression of the biological endpoints based on TWM would not be possible.

F: fresh media; A: aged media n.a. not applicable

Table 10.2.1/02-2: Summary of nominal and mean measured concentrations of geraniol during the 21-day study

Nominal concentration (mg geraniol/L)	Measured concentration (mg geraniol/L)					
	Control	0.563	0.141	0.352	0.880	2.20
Range (min - max)	-	<LOD – 0.0241	<LOD – 0.0652	<LOD – 0.187	<LOD – <LOD	<LOQ – 1.53
Mean*	-	0.0228	0.0639	0.183	0.415	1.269
% of nominal	-	93.5	93.6	95.8	77.5	84.7

* time weighted mean

The test item concentrations were not stable in the test solutions and decreased to measured concentrations at <80% of nominal. The measured concentrations in the freshly-prepared test solutions were between 84.8 and 103% of nominal, indicating appropriate application of the test item. The measured concentrations in the aged test solutions were below the LOD/LOQ or at least considerably lower than in the respective fresh test solutions. It is therefore considered appropriate to express the biological endpoints based on nominal and TWM of the measured concentrations.

In order to calculate time-weighted mean measured concentrations (TWM) for the values below LOQ/LOD, the respective measured concentrations were replaced by 1/2 of the LOQ (0.0120 mg/L/2=0.00600 mg/L) or 1/2 of the LOD (0.0036 mg/L/2 = 0.0018 mg/L), respectively. This approach is considered justified, since it integrates the decreased concentrations in the aged test solutions, and since otherwise the calculation of TWM, and consequently the expression of the biological endpoints based on TWM would not be possible.

Summary of the TWM measured concentrations of the test item throughout the test

Treatment	Nominal concentration [mg test item/L]	Time-weighted mean concentration [mg/L]	Recovery [% of nominal]
C1	0.0244	0.00820	33.6
C2	0.0683	0.0174	25.5
C3	0.191	0.0392	20.5
C4	0.536	0.119	22.2
C5	1.50	0.571	38.1

Biological results

Summaries of the effects of geraniol on parental daphnid survival/immobility and the fecundity of the introduced and surviving parent daphnids are presented in the tables below.

Table 10.2.1/02-3: Total mobility/immobility of parental daphnids at the end of the 21-day study

Nominal concentration (mg geraniol/L)	Mean measured concentration (mg geraniol/L)	Number of daphnids			Immobility	Immobility corrected ^a [%]
		Introduced	Mobile	Immobile		
Control	-	10	9	1	10%	0.0
0.0244	0.00820	10	10	0	0%	0.0
0.0683	0.0174	10	9	1	10%	0.0
0.191	0.0392	10	9	1	10%	0.0
0.536	0.119	10	9	1	10%	0.0
1.50	0.571	10	4	6	60% *	55.6±

^a immobility corrected using Abbott's formula

* Statistical significant difference compared to the control (p<0.05)

For immobile parental daphnids, a weak concentration-response relationship was observed, although immobility at 1.50 mg test item/L was confirmed to be statistically significant (p<0.05). The NOEC value for parental immobility was determined to be 0.536 mg geraniol/L (nominal), corresponding to 0.119 mg

geraniol/L (mean measured). A few sublethal effects were observed in the living parental daphnids at all concentration levels, but no concentration-response relationship based on sublethal effects in living parental daphnids was determined.

Table 10.2.1/02-4: Summary of effects of geraniol on the total number of living offspring per surviving *Daphnia magna* parent after 21-days' exposure

Nominal concentration (mg geraniol/L)	Mean measured concentration (mg geraniol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
Control	-	1	81	96.9	-
		2	45		
		3	97		
		4	112		
		5	84		
		6	88		
		7	117		
		8	116		
		9	132		
		10	0		
0.0244	0.00820	1	114	110.2	-13.7
		2	98		
		3	104		
		4	118		
		5	105		
		6	114		
		7	121		
		8	118		
		9	107		
		10	103		
0.0683	0.0174	1	111	104.4	-7.8
		2	106		
		3	109		
		4	60		
		5	88		
		6	109		
		7	100		
		8	135		
		9	109		
		10	122		
0.191	0.0392	1	130	116.3	-20.1
		2	121		
		3	74		
		4	136		
		5	109		
		6	120		
		7	112		
		8	121		
		9	124		
		10	0		
0.536	0.119	1	77	67.9	29.9
		2	71		
		3	62		
		4	72		
		5	31		
		6	78		
		7	69		

Nominal concentration (mg geraniol/L)	Mean measured concentration (mg geraniol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
1.50	0.571	8	54	39.8	52.0
		9	63		
		10	65		
		1	38		
		2	11		
		3	13		
		4	17		
		5	26		
		6	83		
		7	16		
		8	5		
		9	33		
		10	13		

- = inadvertent mortality (unknown cause): offspring excluded from statistical analysis

* % offspring reduction compared to control (negative values = higher number than control)

The total number of living offspring was evaluated per surviving parental daphnid and per introduced parent daphnid, which did not die accidentally or inadvertently during the test. Endpoints are based on the former parameter.

The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.278 mg geraniol/L, 0.415 mg geraniol/L and 0.898 mg geraniol/L (nominal), respectively. The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.0520 mg geraniol/L, 0.0913 mg geraniol/L and 0.268 mg geraniol/L (mean measured), respectively.

The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.191 mg geraniol/L and the corresponding 21-day LOEC value was determined to be 0.536 mg geraniol/L (nominal). The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.0392 mg geraniol/L and the corresponding 21-day LOEC value was determined to be 0.199 mg geraniol/L (mean measured).

Summary of biological results

Nominal concentration [mg/L]	Control	0.0244	0.0683	0.191	0.536	1.50	
TWM concentration [mg/L]	Control	0.00820	0.0174	0.0392	0.119	0.571	
Number of replicates	n	10	10	10	10	10	
Mean living offspring per introduced	mean	87.2	110.2	104.9	104.7	64.2	25.5
	offspring [% of control]	--	126.4	120.3	120.1	73.6	29.2
	inhibition [% of control]	--	-26.4	-20.3	-20.1	26.4	70.8
Adult immobility	[% of initial number]	10.0	0.0	10.0	10.0	10.0	60.0
	[% corrected for control immobility]	0.0	0.0	0.0	0.0	0.0	55.6
Mean living offspring per survivor	mean	96.9	110.2	104.4	116.3	67.9	39.8
	offspring [% of control]	--	113.7	107.8	120.1	70.1	41.0
	inhibition [% of control]	--	-13.7	-7.8	-20.1	29.9	59.0

Validity

All validity criteria were met in accordance with OECD test guideline 211 (2012):

- The mortality of the parent animals (female *Daphnia*) in the controls does not exceed 20% at the end of the test; (actual value: 10%)
- The mean number of living offspring produced per surviving parent animal in the controls at the end of the test is >60 (actual value: 96.9)
- Analytical measurement of test concentrations was included.

Conclusion

The 21-day chronic toxicity of geraniol to *Daphnia magna* was studied under static-renewal conditions in accordance with OECD test guideline 211 (2012).

The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.278 mg geraniol/L, 0.415 mg geraniol/L and 0.898 mg geraniol/L (nominal), respectively. The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.0520 mg geraniol/L, 0.0913 mg geraniol/L and 0.268 mg geraniol/L (mean measured), respectively.

The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.191 mg geraniol/L and the corresponding 21-day LOEC value was determined to be 0.536 mg geraniol/L (nominal). The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.0392 mg geraniol/L and the corresponding 21-day LOEC value was determined to be 0.199 mg geraniol/L (mean measured).

Parameter	Endpoints based on measured concentrations [mg test item/L]				
	EC ₁₀	EC ₂₀	EC ₅₀	NOEC	LOEC
Reproduction based on surviving F ₀ daphnids	0.0520	0.0913	0.268	0.0392	0.119
Reproduction based on introduced F ₀ daphnids	0.0493	0.0826	0.221	0.0392	0.119
Mortality/immobility of F ₀ daphnids	-	-	0.459	0.119	0.571

Study 3

Comments of zRMS:	<p>The study was performed in line with OECD 211 with no deviations.</p> <p>The test item concentrations were measured in fresh and aged media, but not at each renewal, but once a week. This is considered acceptable, since from performed analyses sufficient data for determination of the mean measured concentrations are available.</p> <p>All the validity criteria were met and the study is considered acceptable with following endpoints are relevant for the risk assessment:</p> <p>NOEC (reproduction) = 0.137 mg a.s./L (based on mean measured concentrations) EC₁₀ = 0.292 mg a.s./L (with CI of 0.1283-0.6645 mg a.s./L, based on mean measured concentrations) NOEC (immobilisation) = 0.137 µg a.s./L (based on mean measured concentrations)</p> <p>Reliability of the EC₁₀ value has been evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> • NW (normalised width) of 1.84 was calculated, which results with rating “poor” in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC₁₀ (0.292 mg a.s./L) is higher than EC_{20,low} (0.238 mg a.s./L), • the dose-response curve is shallow with steepness of 0.16 (i.e. <0.33).
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	Taking into account the provided above indications, the calculated EC ₁₀ is considered to be not fully reliable and the NOEC is recommended for the risk assessment purposes.
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.2.1/03
Report	Thymol: A Study on the Chronic Toxicity to <i>Daphnia magna</i> Egeler, P., 2021, report No 20GC2DB
Guideline(s):	Yes. OECD Test Guideline 211 (2012)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	Thymol
Chemical Name:	5-Methyl-2-isopropylphenol; 5-Methyl-2-(1 methylethyl)phenol; 2-Isopropyl-5-methylphenol
Formulation type:	not applicable
Source and lot/batch no.:	THY/02/2019-20
Active substance content:	99.58%
Appearance:	Colourless to white crystals
Expiry date of lot/batch:	31 July 2021
Storage conditions:	Store in cool, dry, well-ventilated and dark place away from direct sunlight, in tightly closed container; stored at test facility in closed container at ambient temperature in the dark

Test organism

Species:	<i>Daphnia magna</i> (Straus) (water flea).
Strain/clone:	M10
Age at study initiation:	<24 hours old
Source:	Originally supplied by KU Leuven, Belgium, cultured at ECT Oekotoxikologie GmbH since December 22, 2011
Feeding during test:	Three times per week with fresh algae suspension
Acclimation:	Not applicable

Test conditions

Test concentrations:	0, 0.0563, 0.141, 0.352, 0.880 and 2.20 mg test item/L
Exposure regime:	Semi-static
Replicates:	10 individually held Daphnids per control and test item groups (relevant for semi-static design)
Test medium:	Elendt medium M4
Hardness:	250 - 263 mg/L as CaCO ₃
Test temperature:	20.6 – 21.5 °C*
pH:	7.7 – 9.0*

Dissolved oxygen:	8.9 – 11.7 mg/L*
Photoperiod:	16 hours of light / 8 hours of dark
Light intensity:	15.13 – 16.74 $\mu\text{E m}^{-2} \text{ s}^{-1}$

*Measured (x 2) on days 0, 2, 5, 7, 9, 14 and 16 of the test

Ten replicate vessels, each a glass beaker containing a single daphnid in 50-60 mL medium, were allocated to each test concentration and control. Test solutions were renewed three times per week (semi-static test system). The daphnids were fed three times per week after transfer to fresh test solutions. Daily observations were made of the parental daphnids in all test vessels; immobile parental daphnids were removed upon recording. From day 8, onwards, the live offspring (F1 generation) was counted daily and removed from the vessels. Observations of abnormal behaviour of the test animals were recorded.

Samples of test media were taken **once a week** at the start and end of **selected** media renewal cycles, during the 21-day exposure period, and analysed for thymol by GC-MS. Water quality parameters (dissolved oxygen concentration, pH, water hardness and temperature (manual measurement)) were determined once per test week in fresh and aged solutions of the control and the highest test item concentration.

The Cochran-Armitage test procedure was applied with immobility at 21 days to detect an increasing trend in responses (Alpha: 0.050; one-sided greater). Determination of EC_x values for parental immobility by Probit analysis was not possible due to the poor concentration-response relationship. Fisher's Exact Binomial Test with Bonferroni correction was used to determine the threshold concentration for parental immobility. Non-linear regression analysis (3-parameter normal Cumulative Distribution Function (CDF)) was used to determine EC_x values for reproduction. Prior to threshold concentration testing, a qualitative trend analysis by contrasts was applied to check for monotonicity of the concentration-response relationship. The Williams Multiple Sequential t-test Procedure ($p \leq 0.05$) was used to determine the threshold concentrations for reproduction.

The statistical software package ToxRat Professional 3.3.0 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations using the nominal concentrations.

Results and discussions

Analytical results

The GC-MS analytical method for the determination of thymol in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for thymol in the control sample. The analytical calibration was shown to be linear ($r^2 \geq 0.9991$) over the range of 0.0084 mg thymol/L to 0.200 mg thymol/L. Accuracy was confirmed with recovery determined by fortification of thymol at 0.028 and 2.20 mg thymol/L; all recoveries were within the range of 91-105% and mean recoveries were within 96-98% (i.e. within the guideline range of 70-110%). Precision was confirmed with five determinations made at each fortification level; the relative standard deviation was between 1.9-5.5% (i.e. within the guideline limit of $\geq 20\%$). The limit of quantification (LOQ) was 0.0280 mg thymol/L (i.e. below the biological NOEC value). The limit of detection (LOD) was 0.084 mg thymol/L in test medium. All samples were analysed within 24 h after extraction, therefore the stability of thymol in the final extracts was not assessed.

The measured concentrations of thymol during the 21-day exposure period of the *Daphnia magna* toxicity study are summarised in the tables below.

Table 10.2.1/03-1: Nominal and measured concentrations of thymol in each replicate during the 21-day study

Nominal concentration (mg thymol/L)	Measured concentration (mg thymol/L)							Percentage of nominal (%)						
	Day						Mean ^a	Day						Mean ^a
	2F	5A	7F	9A	14F	16A		2F	5A	7F	9A	14F	16A	
Control	<LOD	-	-	-	-	<LOD	-	-	-	-	-	-	-	-
0.0563	0.0507	0.043	0.0523	0.0404	<LOD ^b	<LOD ^b	0.0344	90.1	76.4	92.9	71.8	n.a.	n.a.	61.1
0.141	0.131	0.119	0.135	0.122	0.177	0.153	0.137	92.9	84.4	95.7	86.5	126	109	97.2
0.352	0.330	0.290	0.340	0.270	0.270	0.290	0.300	93.8	82.4	96.6	76.7	76.7	82.4	85.2
0.880	0.840	0.680	0.840	0.730	0.800	0.733	0.767	95.5	77.3	95.5	83.0	90.9	83.3	87.2
2.20	2.110	1.990	2.030	1.880	2.110	1.81	2.00	95.9	90.5	92.3	85.5	95.9	82.3	90.9

^a time weighted mean

^b Measured concentrations <LOD indicate a possible error in preparation of the C1 solution on days 14 and 16. To calculate time-weighted mean measured concentrations (TWM) for the C1 treatment group, the measured concentrations days 14 and 16 were replaced by 1/2 of the LOD (0.0084 mg/L/2 = 0.0042 mg/L), equivalent to 7% of the nominal concentration of 0.0563 mg/L. This is considered a robust approach as it is expected that this error occurred only for the single renewal period of days 14-16 of the study.

F: fresh A: aged media

Biological results

Summaries of the effects of thymol on parental daphnid survival/immobility and the fecundity of the introduced and surviving parent daphnids are presented in the tables below.

Table 10.2.1/03-2: Total mobility/immobility of parental daphnids at the end of the 21-day study

Nominal concentration (mg thymol/L)	Mean measured concentration (mg thymol/L)	Number of daphnids			Immobility [%]
		Introduced	Mobile	Immobile	
Control	-	10	10	0	0.0
0.0563	0.0344	10	10	0	0.0
0.141	0.137	10	10	0	0.0
0.352	0.300	10	10	0	0.0
0.880	0.767	10	10	0	0.0
2.20	2.00	10	9	1	10.0

For immobile parental daphnids, a concentration-response relationship could not be determined. A few sublethal effects were observed in the living parental daphnids at all concentration levels, but no concentration-response relationship based on sublethal effects in living parental daphnids was determined.

Table 10.2.1/03-3: Summary of effects of thymol on the total number of living offspring per surviving *Daphnia magna* parent after 21-days' exposure

Nominal concentration (mg thymol/L)	Mean measured concentration (mg thymol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
Control	-	1	112	135.4	-
		2	127		
		3	109		
		4	122		
		5	149		
		6	164		
		7	124		
		8	130		
		9	145		
		10	172		
0.0563	0.0344	1	136	135.7	-0.2

Nominal concentration (mg thymol/L)	Mean measured concentration (mg thymol/L)	Replicate	Cumulative number of live juveniles per surviving parent at 21 days		
			Per test vessel	Mean	% reduction relative to control*
		2	146		
		3	129		
		4	177		
		5	135		
		6	117		
		7	144		
		8	127		
		9	101		
		10	145		
		0.141	0.137		
2	137				
3	103				
4	139				
5	158				
6	151				
7	99				
8	138				
9	134				
10	171				
0.352	0.300	1	128	114.8**	15.2
		2	85		
		3	152		
		4	112		
		5	75		
		6	102		
		7	109		
		8	121		
		9	126		
		10	138		
0.880	0.767	1	103	103.9**	23.3
		2	96		
		3	135		
		4	89		
		5	106		
		6	119		
		7	116		
		8	68		
		9	121		
		10	86		
2.20	2.00	1	55	65.0**	52.0
		2	73		
		3	-		
		4	45		
		5	57		
		6	72		
		7	67		
		8	71		
		9	109		
		10	36		

- = inadvertent mortality (unknown cause): offspring excluded from statistical analysis

* % offspring reduction compared to control (negative values = higher number than control)

** Statistically significant difference compared to the control (Williams t test, alpha 0.050, one-sided)

The total number of living offspring was evaluated per surviving parental daphnid and per introduced parent daphnid, which did not die accidentally or inadvertently during the test. Endpoints are based on the former parameter.

The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.292 mg thymol/L, 0.554 mg thymol/L and 1.88 mg thymol/L (mean measured), respectively.

The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.137 mg thymol/L and the corresponding 21-day LOEC value was determined to be 0.300 mg thymol/L (mean measured).

Summary of biological results

Nominal concentration	[mg/L]	Control	0.0563	0.141	0.352	0.880	2.20
TWM concentration	[mg/L]	Control	0.0344	0.137	0.300	0.767	2.00
Number of replicates	n	10	10	10	10	10	10
Mean living offspring per introduced	mean	135.4	135.7	139.1	114.8	103.9	61.5
	offspring [% of control]	--	100.2	102.7	84.8	76.7	45.4
	inhibition [% of control]	--	-0.2	-2.7	15.2	23.3	54.6
Adult immobility	[% of initial number]	0.0	0.0	0.0	0.0	0.0	10.0
Mean living offspring per survivor	mean	135.4	135.7	139.1	114.8	103.9	65.0
	offspring [% of control]	--	100.2	102.7	84.8	76.7	48.0
	inhibition [% of control]	--	-0.2	-2.7	15.2	23.3	52.0

Validity

All validity criteria were met in accordance with OECD test guideline 211 (2012):

- The mortality of the parent animals (female *Daphnia*) in the controls does not exceed 20% at the end of the test; (actual value: 0%)
- The mean number of living offspring produced per surviving parent animal in the controls at the end of the test is >60 (actual value: 135.4)
- Analytical measurement of test concentrations was included.

Conclusion

The 21day chronic toxicity of thymol to *Daphnia magna* was studied under static-renewal conditions in accordance with OECD test guideline 211 (2012).

The 21-day EC₁₀, EC₂₀ and EC₅₀ values for *Daphnia magna*, based on the total number of living offspring per surviving parental daphnids were determined to be 0.292 mg thymol/L, 0.554 mg thymol/L and 1.88 mg thymol/L (mean measured), respectively.

The 21-day NOEC value for *Daphnia magna*, based on the total number of living offspring per surviving *Daphnia magna* parental daphnids, was determined to be 0.137 mg thymol/L and the corresponding 21-day LOEC value was determined to be 0.300 mg thymol/L (mean measured).

Parameter	Endpoints based on measured concentrations [mg test item/L]				
	EC ₁₀	EC ₂₀	EC ₅₀	NOEC	LOEC
Reproduction based on surviving F ₀ daphnids	0.292	0.554	1.88	0.137	0.300
Reproduction based on introduced F ₀ daphnids	0.303	0.553	1.75	0.137	0.300
Mortality/immobility of parental daphnids	n.d.	n.d.	n.d.	2.00	>2.00

n.d.: not determined due to lacking concentration-response relationship.

Study 4

Comments of zRMS:	<p>The study was performed in line with OECD 201 with no deviations.</p> <p>Although the mean measured concentrations of the test item in test solutions was maintained at 80-120% of nominal, the results are expressed in terms of mean measured concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with following endpoints are relevant for the risk assessment:</p> <p>E_rC₅₀ = 9.51 mg a.s./L (based on mean measured concentrations) E_vC₅₀ = 5.41 mg a.s./L (based on mean measured concentrations) E_bC₅₀ = 5.84 mg a.s./L (based on mean measured concentrations)</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.2.1/04
Report	Geraniol: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth inhibition Test. Siedel, U., Emnet, P., 2021, report No 155771210
Guideline(s):	Yes. OECD Test Guideline 201 (2011)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	geraniol
Source and lot/batch no.:	L4363091
Active substance content:	98.91% (analysed)
Expiry date of lot/batch:	December 2021
Storage conditions:	At 20 ± 5 °C, in the dark.

Test organism

Species:	Alga <i>Pseudokirchneriella subcapitata</i> (KORSHIKOV), formerly known as <i>Selenastrum capricornutum</i> , and recently renamed as <i>Raphidocelis subcapitata</i> (KORSHIKOV)
Strain/clone:	Strain No. 61, 8l SAG
Source:	Originally from Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzen-wissenschaften, Universität Göttingen, 37073

	Göttingen, Germany; cultivated in the laboratories of ibacon under standardised conditions.
Feeding during test:	Not applicable.
Acclimation:	Algal cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test start under the same conditions as in the test
Initial cell concentration:	5000 cells/mL
<i>Test conditions</i>	
Test concentrations:	0, 1.3, 3.2, 8.0, 20.0 and 50.0 mg test item/L
Exposure regime:	Semi-static
Replicates:	6 for control, 3 per test concentration
Hardness:	24 mg/L as calcium carbonate
Test temperature:	21.4 to 23.0 °C
pH:	8.0 – 8.2 at the start (control and test item treatments) 8.3 – 9.8 at the end (control and test item treatments) The pH in the control increased by slightly more than 1.5 units. However, this does not invalidate the test since the validity criteria were met.
Photoperiod:	Continuous illumination
Light intensity:	4820 to 5380 Lux. 5102 Lux (mean value)

The toxicity of geraniol to *Pseudokirchneriella subcapitata* was tested in an algal growth inhibition test. Since geraniol is poorly soluble in test water, to obtain the desired test concentrations, a supersaturated stock solution of 50 mg geraniol/L (nominal) was prepared by suspending 57 µL geraniol in 1000 mL test water. This stock suspension was stirred for 2 hours 15 minutes at room temperature to dissolve as much geraniol as possible. After cessation of mixing and a following period (15 minutes) of settling to allow phase separation, the aqueous phase, i.e. the water soluble fraction, was drawn off carefully and used as the highest test concentration and to prepare the remaining test concentrations in the series.

Five nominal test concentrations were tested: 50, 20, 8.0, 3.2 and 1.3 mg geraniol/L, plus a test water control, with three replicates of each test concentration and six replicates for the control.

Exponentially growing cultures of *P. subcapitata* were inoculated at 5000 cells/mL and cultured for 72 hours at temperatures of 21.4 to 23.0 °C. Light intensity ranged between 4820 to 5380 lux. The test vessels were 50 mL Erlenmeyer flasks, each containing 30 mL culture medium, which were continuously stirred by magnetic stirrers. These test units were incubated in a water bath, placed in a random order and were repositioned each day to minimize differences in test conditions.

Measured concentrations of geraniol were determined at 0, 24, 48 and 72 hours. The cell density on each observation time was determined by spectrophotometric measurement. Therefore, defined volumes of the algal suspensions from all replicates (and from blanks) were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The algal cell densities were calculated by subtracting the absorption of the blanks, from each of the measured absorption of the test media (with algae). Based on the counted cell densities and the absorption from an algal suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test.

Statistical analysis was performed using ToxRat Professional Version 3.3.0. The 72-hour $E_rC_{50/20/10}$, $E_bC_{50/20/10}$ and $E_yC_{50/20/10}$ values and, where possible, their 95 %-confidence limits were calculated by Weibull analysis. For the determination of the 72-hour LOEC and NOEC values, the calculated growth rates, biomass and yields at each test concentration were tested for significant differences compared to the control values by Bonferroni-Welch t-test (yield, growth rate) and Williams t-test (biomass integral), respectively.

Results and discussions

Analytical results

The GC-MS analytical method for the determination of geraniol in test medium was validated with regards to specificity, linearity, accuracy and precision. The validation results are in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for geraniol in the control sample. The analytical calibration was shown to be linear ($r = 0.9997$) over the range of 3.0 – 300 μg geraniol/L. Accuracy was confirmed with recovery determined by fortification of geraniol at 0.03 and 80 mg geraniol/L; all recoveries were within the range of 85 – 106% and overall mean recovery was 94% (i.e. within the guideline range of 70 – 110%). Precision was confirmed with six determinations made at each fortification level; the relative standard deviation was between 1 – 2% (i.e. within the guideline limit of $\leq 20\%$). The limit of quantification (LOQ) was 0.03 mg geraniol/L (i.e. below the biological E_rC_{50} /NOEC value). The limit of detection (LOD) was 0.7 μg geraniol/L in test medium.

A summary of the measured concentrations of geraniol in the test media is presented in the tables below.

Table 10.2.1/04-1: Nominal and measured concentrations of geraniol in each replicate

Nominal concentration (mg geraniol/L)	Nominal concentration (µg geraniol/L) ¹	Rep	Measured conc. (µg geraniol/L) ¹				Mean ² (mg geraniol/L)	Percentage of nominal				
			0 hours	4 hours	24 hours	72 hours		0 hours	4 hours	24 hours	72 hours	Mean ²
Water control	Water control	1 2 3 4	<LOD	<LOD	<LOD	<LOD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1.3	1278.772	1 2	1096.209 1090.556	1069.667 1116.572	1122.587 1091.306	883.876 885.438	1.02	86 85	84 87	88 85	69 69	80
3.2	3205.128	1 2	2836.230 2915.039	2889.232 2831.033	2798.379 2911.071	2426.449 2351.838	2.69	88 91	90 88	87 91	76 73	84
8.0	7936.508	1 2	6856.017 6854.627	6858.408 6900.343	6666.492 6624.629	6089.820 6176.354	6.51	86 86	86 87	84 83	77 78	82
20	20000	1 2	17936.731 17575.180	17679.963 18049.175	18296.972 18058.986	17033.958 17365.782	17.8	90 88	88 90	91 90	85 87	89
50	50000	1 2	47476.849 46925.132	48283.728 46753.377	46501.502 45531.764	47339.272 47792.631	46.8	95 94	97 94	93 91	95 96	94

Rep = Sample replicate; LOQ = 0.03 mg geraniol/L; LOD = 0.7 µg geraniol/L

¹ The tabulated results represent rounded results calculated on the exact raw data

² Geometric mean of the 8 measurements for each treatment group (duplicate samples at 0, 4, 24 and 74 hours)

n.a. = not applicable

Table 10.2.1/04-2: Summary of nominal and mean measured concentrations of geraniol for 72 hours

Nominal concentration (µg geraniol/L) ¹	Water control	1300	3200	8000	20000	50000
Measured concentration (µg geraniol/L) ¹	Water control	1278.772	3205.128	7936.508	20000	50000
Range (min: max) (µg geraniol/L)	n.a.	883.876 - 1122.587	2351.838 - 2915.039	6089.820 - 6900.343	17033.958 - 18296.972	45531.764 - 48283.728
Median (µg geraniol/L)	n.a.	1090.931	2833.632	6760.559	17808.347	47132.202
Mean ² (mg geraniol/L)	n.a.	1.02	2.69	6.51	17.8	46.8
% of nominal (ref. to mean)	n.a.	80	84	82	89	94

¹ The tabulated results represent rounded results calculated on the exact raw data

² Geometric mean of the 8 measurements for each treatment group (duplicate samples at 0, 4, 24 and 72 hours)

n.a.: not applicable

Analytical verification of test concentrations confirmed that measured concentrations of all fresh and aged samples were between 69 and 96% of nominal concentrations. Biological endpoints are therefore reported based on mean measured concentrations of geraniol.

Biological results

A summary of the effects of geraniol on cell density, growth rate and yield of *Pseudokirchneriella subcapitata* after 72 hours exposure is presented in the tables below.

Table 10.2.1/04-3: Algae cell densities during the test period of 72 hours

Nominal concentration (mg geraniol/L)	Geometric mean concentration (mg geraniol/L)	Replicate	Cell density (10000 cells/mL)		
			24 h	48 h	72 h
Water Control	Water Control	1	3.538	24.151	122.532
		2	3.973	25.982	132.198
		3	3.756	25.295	122.284
		4	3.756	26.897	127.985
		5	3.756	28.728	128.728
		6	3.973	28.956	133.685
Mean value			3.792	26.668	127.902
Standard Deviation			0.163	1.909	4.754
1.3	1.02	1	3.538	24.838	135.668
		2	3.973	25.982	118.138
		3	3.538	21.863	114.104
Mean value			3.683	24.228	122.697
Standard Deviation			0.251	2.126	11.429
3.2	2.69	1	3.756	25.982	106.668
		2	3.973	25.524	114.352
		3	4.407	24.609	109.643
Mean value			4.045	25.372	110.221
Standard Deviation			0.332	0.699	3.874
8.0	6.51	1	2.887	12.482	44.207
		2	3.104	14.770	50.651
		3	2.887	12.939	44.207
Mean value			2.959	13.397	46.355
Standard Deviation			0.125	1.211	3.721
20	17.8	1	1.584	1.270	0.500
		2	2.018	1.728	0.500
		3	1.801	1.040	0.500
Mean value			1.801	1.346	0.500
Standard Deviation			0.217	0.350	0.000
50	46.8	1	1.149	1.956	0.500
		2	1.367	1.270	0.500
		3	1.149	1.270	0.500
Mean value			1.222	1.499	0.500
Standard Deviation			0.125	0.396	0.000

At test start nominal 5000 algal cells/mL were inoculated
 Values lower than the initial cell density were set to the initial cell density

Table 10.2.1/04-4: Effect of geraniol on the growth rate and yield of *Pseudokirchneriella subcapitata* during the 72-hour test period

Nominal conc. (mg geraniol/L)	Geometric mean conc. (mg geraniol/L)	Growth rates μ [1/day]			% of inhibition of μ			Yields y [10000 cells/mL]			% of inhibition of μ		
		0 – 24 hours	0 – 48 hours	0 - 72 hours	0 – 24 hours	0 – 48 hours	0 - 72 hours	0 – 24 hours	0 – 48 hours	0 - 72 hours	0 – 24 hours	0 – 48 hours	0 - 72 hours
Water Control	Water Control	2.025	1.987	1.848	n.a.	n.a.	n.a.	3.292	26.168	127.402	n.a.	n.a.	n.a.
1.3	1.02	1.995	1.939	1.833	1.5	2.4	0.8	3.183	23.728	122.197	3.3	9.3	4.1
3.2	2.69	2.088	1.963	1.798	-3.1	1.2	2.7*	3.545	24.872	109.721	-7.7	5.0	13.9*
8.0	6.51	1.778	1.643	1.509	12.2*	17.3*	18.3*	2.459	12.897	45.855	25.3*	50.7*	64.0*
20	17.8	1.277	0.484	0.000	37.0*	75.6*	100.0*	1.301	0.846	0.000	60.5*	96.8*	100.0*
50	46.8	0.890	0.538	0.000	56.1*	72.9*	100.0*	0.722	0.999	0.000	78.1*	96.2*	100.0*

Validity

All validity criteria were met in accordance with OECD test guideline 201 (2011):

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period (actual value: 255.8-fold increase).
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35% (actual value: 13.4%).
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests (actual value: 0.7%).

After 72 hours' exposure to geraniol, statistically significant effects were recorded in growth rate, at all but the lowest test concentration, when compared to the controls ($p < 0.05$). Statistically significant effects were also recorded in yield at all but the lowest test concentrations, after 72 hours, when compared to the controls. No abnormalities in appearance of the algae at the start and at the end of the test were observed.

The 72-hour $E_r C_{50/20/10}$ values for *Pseudokirchneriella subcapitata*, based on growth rate, were calculated to be 9.51 mg geraniol/L (95% confidence interval of 8.89 - 10.2 mg geraniol/L), 6.68 mg geraniol/L (95% confidence interval of 6.48 - 6.89 mg geraniol/L) and 5.29 mg geraniol/L (95% confidence interval of 5.03 - 5.56 mg geraniol/L), respectively, based on mean measured concentrations. The corresponding 72-hour NOEC and LOEC values, based on growth rate were determined to be 1.02 and 2.69 mg geraniol/L, respectively, based on mean measured concentrations.

The 72-hour $E_y C_{50/20/10}$ values for *Pseudokirchneriella subcapitata*, based on yield, were calculated to be 5.41 mg geraniol/L (95% confidence interval of 5.13 - 5.71 mg geraniol/L), 3.12 mg geraniol/L (95% confidence interval of 2.77 - 3.51 mg geraniol/L) and 2.17 mg geraniol/L (95% confidence interval of 1.82 - 2.59 mg geraniol/L), respectively, based on mean measured concentrations. The corresponding 72-hour NOEC and LOEC values, based on yield were determined to be 1.02 and 2.69 mg geraniol/L, respectively, based on mean measured concentrations.

Parameter (0-72 h)	Yield [mg test item/L]	Growth rate [mg test item/L]	Biomass [mg test item/L]
72-hour EC_{50}	5.41	9.51	5.84
95 % conf. interval	5.13 - 5.71	8.89 - 10.2	5.46 - 6.25
72-hour EC_{20}	3.12	6.68	3.30
95 % conf. interval	2.77 - 3.51	6.48 - 6.89	2.92 - 3.73
72-hour EC_{10}	2.17	5.29	2.26
95 % conf. interval	1.82 - 2.59	5.03 - 5.56	1.88 - 2.72
72-hour NOEC	1.02	1.02	< 1.02
72-hour LOEC	2.69	2.69	≤ 1.02

Values refer to geometric mean measured test concentrations

Conclusion

In a 72-hour toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to geraniol at nominal concentrations of 50, 20, 8.0, 3.2 and 1.3 mg geraniol/L under static test conditions, in accordance with the OECD test guideline 201 (2011). Analytical verification of test concentrations confirmed that measured concentrations of all fresh samples were between 69 and 95% of nominal concentrations, after 72 hours. The biological endpoints are therefore based on mean measured concentrations of geraniol.

The 72-hour $E_rC_{50/20/10}$ values for *Pseudokirchneriella subcapitata*, based on growth rate, were calculated to be 9.51 mg geraniol/L (95% confidence interval of 8.89 - 10.2 mg geraniol/L), 6.68 mg geraniol/L (95% confidence interval of 6.48 - 6.89 mg geraniol/L) and 5.29 mg geraniol/L (95% confidence interval of 5.03 - 5.56 mg geraniol/L), respectively, based on mean measured concentrations. The corresponding 72-hour NOEC and LOEC values, based on growth rate were determined to be 1.02 and 2.69 mg geraniol/L, respectively, based on mean measured concentrations.

The 72-hour $E_yC_{50/20/10}$ values for *Pseudokirchneriella subcapitata*, based on yield, were calculated to be 5.41 mg geraniol/L (95% confidence interval of 5.13 - 5.71 mg geraniol/L), 3.12 mg geraniol/L (95% confidence interval of 2.77 - 3.51 mg geraniol/L) and 2.17 mg geraniol/L (95% confidence interval of 1.82 - 2.59 mg geraniol/L), respectively, based on mean measured concentrations. The corresponding 72-hour NOEC and LOEC values, based on yield were determined to be 1.02 and 2.69 mg geraniol/L, respectively, based on mean measured concentrations.

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
A 2.3.1.1.2	KCP 10.3.1.1.2	Acute contact toxicity to bees
A 2.3.1.2	KCP 10.3.1.2.	Chronic toxicity to bees

Comments of zRMS:	<p>The study was performed in line with OECD 201 with no deviations.</p> <p>The temperature of 29.0-34.0°C and the relative humidity of 37.3-63.1% were outside the range recommended by the test guideline (31-35°C and 50-70% respectively). Deviations were noted only during observations, when the door of the incubator had to be opened to perform observations and treatments and lasted less than 2 hours. In line with the test guideline, such deviations are unavoidable and are considered to have no impact on the integrity or outcome of the test.</p> <p>All the validity criteria were met and the study is considered acceptable with following endpoints are relevant for the risk assessment:</p> <p>LDD₅₀ = 123.53 µg product/bee/day NOEDD = 66.96 µg product/bee/day</p> <p>It is noted that compliance with the GLP regulations is confirmed only by the statement of the study director and copy of the GLP certificate is not included in the study report. However, the laboratory (BioTecnologie BT S.r.l., Italy) is listed on the list of certified laboratories available on the website of the Italian Ministry of Health. The area of certification includes toxicity testing on terrestrial non-target organisms.</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.3.1.2/01
Report	Chronic oral effects of ARAW on adult worker Honey bees <i>Apis mellifera</i> L., 10-day feeding laboratory test. Pecorari, F., 2019a, report No BT059/19
Guideline(s):	Yes. OECD Guideline for the testing on chemicals 245 “Honey bee (<i>Apis mellifera</i> L.), Chronic Oral Toxicity test (10-day feeding test in the laboratory)”.
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	ARAW (Tradename for Mevalone)
Formulation type:	CS
Lot/batch no.:	04017005
Active substance content:	Eugenol: 3.29%; Geraniol: 6.46%; Thymol: 6.57% (w/v)
Expiry date of lot/batch:	13 th October 2020

Toxic reference

Name:	Dimethoate
Lot/batch no.:	779155
Active substance content:	99.5%
Expiry date of lot/batch:	1 st February 2022

Test organism

Species:	Honey bee <i>Apis mellifera ligustica</i>
Age at study initiation:	Young workers (maximum 2 days old)
Source:	Reared from capped brood combs with emerging bees taken from as far as possible disease-free and queen-right colonies obtained from BioTecnologie BT S.r.l. No chemical substances were administered in the hives for at least one month prior to the test.
Feeding during test:	Yes, <i>ad libitum</i> with sucrose solution
Acclimation:	One day under test conditions

Test conditions

Test concentrations:	0 (control), 0 (xanthan gum control), 1300, 3200, 8000, 20000 and 50000 ppm (corresponding to 0, 0, 37.66, 66.96, 149.79, 206.43 and 259.44 µg product/bee/day) Toxic standard: 1.0 ppm (corresponding to 0.02 µg a.s./bee/day)
Replicates:	3 per test group and controls with 10 bees each
Test temperature:	29.0 - 34.0 °C (continuous monitoring), average 32.5 °C
Relative humidity:	37.3 - 63.1 % (continuous monitoring), average 58.6%
Photoperiod:	24 hour darkness, except during application and assessments

Two days prior to test start, young *Apis mellifera ligustica* workers were collected from the capped brood combs and distributed into the test cages. Each test cage contained 10 bees. Test units comprised well-ventilated and disposable cardboard cages of 5.0 x 9.5 x 6.5 cm. Each cage was equipped with a frontal transparent acetate lid.

The following treatment groups were included in the test: one untreated control [50% (w/v) aqueous sucrose solution]; one control with 0.2% of xanthan gum, the reference item exposed to a single dose (1 mg dimethoate/kg feeding solution) and five nominal product concentrations 1300, 3200, 8000, 20000 and 50000 mg product/bee /kg feeding solution.

The product was dispersed directly in 50% w/v sugar-water solution (feeding solution) for preparation of treatments. A hydrocolloid agent (xanthan gum) was used for the preparation of feeding solutions at the concentration of 0.2% w/w in order to keep the solutions homogeneous.

The feeding solutions were prepared freshly every day by adding an amount of product to a defined quantity of 50% (w/v) aqueous sucrose solution added with 0.2% w/w of xanthan gum and were observed for homogeneity (e.g. signs of precipitation) at the start and at the end of each feeding interval (about 24 hours).

The feeding solutions were offered *ad libitum* to the honey bees in plastic syringes of 2.5 mL without tips, filled with approx. 2 mL per day and replaced daily with new feeders. Each feeding interval was of 24 ± 2 hours and the amount of consumed feeding solution was determined by weighing the feeders before and after administration with a calibrated balance. At the end of each feeding period there was always some feeding solution remaining in the feeders, in order to guarantee *ad libitum* feeding. The bees were continuously exposed to the feeding solutions over a period of 10 days.

An adjustment for evaporation of the test solutions from the feeders was carried out using additional test cages without bees, but only with feeders containing the untreated feeding solution (3 replicates).

These cages were placed in the test environment alongside the test units and the feeders were replaced daily, weighing them before and after each feeding interval. The mean evaporation figure was calculated and reported per feeder for each feeding period.

The concentrations of the active substances eugenol, geraniol and thymol in feeding solutions were analysed in the lowest and highest product feeding solutions.

Mortality and behavioural abnormalities were recorded every 24 ± 2 hours after the start of feeding, for a duration of 10 days.

For data evaluation the statistical programme ToxRat Professional 3.3.0 was used. The Step-down Cochran-Armitage test procedure (step down test to detect an increasing trend in response – alpha 0.05) was performed in order to verify the significance of the data and evaluate the NOEC/NOEDD value. A Weibull analysis (with linear maximum likelihood regression) was used to evaluate the LDDx and LCx values.

Results and discussions

Analytical results

The HPLC-DAD analytical method for the determination of eugenol, geraniol and thymol in 50% aqueous sucrose solution was validated with regards to specificity, linearity and accuracy in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for Mevalone in the control sample. The analytical calibration was shown to be linear ($r \geq 0.9998$) over the range of 1.0477-73.3373 mg/L for eugenol, ($r \geq 0.9999$) over the range of 0.5138-35.9967 mg/L for geraniol and ($r \geq 0.9998$) over the range of 1.0030-70.2097 mg/L for thymol.

The measured concentrations of eugenol, geraniol and thymol in each feeding solution (control and lowest and highest product solutions) are summarised in the table below.

Table 10.3.1.2/01-1: Nominal and measured concentrations of eugenol in feeding solution

Nominal concentration (mg eugenol/kg f.s.)	Measured concentration (mg eugenol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
41.70	45.1636	45.5388	109
	45.9140		
1601.80	1735.0739	1737.2604	108
	1739.4469		

*arithmetic mean
 f.s.; feeding solution.

Table 10.3.1.2/01-2: Nominal and measured concentrations of geraniol in feeding solution

Nominal concentration (mg geraniol/kg f.s.)	Measured concentration (mg geraniol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
81.80	84.3873	83.6220	102
	82.8567		
3145.10	3430.2616	3451.4327	110
	3472.6038		
	3243.5204		

*arithmetic mean
 f.s.; feeding solution.

Table 10.3.1.2/01-3: Nominal and measured concentrations of thymol in feeding solution

Nominal concentration (mg thymol/kg f.s.)	Measured concentration (mg thymol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
83.20	82.1822	81.0797	97
	79.9771		
3198.60	3243.4105	3243.4655	101
	3243.5204		

*arithmetic mean
f.s.; feeding solution.

Analytical verification of the lowest and highest feeding solutions confirmed that measured concentrations of eugenol, thymol and geraniol were all within 97 - 110% of nominals. Therefore, biological endpoints are reported in terms of nominal concentrations of Mevalone (and corresponding nominal concentrations of each active substance taking into account the a.s. content of the Mevalone); and in terms of actual consumed doses based on nominal concentrations, taking into account the mean food consumption.

Biological results

A summary of the mean consumption of feeding solution per bee, accounting for the number of dead bees per replicate and the evaporation control, over the 10-day exposure period is presented in the table below.

Table 10.3.1.2/01-4: Consumption of feeding solution by adult *Apis mellifera*

Groups	Concentrations	No. bees/cage	Mean uptake of feeding solution	Mean uptake of Mevalone
	mg product/bee /kg f.s.		mg f.s./bee/day*	µg product/bee /bee/day
Untreated control	0.0	10	35.83	0.00
Control 0.2% of xanthan gum	0.0	10	28.14	0.00
T1	1300.0	10	28.97	37.66
T2	3200.0	10	20.93	66.96
T3	8000.0	10	18.72	149.79
T4	2000.0	10	10.32	206.43
T5	50000.0	10	5.19	259.44
Ref. item	1.0	10	17.68	0.02

*adjusted for evaporation from the feeders
Ref.: reference;f.s.; feeding solution.

A summary of the effects of Mevalone on cumulative mortality and behavioural abnormalities of adult honey bees over a 10-day exposure period is presented in the table below.

Table 10.3.1.2/01-5: Effect of Mevalone on adult mortality at the end of the test (on day 10)

Groups	Concentrations	Doses	Cumulative Mortality		
	mg product/bee /kg f.s.	µg prod./bee/day	Mortality	±SD	Mean Corrected Mortality (%CM)
Untreated control	0.0	0.00	13.3	0.6	-
Control 0.2% of xanthan gum	0.0	0.00	13.3	0.6	-
T1	1300.0	37.66	20.0	1.0	7.7
T2	3200.0	66.96	16.7	1.5	3.9
T3	8000.0	149.79	73.3	2.9	69.2*
T4	2000.0	206.43	96.7	0.6	96.2*
T5	50000.0	259.44	100.0	0.0	100.0*
Ref. item	1.0	0.02	100.0	0.0	100.0

*The Step-down Cochran-Armitage test procedure evidenced that the product had lethal effects on adult Honey bees after being administered for ten consecutive days starting from the concentration of 8000 mg prod./kg diet, corresponding to a dose of 149.79 µg prod./bee/day (related to the mean food consumption).

Ref.: reference; Prod.: product, f.s.; feeding solution.

The 10-day NOEC value was 3200.0 mg product/kg diet (equivalent to 102.5 mg of eugenol, 201.3 mg of geraniol and 204.7 mg of thymol/kg diet), corresponding to a NOEDD value of 66.96 µg product/bee /bee/day.

The 10-day LC₅₀ value was calculated as 7075.32 mg product/kg feeding solution, (confidence limits CL: 4161.92-12028.14 mg product/kg feeding solution) equivalent to 114.43 mg of eugenol, 224.68 mg of geraniol and 228.51 mg of thymol/kg feeding solution; corresponding to a LDD₅₀ value of 123.53 µg product/bee /bee/day.

The 10-day LDD₁₀ values was 64.62 µg product/bee /bee/day (confidence limits CL: 48.79-85.59 µg product/bee /bee/day) equivalent to 2.07 µg of eugenol, 4.06 µg of geraniol and 4.13 µg of thymol/bee/day.

The 10-day LDD₂₀ value was 83.65 µg product/bee /bee/day (confidence limits CL: 67.52-103.63 µg product/bee/day), equivalent to 2.68 µg of eugenol, 5.26 µg of geraniol and 5.35 µg of thymol/bee/day.

The 10-day LDD₅₀ value was 123.53 µg product/bee/day (confidence limits CL: 108.46÷140.70 µg product/bee/day), equivalent to 3.96 µg of eugenol, 7.77 µg of geraniol and 7.90 µg of thymol/bee/day.

Validity

All validity criteria were met in accordance with OECD test guideline 245 (2017):

- Mean mortality in the untreated and solvent controls at test end was ≤15% (actual values: 13.3% in untreated control; 10.0% in solvent control).
- Mean mortality in the toxic reference at test end was ≥50% (actual value: 100%).

Conclusion

The 10-day chronic oral toxicity of Mevalone to adult honey bees (*Apis mellifera*) was tested in line with OECD test guideline 245 (2017). The analytical results demonstrate that the active substances' content in feeding solutions was in the range of ± 20% of nominal concentrations that were therefore used for calculating the endpoints of the test.

The 10-day NOEC value was 3200.0 mg product /kg feeding solution (equivalent to 102.5 mg of eugenol, 201.3 mg of geraniol and 204.7 mg of thymol/kg feeding solution), corresponding to a NOEDD value of 66.96 µg product/bee /bee/day.

The 10-day LC₅₀ value was calculated as 7075.32 mg product /kg feeding solution, (confidence limits CL: 4161.92-12028.14 mg product/kg feeding solution) equivalent to 114.43 mg of eugenol, 224.68 mg of geraniol and 228.51 mg of thymol/kg feeding solution; corresponding to a LDD₅₀ value of 123.53 µg product/bee/day.

The 10-day LDD₁₀ values was 64.62 µg product /bee/day (confidence limits CL: 48.79-85.59 µg product/bee/day) equivalent to 2.07 µg of eugenol, 4.06 µg of geraniol and 4.13 µg of thymol/bee/day.

The 10-day LDD₂₀ values was 83.65 µg product /bee/day (confidence limits CL: 67.52-103.63 µg product/bee/day), equivalent to 2.68 µg of eugenol, 5.26 µg of geraniol and 5.35 µg of thymol/bee/day.

The 10-day LDD₅₀ value was 123.53 µg product/bee/day (confidence limits CL: 108.46÷140.70 µg product/bee/day), equivalent to 3.96 µg of eugenol, 7.77 µg of geraniol and 7.90 µg of thymol/bee/day.

This study is considered acceptable and satisfies the guideline requirements for a chronic oral toxicity study with adult honey bees (OECD test guideline 245, 2017).

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 was performed in line with OECD 201 with no deviations.</p> <p>The temperature of 33.2-35.1°C was outside the range recommended by the test guideline (34-35°C). Deviations in range of relative humidity lasting <2 hours (with exceptions of day 3 and day 4, when deviation lasted about 4 hours) were also observed during the larval stage (86.7-98.1% vs. 90-100% recommended by the guideline). Deviations were noted only after opening of the desiccator for the daily operations and are in general unavoidable. Since deviations were short-term and all validity criteria were met, they are considered to have no impact on the test results.</p> <p>All the validity criteria were met and the study is considered acceptable with following endpoints are relevant for the risk assessment:</p> <p>NOED = 1300 µg product/larvae/developmental period</p> <p>It is noted that compliance with the GLP regulations is confirmed only by the statement of the study director and copy of the GLP certificate is not included in the study report. However, the laboratory (BioTecnologie BT S.r.l., Italy) is listed on the list of certified laboratories available on the website of the Italian Ministry of Health. The area of certification includes toxicity testing on terrestrial non-target organisms.</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.3.1.3/01
Report	Effects of ARAW on Honey bees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure. Pecorari, F., 2019b, report No BT060/19
Guideline(s):	Yes. OECD guidance document 239 “Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity test, Repeated Exposure (15-Jul-2016)”.
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	ARAW (Mevalone)
Formulation type:	CS
Lot/batch no.:	4017005
Active substance content:	eugenol: 3.29%; geraniol: 6.46%; thymol: 6.57% (w/v)
Expiry date of lot/batch:	13 th October 2020
Storage conditions:	Deep frozen (≤ -20 °C), dark, dry

Toxic reference

Name:	dimethoate
Formulation type:	-
Lot/batch no.:	779155
Active substance content:	99.5%

Expiry date of lot/batch: 1st February 2022
Storage conditions: Cool (1 - 10 °C), dark, dry

Test organism

Species: *Apis mellifera ligustica*
Age at study initiation: 3 day old larvae
Source: Larvae collected from three healthy colonies maintained at BioTecnologie BT S.r.l. The colonies were adequately fed, healthy, disease-free and with known history and pathological status. No pesticides were used in those hives one month before the collection.
Feeding during test: Yes (dependent on developmental stage)
Acclimation: Not applicable

Test conditions

Test concentrations: 0 (control), 81.3, 162.5, 325, 650 and 1300 µg product/larvae
Toxic standard: 7.39 µg a.s./larvae
Replicates: 3 per test group and controls with 12 larvae each
Test temperature: 33.2 - 35.1 °C (recorded continuously)
Relative humidity: 86.7 - 98.1% (avg. 96.1%) from D1 to D8
77.0-84.6% (avg. 79.6%) from D8 to D15;
57.0-65.3% (avg. 59.9%) from D15 to D22.
(Recorded continuously)
Photoperiod: darkness (except during observations).

The larval diet was prepared with deionised, autoclaved water using the following ingredients:

Diet A (D1): 50% weight fresh royal jelly + 50% weight of an aqueous solution (containing 2% weight yeast extract, 12% weight glucose and 12% weight fructose).

Diet B 50% (D3): weight fresh royal jelly + 50% weight of an aqueous solution (containing 3% weight yeast extract, 15% weight glucose and 15% weight fructose).

Diet C (from D4 to D6): 50% weight fresh royal jelly + 50% weight of an aqueous solution (containing 4% weight yeast extract, 18% weight glucose and 18% weight fructose).

The diets A, B and C prepared in this way have a density of about 1.1 mg/µL (e.g. 20 µL diet corresponds to 22 mg diet). The volume of the product stock solutions mixed with the diet did not exceed 10% of the final diet volume (reached value: 8.44%).

The larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm. The cells were sterilized by immersion in 70% ethanol solution for 30 minutes and dried in a laminar-flow hood. Each cell was placed into a well of a 48-well plate. The top of each grafting cell was maintained at the level of the plate by placing a piece of dental roll, wetted with 500 µL of sterilizing solution enhanced with 15% weight/volume glycerol, at the bottom of the wells. These plates were placed into a hermetic Plexiglas desiccator placed into an incubator with a forced air circulation system at 34-35 °C.

On day 1 (D1), the combs containing first instar larvae were carried from the hives to the laboratory. A volume of 20 µL of diet A was dropped into each cell of a 48-well plate, then one larva was transferred from the comb onto the surface of the diet of each cell, using a grafting tool. Thus, each plate contained larvae from the same colony, each colony being the origin of one of the three replicates used in the test. On D2, no food was provided to the larvae.

On D3, twelve well-fed larvae from each of the three replicates were selected and placed in the same plate: the grafting cells containing living larvae were transferred from the plates prepared on D1 to new plates and arranged in order to clearly distinguish the three replicates. All larvae on one plate received the same treatment.

The product was mixed with deionised water in order to get a stock solution (S5). The other stock solutions (from S4 to S1) were each obtained by diluting the previous higher concentrated solution with deionised water. The stock solutions were prepared every day and used to treat the diets (also named feeding solutions). The reference item stock solution was prepared in deionised water once and stored at about 4 °C. The treated diets were prepared every day just before their administration and used after being warmed in an incubator. The diet was dropped into each cell using a grafting tool.

Product was administered at nominal concentrations of 527.6, 1055.2, 2110.4, 4220.8 and 8441.6 mg product/kg feeding solution, equivalent to nominal doses of 81.3, 162.5, 325.0, 650.0 and 1300.0 µg product/larva/developmental period. The reference item dimethoate was tested at the single concentration of 48.0 mg a.s./kg (corresponding to a dose of 7.39 µg a.s./larva).

The concentrations of the active substances geraniol, eugenol and thymol in the product stock solutions were analysed. Specimens of the highest and the lowest concentrated product stock solutions were taken on each of the treatment dates and stored in a freezer at a temperature $\leq -18^{\circ}\text{C}$ until analysis.

For data evaluation the statistical programme ToxRat Professional 3.3.0 was used. The Chi² 2x2 Table with Bonferroni Correction ($\alpha = 0.05$, one-sided greater) was performed in order to evaluate the NOED/NOEC values on D8 and D22, respectively. No statistical analysis was used to evaluate the ED_x/EC_x values, because the corrected mortality at the highest concentration was very low (<7%).

Results and discussions

Analytical results

The HPLC-DAD analytical method for the determination of Mevalone in 50% aqueous sucrose solution was validated with regards to specificity, linearity and accuracy in accordance with guideline SANCO/3029/99 rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a peak at the characteristic retention time for Mevalone in the control sample. The analytical calibration was shown to be linear ($r \geq 0.9999$) over the range of 1.5414-35.9667 mg/L for eugenol; ($r \geq 1.0$) over the range of 3.14-73.3373 mg/L for geraniol and ($r \geq 0.9999$) over the range of 3.0090-70.2097 mg/L for thymol.

The measured concentrations of Mevalone in the lowest and highest stock solutions prepared each day are summarised in the tables below.

Table 10.3.1.3/01-1: Nominal and measured concentrations of eugenol in feeding solution at D3

Nominal concentration (mg eugenol/kg f.s.)	Measured concentration (mg eugenol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.2000	0.2045	0.203	101
	0.2005		
3.2000	3.3400	3.465	108
	3.5890		
	5.9303		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-2: Nominal and measured concentrations of geraniol in feeding solution at D3

Nominal concentration (mg geraniol/kg f.s.)	Measured concentration (mg geraniol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.3900	0.3942	0.394	101
	0.3931		
6.2900	6.374	6.397	102
	6.4193		
	5.9303		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-3: Nominal and measured concentrations of thymol in feeding solution at D3

Nominal concentration (mg thymol/kg f.s.)	Measured concentration (mg thymol/kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.4000	0.3685	0.366	91
	0.3628		
6.400	5.9875	5.959	93
	5.9303		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-4: Nominal and measured concentrations of eugenol in feeding solution at D4

Nominal concentration (mg eugenol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.2000	0.1929	0.193	97
	0.1939		
3.200	3.4648	3.454	108
	3.4429		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-5: Nominal and measured concentrations of eugenol in feeding solution at D4

Nominal concentration (mg geraniol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.3900	0.3779	0.384	98
	0.3909		
6.2900	6.6554	6.708	106
	6.7611		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-6: Nominal and measured concentrations of eugenol in feeding solution at D4

Nominal concentration (mg thymol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.4000	0.356	0.354	88
	0.3524		
6.400	6.1937	6.18	96
	6.1664		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-7: Nominal and measured concentrations of eugenol in feeding solution at D5

Nominal concentration (mg eugenol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.2000	0.2307	0.230	115
	0.2293		
3.200	3.4019	3.475	109
	3.5472		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-8: Nominal and measured concentrations of geraniol in feeding solution at D5

Nominal concentration (mg geraniol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.3900	0.4648	0.461	118
	0.4568		
6.2900	6.7058	6.733	107
	6.7603		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-9: Nominal and measured concentrations of thymol in feeding solution at D5

Nominal concentration (mg thymol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.4000	0.4204	0.426	106
	0.4316		
6.400	6.2483	6.219	97
	6.1896		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-10: Nominal and measured concentrations of eugenol in feeding solution at D6

Nominal concentration (mg eugenol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.200	0.2114	0.212	106
	0.2132		
3.200	3.2929	3.292	103
	3.2901		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-11: Nominal and measured concentrations of geraniol in feeding solution at D6

Nominal concentration (mg geraniol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.3900	0.4004	0.413	105
	0.4248		
6.2900	6.5672	6.606	105
	6.644		

*arithmetic mean
 f.s.; feeding solution

Table 10.3.1.3/01-12: Nominal and measured concentrations of thymol in feeding solution at D6

Nominal concentration (mg thymol/kg f.s.)	Measured concentration (mg /kg f.s.)	Mean conc. Measured*	Percentage of nominal
0.4000	0.4004	0.392	98
	0.3842		
6.400	6.3105	6.223	97
	6.1357		

*arithmetic mean
 f.s.; feeding solution

The analysis of the stock solutions used to treat the diet administered to the larvae demonstrates that the product content was in the range of 91 - 115% of nominal concentrations, so it was demonstrated that the larvae were treated with the corresponding dose of product and the endpoints were calculated on the basis of the nominal doses of product (Mevalone).

Biological results

A summary of the effects of Mevalone on the larval development and subsequent adult emergence of honey bees (*Apis mellifera* L.) is presented in the table below.

Table 10.3.1.3/01-13: Mean mortality (M %) and mean corrected mortality (CM %) of larvae on D8 following repeated exposure to Mevalone

Nominal doses µg product/larva	Nominal concentrations mg product/kg f.s.	Larval mortality	
		Mean mortality M%	Corrected mortality CM%
Control	-	2.78	0.0
81.3	5127.6	8.33	5.71
162.5	1055.2	2.78	0.00
325	2110.4	0.00	0.00
650	4220.8	5.56	2.86
1300	8441.6	8.33	5.71
Reference item	7.39	100	100

* No significant effects were observed

Table 10.3.1.3/01-14: Mean mortality (M %) of pupae

Nominal Doses µg product/larva	Concentrations mg product/kg f.s.	Pupal mortality from D8 to D15	Pupal mortality from D8 to D22
		M% ¹	M% ²
Control	-	0.00	0.000
81.3	5127.6	0.00	3.03
162.5	1055.2	0.00	5.71
325	2110.4	2.78	8.33
650	4220.8	0.00	5.88
1300	8441.6	3.03	6.06
Reference item	7.39	100	100

¹Calculated in percentage comparing the number of dead pupae from D8 to D15 to the number of alive pupae on D8

²Calculated in percentage comparing the number of dead pupae from D8 to D22 to the number of alive pupae on D8

Table 10.3.1.3/01-15: Mean mortality (M %) and Mean corrected mortality (CM %) from D3 to D22 and adult emergence on D22

Doses µg product/ /larva	Concentrations mg product/ /kg f.s.	Total Mortality (larvae + pupae)		Adult emergence	
		M%	CM%	%Emerged	% inhibition
Control	-	8.33	0.00	91.67	n.a.
81.3	5127.6	11.11	3.03	88.89	3.03
162.5	1055.2	8.33	0.00	91.67	0.00
325	2110.4	8.33	0.00	91.67	0.00
650	4220.8	11.11	3.03	88.89	3.03
1300	8441.6	13.89	6.06	86.11	6.06
reference item	7.39	100	100	0.00	100

* No significant effects were observed

n/a = not applicable; “+” = significant; “-” = not-significant;

At D8, the product did not cause significant effects on mortality at all tested doses. Accordingly, the 8-day NOED value was 1300.0 µg product/larva/developmental period (nominal), corresponding to a 8-day NOEC value of 8441.6 mg product/kg f.s. The 8-day LD₅₀ value was estimated to be > 1300.0 µg product/larva, corresponding to an 8-day LC₅₀ value >8441.6 mg product/kg f.s.

The adult emergence on D22 was not affected by the product administration to the larvae at all the tested doses. Thus, the 22-day NOED value was 1300.0 µg product/larva/developmental period, corresponding to a 22-day NOEC value of 8441.6 mg product/kg f.s.

The 22-day ED₅₀ value was estimated to be > 1300.0 µg product/larva, corresponding to a 22-dayEC₅₀ value of >8441.6 mg product/kg f.s.

Validity

All validity criteria were met in accordance with OECD guidance document 239 (2016):

- Cumulative larval mortality from Days 3 to 8 was ≤15% across all control replicates (actual mean value: 2.78%).
- Adult emergence at Day 22 was ≥70% across all control replicates (actual mean value: 91.67%).
- Cumulative larval mortality at Day 8 was ≥50% across all toxic reference (dimethoate) replicates (actual mean value: 100%).

Conclusion

The 22-day chronic oral toxicity of Mevalone to honey bee (*Apis mellifera*) larvae was tested in accordance with OECD guidance document 239 “Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure” (2016). Analytical verification confirmed that mean measured concentrations of eugenol, geraniol and thymol in daily stock solutions used to prepare the larval diets were between 96 and 108% of nominals, and biological endpoints are therefore reported based on nominal concentrations of Mevalone.

Regarding the effects on adult emergence on D22, the product did not cause adverse effects at all the tested doses. The NOED and the NOEC for adult emergence rate were determined to be 1300.0 µg product/larva/developmental period and 8442.0 mg product/kg f.s., respectively. The ED₅₀ was estimated to be greater than 1300.0 µg product/larva (eugenol: >43.9, geraniol: >86.2 and thymol: >87), corresponding to an EC₅₀ greater than 8441.6 mg product/kg f.s. (eugenol: >285.2, geraniol: >560.0 and thymol: >570.0 mg/kg f.s.). The mortality data did not allow the extrapolation of the ED_{10/20} values.

- | | | |
|------------------|---------------------|-----------------------------------|
| A 2.3.1.4 | KCP 10.3.1.4 | Sub-lethal effects |
| A 2.3.1.5 | KCP 10.3.1.5 | Cage and tunnel tests |
| A 2.3.1.6 | KCP 10.3.1.6 | Field tests with honeybees |

- A 2.3.2 KCP 10.3.2. Effects on non-target arthropods other than bees**
- A 2.3.2.1 KCP 10.3.2.1. Standard laboratory testing for non-target arthropods**
- A 2.3.2.2 KCP 10.3.2.2. Extended laboratory testing, aged residue studies with non-target arthropods**
- A 2.3.2.3 KCP 10.3.2.3. Semi-field studies with non-target arthropods**
- A 2.3.2.4 KCP 10.3.2.4. Field studies with non-target arthropods**
- A 2.3.2.5 KCP 10.3.2.5. Other routes of exposure for non-target arthropods**

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>The test design was suitable to derive both, NOEC and EC_x values.</p> <p>Reliability of the EC₁₀ value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> • NW (normalised width) of 2.3 was calculated, which results in rating “bad” in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC₁₀ (86.8 mg/kg soil dw) is greater than EC_{20,low} (39.6 mg/kg dw), • the dose-response curve could not be calculated because EC₅₀ value was not determined in the study (it was greater than the maximum concentration tested). <p>Taking the above results into account, the calculated EC₁₀ is considered to be not reliable.</p> <p>The following endpoints are relevant for the risk assessment:</p> <p>NOEC (reproduction) = 52.9 mg product/kg soil dw NOEC (mortality) = 556.0 mg product/kg soil dw</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.4.1.1/01
Report	Mevalone: Effects on Reproduction and Growth of Earthworms <i>Eisenia andrei</i> in Artificial Soil Straube, D., 2021, report No 155781022
Guideline(s):	OECD Guideline 222 (2016); ISO 11268-2 (2012)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	Mevalone
Formulation type:	CS
Lot/batch no.:	11001
Active substance content:	Eugenol: Nominal: 3.2%, Analysed: 3.15% w/w Geraniol: Nominal: 6.4%, Analysed: 6.93% w/w Thymol: Nominal: 6.4%, Analysed: 6.23% w/w Total Terpene: Nominal: 16.0% Analysed 16.3%
Expiry date of lot/batch:	July 2022
Storage conditions:	At 20 ± 5 °C, in the dark
Test concentrations:	0, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg dws

Toxic reference

Name: Carbendazim
Lot/batch no.: Not reported
Active substance content: 500 g/L nominal

Test organism

Species: Earthworm *Eisenia andrei* (Annelida: Oligochaeta)
Age at study initiation: Approximately 9 months old with well-developed clitellum, age range between test individuals not differing by more than 4 weeks.
Weight at study initiation: 303 mg to 598 mg
Source: Bred under standardised conditions at Ibacon laboratories in a breeding medium of cattle manure, peat, sand, calcium carbonate and straw, fed with cattle manure, stored at room temperature.
Feeding during test: Yes. Finely ground and air-dried cattle manure was used as food. 5 g/container was scattered on the soil surface at day 1 after application and was moistened with 5 g deionised water; 5 g/container (moistened with 2 g deionised water) was added each week for the first 28 days of the experiment, when the food of the previous week had almost been consumed. If the food was not quite fully consumed, the added amount of food was adjusted to replace the visually estimated consumption. Four weeks after application, the food was mixed into the substrate following removal of the adult earthworms.
Acclimation: 2 days, in artificial soil, under test conditions.
Replicates: 8 per control and 4 per test item group with 10 earthworms each.

Test conditions

Test medium: 10% sphagnum-peat, air-dried and finely ground (<2 mm, with no visible plant remains);
20% Kaolin clay (Kaolinite content >30%)
69.6% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm;
0.4% Calcium carbonate (CaCO₃) was added to adjust pH to 6.0 ± 0.5
The artificial soil was moistened to approximately half of the final water content 1 day before the application. The additional water required to achieve the final water content was added when applying the test item.
Test temperature: within the range 18 – 22 °C
Soil pH: 5.7- 5.7 (test start); 6.2 – 6.5 (test end)
Photoperiod: 16 hours light: 8 hours dark
Light intensity: within the range 400 – 800 lux

Test units comprised plastic vessels (18.3 cm x 13.6 cm x 6 cm, tapered towards the bottom, with a soil surface of approximately 189.75 cm²), with perforated transparent lids to enable exchange of air, to minimise evaporation from the artificial soil, and to prevent the earthworms from escaping. Each container was filled with 627.3 g ± 1 g of the prepared soil (500 g dry weight plus deionised water). The depth of the soil layer in the containers was approximately 5 cm.

A soil water content of 49% of the maximum water holding capacity (WHC_{max}) was maintained by adding a suitable amount of deionised water to dry artificial soil once per week (ensuring the difference in water content between test start and end was <10%).

A stock solution was prepared by mixing Mevalone into deionised water, which was then prepared further

into a dilution series of stock solutions. Appropriate amounts of these stock solutions were added to 2100 g of dry artificial soil to obtain the target nominal concentrations of the product in the soil. There were no significant deviations to the nominal target concentration (< 5%). The control was left untreated. While mixing the artificial soil in a laboratory mixer for approximately 5 minutes the soil of each treatment group (including the control) was moistened with deionised water. Each group was treated in one batch (two in the control) which was then split into the replicates.

On the day of application, all earthworms were rinsed with tap water, dried with dry paper towels, weighed individually and randomly assigned to batches of 10 earthworms. The different batches were sorted into four classes based on the total weight and one batch of each weight class was assigned to each treatment group (two batches for the control) to ensure weights were homogeneous. The earthworms were placed on the surface of the artificial soil after application. There were 8 replicates of 10 earthworms each for the control and 4 replicates of 10 earthworms for each test treatment.

After 28 days, the artificial soil was transferred to a tray and adult earthworms were counted, removed and weighed per replicate after being rinsed under tap water and dried on paper towels. Missing earthworms and earthworms that failed to respond to gentle stimulation were considered dead. The remaining soil (without the adult earthworms) was then returned to the respective test containers.

After a further 28 days, juveniles were removed by placing the test units in a water bath at 50 - 60 °C and counting all emerging earthworms. In addition, the soil of each container was emptied out onto a tray and checked visually for any remaining juvenile earthworms.

The numbers of dead adult earthworms, sub-lethally affected earthworms, body weights and the amount of food added to each test container (which approximately reflects the amount of food eaten) were listed for each replicate. The number of offspring was recorded for each test replicate and treatment.

For data evaluation the statistical programme ToxRat Professional Version 3.3.0 was used. Mortality data were analysed for significance by using the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.01$) using the Shapiro-Wilk's test and the Levene's test, respectively. Since the weight change data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend), the Dunnett's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, two-sided). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend), the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values.

The EC_x values and their 95% confidence limits for reproduction were calculated by applying Probit Analysis.

The reference item carbendazim (nominally 500 g/L), was tested in a separate study at 0, 1.09, 1.56, 2.25, 3.24 and 4.87 mg test item/kg soil d.w., (equivalent to 0, 0.482, 0.694, 1.00, 1.44 and 2.07 mg carbendazim/kg soil d.w.).

Results and discussions

Biological results

A summary of the effects of the product Mevalone on mortality of adult earthworms after 28 days exposure is presented in the table below.

Table 10.4.1.1/02-01: Effect of the product Mevalone on adult mortality of *Eisenia Andrei*

Nominal concentration (mg product/kg soil d.w.)	Replicate number	Number of adults at test start	Day 28		
			Number dead per replicate	% mortality per replicate	% mean mortality per treatment (± SD ¹)
Control	1	10	0	0	2.5 (± 4.6)
	2	10	1	10	
	3	10	0	0	
	4	10	0	0	
	5	10	1	10	
	6	10	0	0	
	7	10	0	0	
	8	10	0	0	
16.3	1	10	0	0	0.0 (± 0.0)
	2	10	0	0	
	3	10	0	0	
	4	10	0	0	
29.4	1	10	1	10	7.5 (± 5.0)
	2	10	1	10	
	3	10	0	0	
	4	10	1	10	
52.9	1	10	1	10	5.0 (± 5.8)
	2	10	1	10	
	3	10	0	0	
	4	10	0	0	
95.3	1	10	0	0	5.0 (± 10.0)
	2	10	0	0	
	3	10	0	0	
	4	10	2	20	
171	1	10	0	0	5.0 (± 5.8)
	2	10	0	0	
	3	10	1	10	
	4	10	1	10	
309	1	10	1	10	7.5 (± 5.0)
	2	10	0	0	
	3	10	1	10	
	4	10	1	10	
556	1	10	0	0	2.5 (± 5.0)
	2	10	0	0	
	3	10	0	0	
	4	10	1	10	
1000	1	10	1	10	17.5* (± 17.1)
	2	10	2	20	
	3	10	0	0	
	4	10	4	40	

SD = Standard deviation

¹ mean ± standard deviation of 4 replicates (8 in the control)

* = significantly different compared to the control, Williams t-test, $\alpha = 0.05$, one-sided smaller

After 28 days' exposure to the product Mevalone, there were no statistically significant differences in adult mortality at any of the test concentrations up to and including 556 mg product/kg soil d.w., compared to the control group (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$). At the test concentration of 1000 mg product/kg soil d.w., adult mortality was reported to be 17.5%, which was statistically significantly different compared to the control. Therefore, the 28-day NOEC value for adult mortality was determined to be 556 mg product/kg soil d.w. The 28-day LOEC value for adult mortality was determined to be 1000 mg product/kg soil d.w. The 28-day LC₅₀ value for adult mortality was estimated to be >1000 mg product/kg soil d.w.

A summary of the effects of the product Mevalone on body weight change of adult earthworms after 28 days' exposure is presented in the table below.

Table 10.4.1.1/02-02: Effect of the product Mevalone on adult body weight of *Eisenia andrei*

Nominal concentration (mg product/kg soil d.w.)	Replicate number	Per replicate				Per treatment	
		Mean body weight (mg)		Mean body weight change (mg)	Mean % body weight change	Mean body weight change	
		Day 0	Day 28	Day 0 – Day 28	Day 0 – Day 28	mg	%
						(± SD ¹)	(± SD ¹)
Control	1	418.9	571.9	153.0	36.5	90 (± 44)	20.1 (±10.9)
	2	438.8	581.7	142.9	32.6		
	3	441.1	539.3	98.2	22.3		
	4	457.3	553.3	96.0	21.0		
	5	460.7	555.6	94.9	20.6		
	6	474.7	507.4	32.7	6.9		
	7	478.6	546.0	67.4	14.1		
	8	521.3	555.5	34.2	6.6		
16.3	1	430.7	538.6	107.9	25.1	79 (± 32)	17.4 (± 7.7)
	2	457.2	559.3	102.1	22.3		
	3	466.4	532.0	65.6	14.1		
	4	500.2	541.0	40.8	8.2		
29.4	1	432.1	543.6	111.5	25.8	76 (± 46)	17 (± 10.7)
	2	453.8	572.9	119.1	26.2		
	3	466.4	517.3	50.9	10.9		
	4	499.9	524.3	24.4	4.9		
52.9	1	432.2	554.1	121.9	28.2	98 (± 20)	21.4 (± 5.5)
	2	452.5	557.3	104.8	23.2		
	3	468.5	543.6	75.1	16.0		
	4	496.5	586.2	89.7	18.1		
95.3	1	432.6	536.8	104.2	24.1	91 (± 40)	19.6 (± 8.2)
	2	449.2	496.4	47.2	10.5		
	3	469.2	540.3	71.1	15.2		
	4	491.9	632.0	140.1	28.5		
171	1	432.9	511.6	78.7	18.2	83 (± 27)	18.2 (± 6.2)
	2	447.0	560.9	113.9	25.5		
	3	472.2	561.3	89.1	18.9		
	4	485.1	534.7	49.6	10.2		
309	1	436.6	554.4	117.8	27.0	96 (± 20)	21.1 (± 5.3)
	2	446.2	552.3	106.1	23.8		
	3	472.3	559.1	86.8	18.4		
	4	483.4	556.4	73.0	15.1		
556	1	437.5	523.2	85.7	19.6	66 (± 17)	14.4 (± 4.3)
	2	445.3	515.6	70.3	15.8		
	3	473.0	535.1	62.1	13.1		
	4	481.3	526.0	44.7	9.3		
1000	1	438.2	554.0	115.8	26.4	113 (± 45)	25.0 (± 10.6)
	2	442.4	614.6	172.2	38.9		
	3	473.2	573.1	99.9	21.1		
	4	479.5	545.0	65.5	13.7		

SD = Standard deviation

¹ mean ± standard deviation of 4 replicates (8 in the control)

There were no statistically significant differences in body weight changes in the test item treated groups compared to the control, up to and including the highest test concentration of 1000 mg product/kg soil d.w. (Dunnett's t-test, $\alpha = 0.05$, two-sided). The 28-day NOEC value for adult body weight change was therefore determined to be 1000 mg product/kg soil d.w. The 28-day LOEC value for adult body weight change was estimated to be >1000 mg product/kg soil d.w.

A summary of the effects of the product Mevalone on earthworm (*Eisenia andrei*) reproductive output (number of juvenile earthworms after 56 days exposure) is presented in the table below.

Table 10.4.1.1/02-03: Effect of the product Mevalone on reproductive output (number of juvenile earthworms after 56 days exposure) of *Eisenia andrei*

Nominal concentration (mg product/kg soil d.w.)	Replicate number	Day 56			
		Number of juvenile worms per replicate	Mean number of juvenile worms per replicate per treatment (\pm SD ¹)	Coefficient of variation (%)	% reduction in reproductive output relative to control ²
Control	1	109	120 (\pm 15)	12.5	n.a.
	2	113			
	3	127			
	4	115			
	5	127			
	6	140			
	7	135			
	8	96			
16.3	1	132	128 (\pm 11)	8.6	-6.4
	2	112			
	3	132			
	4	136			
29.4	1	115	137 (\pm 20)	14.6	-14.1
	2	148			
	3	126			
	4	160			
52.9	1	127	114 (\pm 23)	20.2	5.0
	2	138			
	3	86			
	4	106			
95.3	1	74	95 (\pm 22)	23.2	21.2*
	2	94			
	3	85			
	4	126			
171	1	90	94 (\pm 8)	8.5	22.0*
	2	92			
	3	88			
	4	105			
309	1	115	102 (\pm 26)	25.5	15.6*
	2	112			
	3	116			
	4	63			
556	1	95	97 (\pm 7)	7.2	19.8*
	2	103			
	3	101			
	4	87			
1000	1	59	64 (\pm 12)	18.8	47.2*
	2	81			
	3	56			
	4	58			

SD = Standard deviation

¹ mean \pm standard deviation of 4 replicates (8 in the control)

² = % reduction in reproduction compared to the control (56 days). Negative values represent an increase relative to the control.

n.a. = not applicable

* = significantly different compared to the control, Williams t-test, $\alpha = 0.05$, one-sided smaller

There were no statistically significant differences in the number of juvenile earthworms (*Eisenia andrei*) compared to the control, up to and including the test concentration of 52.9 mg product/kg soil d.w. (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentrations of 95.3 mg product/kg soil d.w. and above, reproduction rates were statistically significantly decreased compared to the control. Therefore, the 56-day NOEC value (reproduction) was determined to be 52.9 mg product/kg soil d.w. The 56-day LOEC value (reproduction) was determined to be 95.3 mg product/kg soil d.w. The 56-day EC₁₀ (reproduction) value was determined to be 86.8 mg product/kg soil d.w. (95% confidence intervals of 0.77 to 203.2 mg product/kg soil d.w.) and the EC₂₀ value (reproduction) was

determined to be 252.1 mg product/kg soil d.w. (95% confidence intervals of 39.6 to 524.2 mg product/kg soil d.w.). The EC₅₀ value (reproduction) could not be determined statistically and was therefore estimated to be >1000 mg product/kg soil d.w.

The toxic reference chemical carbendazim was tested in a separate study. There were statistically significant effects on reproduction at a concentration of 0.694 mg a.s./kg soil d.w. and above (tested up to 2.07 mg a.s./kg soil d.w.), which is in line with the test guideline OECD 222, 2016 (effects should be observed between 1 and 5 mg a.s./kg soil d.w.). The EC₅₀ value (reproduction) was calculated as 0.88 mg a.s./kg soil d.w., confirming the sensitivity of the test system.

Validity

All validity criteria were met in accordance with OECD test guideline 222 (2016):

- Each control replicate (containing 10 adult earthworms) produced ≥30 juveniles by the end of the test (actual values: 96 - 140 juveniles per control replicate).
- The coefficient of variation of reproduction in the control group was ≤30% (actual value: 12.5%).
- Adult mortality in the control group over the initial 28 days was ≤10% (actual value: 2.5%).

Conclusion

The 56-day chronic toxicity of the product Mevalone to earthworm (*Eisenia andrei*) was studied in artificial soil according to OECD test guideline 222 (2016).

The 56-day NOEC value for earthworm (*Eisenia andrei*) based on reproductive output was determined to be 52.9 mg product/kg soil d.w. (equivalent to 1.7, 3.7 and 3.3 mg a.s./kg soil d.w. for eugenol, geraniol and thymol, respectively), based on nominal concentrations.

The 56-day EC₁₀ (reproduction) value for earthworm (*Eisenia andrei*) was determined to be 86.8 mg product/kg soil d.w. (95% CI: 0.77 to 203.2 mg test item/kg dws) (equivalent to 2.7, 6.0 and 5.4 mg a.s./kg soil d.w. for eugenol, geraniol and thymol, respectively), based on nominal concentrations. The 56-day EC₂₀ (reproduction) value was determined to be 252.1 mg product/kg soil d.w. (95% CI: 39.6 to 524.2 mg test item/kg dws) (equivalent to 7.9, 17.5 and 15.7 mg a.s./kg soil d.w. for eugenol, geraniol and thymol, respectively), based on nominal concentrations.

This study is considered acceptable and valid.

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

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>The test design was suitable to derive both, NOEC and EC_x values.</p> <p>Reliability of the EC₁₀ value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> • NW (normalised width) of 0.63 was calculated, which results in rating “fair” in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC₁₀ (37.3 mg/kg soil dw) is greater than EC_{20,low} (30.4 mg/kg dw), • the dose-response curve is medium with steepness of 0.62 (i.e. between 0.33 and 0.66). <p>Taking the above results into account, the calculated EC₁₀ is considered to be not fully</p>
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	<p>reliable, mainly due to median EC₁₀ being greater than EC_{20,low}. However, at the concentration set as NOEC, reproduction was reduced by 21% comparing to control and this effect should not be ignored. Taking into account that at 25 mg product/kg soil dw the reproduction was reduced by only 5%, the EC₁₀ of 37.3 mg product/kg soil dw is reasonable, even if not fully reliable. This value is recommended for calculation of the TER values as being lower than the NOEC.</p> <p>The following endpoints are relevant for the risk assessment:</p> <p>EC₁₀ = 37.3 mg product/kg soil dw NOEC (reproduction) = 45.0 mg product/kg soil dw NOEC (mortality) = 45.0 mg product/kg soil dw</p>
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This study has also been submitted within the AIR dossier submitted 28th February 2021 (RMS: Spain).

Reference:	KCP 10.4.2.1/01
Report	Mevalone: Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil Straube, D., 2020, Report No 155781016
Guideline(s):	OECD Guideline 232 (2016) and ISO 11267 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable, not a vertebrate study

Materials and methods

Test material

Name:	Mevalone
Formulation type:	CS
Source and lot/batch no.:	11001
Active substance content:	Eugenol: 3.2% w/w (nominal); 3.15% w/w (analysed) Geraniol: 6.4% w/w (nominal); 6.93% w/w (analysed) Thymol: 6.4% w/w (nominal); 6.23% w/w (analysed)
Expiry date of lot/batch:	July 2022
Storage conditions:	At 20 ± 5 °C, in the dark
Test concentrations:	0, 1.32, 2.38, 4.29, 7.72, 13.9, 25.0, 45.0 and 81.0 mg product/kg dws

Test organism

Species:	<i>Folsomia candida</i> , Collembola
Age at study initiation:	9-12 days
Source:	The synchronised individuals were bred at Ibacon and were fed with granulated dry yeast and kept under breeding conditions until test start.
Feeding during test:	Yes, after the introduction of the test organisms (day 0), and after 14 days, approximately 2 mg of granulated dried yeast was spread over the soil surface.
Acclimation:	Not reported
Replicates:	8 per control and 4 per test item group with 10 springtails each.

Test medium	5% sphagnum peat; 20% kaolin clay; 74.8% fine quartz-sand;
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0.2% calcium carbonate

Test conditions

Test temperature:	18-22 °C
pH:	At test start: 5.7 to 5.8 At test end: 5.8
Photoperiod:	16 h light: 8 h dark
Light intensity:	400-800 lux
Water content:	44.1-53.6% WHC

Test units comprised glass containers (diameter 5 cm; volume 100 mL) closed tightly with a crew lid to avoid water evaporation. All vessels were ventilated by opening the lids for a short period.

A stock solution was prepared by weighing 70.0 mg of Mevalone using an analytical balance. The test item was transferred into a glass beaker and deionised water was added to obtain a final net weight of 105.1 g. The resulting suspension contained a concentration of 0.6660 mg test item/g. The remaining test item solutions were prepared by dilution with deionised water. The artificial soil was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item, which was homogeneously with the test soil. The water content of the soil was maintained throughout the test. It was not necessary to compensate for loss of water as deviation did not exceed 2% of the initial water content.

10 collembola were introduced in each test unit on the surface of the treated artificial soil. Four replicates were tested per test item treatment and eight control replicates were tested. After 28 days, to determine the number of adult and juvenile *Folsomia* the contents of the test containers were suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. The Collembola drifted to the surface. Adult animals were counted once visually, juvenile animals were counted using FolsomiaCounter, a photo based evaluation software, which automatically determines the number of juvenile animals from a digital photograph (validated counting system, FolsomiaCounter Version 1.23, © 2020 Visionalytics). The extraction efficiency was checked separately in August 2020. Two extraction units with untreated soil were prepared by adding 60 collembolans to each unit. The number of extracted animals was counted. 119 animals out of 120 were recovered giving an extraction efficiency of 99.2%. The numbers of living adult Collembola at day 28 after application were recorded. Missing adult Collembola were recorded as dead as it is assumed that missing adult Collembola had died and degraded during the test period.

Mortality data were statistically analysed using Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). An LC_{50} value and its 95% confidence limits at day 28 was calculated by applying Weibull Analysis. Values were compensated for control mortality using Abbott's formula.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The ECx values for reproduction were calculated by Probit Analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

A separate GLP study was performed with the boric acid as the toxic standard.

Results and discussions

Biological results

A summary of the effects of eugenol on mortality and reproduction of *Folsomia candida* after 28 days exposure are presented in the tables below.

Table 10.4.2.1/01-01: Effect of Mevalone on mortality of *Folsomia candida*

Nominal concentration (mg product/kg soil dw)	Replicate number	Day 28	
		No. dead per replicate	% mean mortality per treatment
Control	1	0	8
	2	1	
	3	1	
	4	1	
	5	1	
	6	1	
	7	0	
	8	1	
1.32	1	2	5
	2	0	
	3	0	
	4	0	
2.38	1	1	8
	2	1	
	3	0	
	4	1	
4.29	1	0	3
	2	0	
	3	0	
	4	1	
7.72	1	0	3
	2	0	
	3	0	
	4	1	
13.9	1	0	5
	2	0	
	3	1	
	4	1	
25.0	1	1	8
	2	0	
	3	0	
	4	2	
45.0	1	2	18
	2	1	
	3	2	
	4	2	
81.0	1	6	78*
	2	10	
	3	9	
	4	6	

* Significant difference compared to the control (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater).

Mortality of *Folsomia candida* was not statistically significantly different compared to the control up to and including the test concentration of 45.0 mg product/kg soil dw (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 81.0 mg product/kg soil dw a statistically significant increased mortality was observed. No abnormal behaviour was observed with the surviving Collembola.

The 28-day LC₅₀ (adult mortality) of Mevalone for *Folsomia candida* in artificial soil was determined to be 69.1 mg product/kg soil dw (95% confidence limits of 62.6 to 74.8 mg product/kg soil dw), corresponding to 2.2 mg eugenol/kg soil dw ,4.8 mg geraniol/kg soil dw and 4.3 mg thymol/kg soil dw.

Table 10.4.2.1/01-02: Effect of Mevalone on reproductive output (number of juvenile springtails after 28 days exposure) of *Folsomia candida*

Nominal concentration (mg product/kg soil dw)	Replicate number	Day 28			
		No. of juveniles per replicate	Mean no. of juveniles per replicate per treatment	Coefficient of variation (%) ^a	% reduction in reproductive output relative to control ^b
Control	1	1353	1339	12.2	-
	2	1379			
	3	1130			
	4	1248			
	5	1216			
	6	1497			
	7	1631			
	8	1260			
1.32	1	790	1402	33.1	-5
	2	1711			
	3	1808			
	4	1300			
2.38	1	1253	1373	16.3	-3
	2	1537			
	3	1583			
	4	1119			
4.29	1	947	1360	31.5	-2
	2	1657			
	3	1794			
	4	1041			
7.72	1	1710	1266	26.6	5
	2	1238			
	3	1227			
	4	890			
13.9	1	1212	1140	5.3	15
	2	1066			
	3	1128			
	4	1153			
25.0	1	1209	1268	21.4	5
	2	1641			
	3	993			
	4	1227			
45.0	1	909	1059	25.3	21
	2	1266			
	3	759			
	4	1303			
81.0	1	594	283*	101	79
	2	2			
	3	83			
	4	454			

^a Coefficient of variation (CV) calculated based on the mean (x) and standard deviation (s) values presented in the report, where $CV = s / x * 100$

^b Negative values represent an increase compared to control

* Statistically significant difference compared to the control group (Williams' test, one-sided smaller, $\alpha = 0.05$)

There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the concentration of 45.0 mg product/kg soil dw (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the concentration of 81.0 mg product/kg soil dw reproduction was statistically significantly reduced compared to the control. No abnormal behaviour was observed with the surviving Collembola.

The 28-day NOEC value for reproduction was determined to be 45.0 mg product/kg soil dw, corresponding to 1.4 mg eugenol/kg soil dw , 3.1 mg geraniol/kg soil dw and 2.8 mg thymol/kg soil dw.

The 28-day EC₁₀ (reproduction) value for *Folsomia candida* in artificial soil was determined to be 37.3 mg product/kg soil dw, corresponding to 1.2 mg eugenol/kg soil dw, 2.6 mg geraniol/kg soil dw and 2.3 mg thymol/kg soil dw. The 28-day EC₂₀ (reproduction) value was determined to be 44.0 mg product/kg soil dw, corresponding to 1.4 mg eugenol/kg soil dw, 3.0 mg geraniol/kg soil dw; 2 and 7 mg thymol/kg soil dw. The 28-day EC₅₀ (reproduction) value was determined to be 60.3 mg product/kg soil dw, corresponding to 1.9 mg eugenol/kg soil dw ,4.2 mg geraniol/kg soil dw and 3.8 mg thymol/kg soil dw.

Table summarising results is given below.

Mevalone [mg/kg soil dry weight]	Control	1.32	2.38	4.29	7.72	13.9	25.0	45.0	81.0
Mortality (day 28) [%]	8	5	8	3	3	5	8	18	78
Significance ¹⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
No. of juveniles (day 28)	1339	1402	1373	1360	1266	1140	1268	1059	283
Reproduction in [%] of control (day 28)	-	105	103	102	95	85	95	79	21
Statistical significance ²⁾	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Endpoints [mg Mevalone/kg soil dry weight]									
NOEC (mortality)	45.0								
LOEC (mortality)	81.0								
LC ₅₀ (mortality) ³⁾	69.1								
NOEC (reproduction)	45.0								
LOEC (reproduction)	81.0								
EC Values (reproduction) ⁴⁾	EC ₁₀			EC ₂₀			EC ₅₀		
	37.3			44.0			60.3		
95% confidence limits	22.3 to 45.7			30.4 to 51.9			50.9 to 71.7		

n.s. = not significantly different compared to the control

* = significantly different compared to the control

¹⁾ Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater

²⁾ Williams t-test, $\alpha = 0.05$, one-sided smaller

³⁾ Weibull Analysis

⁴⁾ Probit Analysis

- not applicable

In a separate study, the reference item boric acid showed statistically significant effects on reproduction at concentrations of ≥ 48.8 mg boric acid/kg soil. The EC₅₀ value for reproduction was calculated to be 104.6 mg boric acid/kg soil.

Validity

All validity criteria were met in accordance with OECD test guideline 232 (2016):

- Mean adult mortality in the control group was $\leq 20\%$ at test end (actual value: 8%).
- The mean number of juveniles per vessel in the control group was ≥ 100 at test end (actual value: 1139).
- The coefficient of variation calculated for the number of juveniles in the control group was $< 30\%$ (actual value: 12.2%).

Conclusion

The 28-day chronic toxicity of Mevalone to *Folsomia candida* was studied in artificial soil with 5% peat

according to OECD guideline 232 (2016).

The 28-day LC₅₀ (mortality) of Mevalone for *Folsomia candida* in artificial soil was determined to be 69.1 mg product/kg soil dw (95% confidence limits of 62.6 to 74.8 mg product/kg soil), corresponding to 2.2 mg eugenol/kg soil dw , 4.8 mg geraniol/kg soil dw and 4.3 mg thymol/kg soil dw .

The overall 28-day NOEC value based on reproductive and mortality outputs was determined to be 45.0 mg product/kg soil dw, corresponding to 1.4 mg eugenol/kg soil dw , 3.1 mg geraniol/kg soil dw and 2.8 mg thymol/kg soil dw .

The overall 28-day LOEC for reproduction and mortality outputs was determined to be 81.0 mg product/kg soil dw, corresponding to 2.6 mg eugenol/kg soil dw , 5.6 mg geraniol/kg soil dw;; and 5.0 mg thymol/kg soil dw.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

- A 2.5 KCP 10.5 Effects on soil nitrogen transformation**
- 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**
- 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**