European Commission

Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Thymol;
5-methyl-2-(propan-2-yl) phenol

Volume 1

Rapporteur Member State: Spain
Co-Rapporteur Member State: Greece

Version History

When	What
July 2022	Initial DRAR – RMS Spain
November 2022	DRAR after CoRMS and Applicant comments
February 2023	RAR updated after EFSA CoCh received on 24/01/2023

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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Level 1

THYMOL

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

The draft assessment report was prepared for the renewal of approval of thymol in accordance with Regulation (EC) No 1107/2009.

Thymol was approved as active substance in accordance with Regulation (EC) No 1107/2009 by Commission Implementing Regulation (EU) No 568/2013 of 18 June 2013. The main notifier for the original EU Inclusion was Eden Research plc and the RMS was United-Kingdom.

Thymol is included in AIR 5 program (SANTE-2018- 10048–rev 3, February 2020). Eden Research plc is the main notifier for the renewal of approval of thymol, the RMS is Spain and the co-RMS is Greece (Commission Implementing Regulation (EU) No 2018/155 of 31 January 2018).

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.

The content of the submitted dossier was in compliance with the data requirements described in Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 and according to Commission Regulation (EU) No 844/2012 and prepared according to SANCO/10181/2013 – rev. 5 (12 June 2019, Guidance Document For Applicants On Preparing Dossiers For The Approval Of A Chemical New Active Substance And For The Renewal Of Approval Of A Chemical Active Substance According To Regulation (Eu) No 283/2013 And Regulation (Eu) No 284/2013).

The active substance is for use as a fungicide, for application on grapes and pome fruits. The representative product is Mevalone, a CS formulation containing 33 g/L eugenol, 66 g/L geraniol and 66 g/L thymol. Mevalone is currently authorised in various EU Member States. Mevalone is the common representative product of the three active substances eugenol, geraniol and thymol intended to be renewed at the same time.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

According to Commission Implementing Regulation (EU) 2018/155 of 31 January 2018 amending Implementing Regulation No 686/2012 allocating to Member States, for the purposes of the renewal procedure, the evaluation of the active substances whose approval expires by 31 December 2024 at the latest, Spain has been designated as the Rapporteur Member State (RMS) and Greece as the Co-rapporteur Member State (Co-RMS).

For the purposes of the renewal procedure, the evaluation of each active substance set out in the first column of the Annex, is allocated to a rapporteur Member State, as set out in the second column of that Annex, and to a co-rapporteur Member State, as set out in the third column of that Annex.

'PART C

ALLOCATION OF THE EVALUATION OF ACTIVE SUBSTANCES WHOSE APPROVAL EXPIRES AFTER 31 DECEMBER 2021 AND NOT LATER THAN 31 DECEMBER 2024

Active substance	Rapporteur Member State	Co-rapporteur Member State
Thymol	ES	EL

RMS has arranged with the Co-RMS a commenting period before sending the RAR to EFSA

1.1.3 EU Regulatory history for use in Plant Protection Products

Refer to 1.1.1.

1.1.4 Evaluations carried out under other regulatory contexts

Thymol is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area.

This substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

Consumer Uses
Thymol is used in the following products: perfumes and fragrances, air care products, washing & cleaning products, cosmetics and personal care products, biocides (e.g. disinfectants, pest control products) and polishes and waxes. Other release to the environment of this substance is likely to occur from: indoor use as processing aid and outdoor use as processing aid.

Thymol is registered in US-EPA as pesticide and it is listed as 40 CFR 180: Pesticide Tolerance Exemptions, Tolerances and exemptions for pesticide chemical residues in food. It is also listed by US-EPA as 2020 CDR TSCA (The Toxic Substances Control Act) Inv Active in the chemicals list which is subject to a data report every 4 years via Chemical Data Reporting (CDR).

References :

EU source: Substance Information - ECHA (europa.eu)
EPA source: System of Registries | US EPA

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Name: Eden Research plc
Address: 67C Innovation Drive
Milton Park
Oxfordshire
OX14 4RQ
UK

Contact: [redacted]
E-Mail:
Phone:

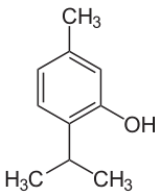
1.2.2 Producer or producers of the active substance

CONFIDENTIAL information – data provided separately-Vol. 4

1.2.3 Information relating to the collective provision of dossiers

Eden Research plc is to the best of their knowledge the only company submitting a dossier for the renewal of thymol. Therefore, a task force is not required for this submission.
All data is owned by Eden Research plc.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Thymol (No ISO common name)
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	5-methyl-2-propan-2-yl-phenol
CA	Phenol, 5-methyl-2-(1-methylethyl)-
1.3.3 Producer's development code number	None
1.3.4 CAS, EEC and CIPAC numbers	
CAS	89-83-8
EC	201-944-8
CIPAC	969
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{10}H_{14}O$
Structural formula	
Molecular mass	150.22 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.7 Specification of purity of the active substance in g/kg	Minimum purity : 990 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
Additives	CONFIDENTIAL information - data provided separately (Volume 4)
Significant impurities	CONFIDENTIAL information - data provided separately (Volume 4)
Relevant impurities	None
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately (Volume 4)

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	Eden Research plc
1.4.2 Producer of the plant protection product	CONFIDENTIAL information – data provided separately-Vol. 4

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product

Trade name: Mevalone
Code number: 3AEY

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product

Composition of the plant protection product

Chemical name	g/L	% w/w
Eugenol (pure, 100%)	33.0 (29.7 – 36.3)	3.21 (2.89 – 3.53)
Eugenol technical (99.0%)	33.3 (30.0 – 36.6)	3.24 (2.92 – 3.56)
Geraniol (pure, 100%)	66.0 (59.4 – 72.6)	6.41 (5.77 – 7.06)
Geraniol technical (98.0%)	67.3 (60.6 – 74.1)	6.54 (5.89 – 7.20)
Thymol (pure, 100%)	66.0 (59.4 – 72.6)	6.41 (5.77 – 7.06)
Thymol technical (99.0%)	66.7 (60.0 – 73.3)	6.48 (5.83 – 7.12)

FAO tolerance: ± 10%

Information on the active substances

Type	Name/Code Number
ISO common name	No ISO common name for Thymol
CAS No.	89-83-8
EC No.	201-944-8
CIPAC No.	969
Salt, ester anion or cation present	No

Type	Name/Code Number
ISO common name	No ISO common name for Eugenol
CAS No.	97-53-0
EC No.	202-589-1
CIPAC No.	967
Salt, ester anion or cation present	Not applicable

Type	Name/Code Number
ISO common name	No ISO common name for Geraniol
CAS No.	106-24-1
EC No.	203-377-1
CIPAC No.	968
Salt, ester anion or cation present	Not applicable

Information on safeners, synergists and co-formulants: CONFIDENTIAL information – data provided separately-Vol. 4

1.4.5 Type and code of the plant protection product: Capsule Suspension [Code: CS]

1.4.6 Function: Fungicide.

1.4.7 Field of use envisaged: Viticulture and Pome fruits.

1.4.8 Effects on harmful organisms	Thymol has action on contact. Due to the lipophilic nature of the active substance, it disrupts the cell walls, membranes or organelles of micro-organisms.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	g a.s /hL min-max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
Grape vines	*MT, ES, IT, PT*	*3AEY (Mevalone)*	*F*	*Botrytis [BOTRCI]*	*CS*	*eugenol 33 g/L geraniol 66 g/L thymol 66 g/L*	*Tractor-mounted / trailed boom or air blast sprayer. Hand-held knapsack sprayer.*	*BBCH 60-89*	*1-4*	*7*	*Per appl. 13.2 (E) 26.4 (G) 26.4 (T)*	*400-1000*	*1 appl. 52.8 - 132 (E) 105.6 - 264 (G) 105.6 - 264 (T)* *4 appl. 211.2 - 528 (E) 422.4 - 1056 (G) 422.4 - 1056 (T)*	*7*	*The concentration in g a.s./hL is kept constant – the higher application rate is diluted in the higher water volume. Preventative and curative control.*
Grape vines	*ES*	*3AEY (Mevalone)*	*F*	*Powdery mildew [UNCINE]*	*CS*	*eugenol 33 g/L geraniol 66 g/L thymol 66 g/L*	*Tractor-mounted / trailed boom or air blast sprayer. Hand-held knapsack sprayer.*	*BBCH 60-89*	*1-4*	*7*	*Per appl. 13.2 (E) 26.4 (G) 26.4 (T)*	*400-1000*	*1 appl. 52.8 - 132 (E) 105.6 - 264 (G) 105.6 - 264 (T)* *4 appl. 211.2 - 528 (E) 422.4 - 1056 (G) 422.4 - 1056 (T)*	*7*	*The concentration in g a.s./hL is kept constant – the higher application rate is diluted in the higher water volume. Preventative and curative control.*

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	g a.s /hL min-max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
Grape vines	*EL, BG, CY*	*3AEY (Mevalone)*	*F*	*Grey mould (Botryotinia fuckeliana, Botrytis cinerea) (BOTRCI)*	*CS*	*eugenol 33 g/L geraniol 66 g/L thymol 66 g/L*	*Tractor-mounted / trailed boom or air blast sprayer. Hand-held knapsack sprayer.*	*BBCH 60-89*	*1-4*	*7*	*Per appl. 13.2 (E) 26.4 (G) 26.4 (T)*	*400-1000*	*1 appl. 52.8 -132 (E) 105.6 - 264 (G) 105.6 - 264 (T)* *4 appl. 211.2 - 528 (E) 422.4 - 1056 (G) 422.4 - 1056 (T)*	*7*	*The concentration in kg a.s./hL is kept constant – the higher application rate is diluted in the higher water volume. Preventative and curative control. Table grapes includes use on grapes grown for raisin production.*
*Pome Fruit**	*Central Zone IE, GB, NL, BE, LU, DE, CZ, AT, SI, SK, HU, RO, PL*	*Mevalone*	*F*	*Post-harvest storage diseases* (Phytophthora spp. PHYTSP mainly P. cactorum PHYTCC or P. syringae PHYTSY, ALTESP, Botrytis cinerea BOTRCI)*	*CS*	*eugenol 33 g/L geraniol 66 g/L thymol 66 g/L*	*Tractor-mounted / trailed boom or air blast sprayer. Hand-held knapsack sprayer.*	*BBCH 75-87*	*1-4*	*7*	*Per appl. 13.2 (E) 26.4 (G) 26.4 (T)*	*600-1000*	*1 appl. 79.2 -132 (E) 158 -264 (G) 158 -264 (T)* *4 appl. 317 -528 (E) 634 - 1056 (G) 634 - 1056 (T)*	*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 volume. Therefore, the higher application rate is diluted in the higher water volume.*

*(apple *Malus domestica* MABSD, pear *Pyrus communis* PYUCO, quince *Cydonia oblonga* CYDOB, crab-apple *Malus sylvestris* MABSY, loquat *Eryobotria japonica* EIOJA, medlar *Mespilus germanica* MSPGE, Nashi pear *Pyrus pyrifolia var. culta* PYUPC, black chokeberry *Aronia melanocarpa* ABOME, mountain ash *Sorbus* sp. SOUSS)

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I) (c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds (d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR), capsule suspension (CS) (e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide	(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl). (j) Growth stage range from first to 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

(f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated	(k) Indicate the minimum and maximum number of applications possible under practical conditions of use (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha (m) PHI - minimum pre-harvest interval

RMS comment: The uses 1 and 3 are the same, only the MSs are different. They were kept separated for internal strategy from the notifier.It should be emphasized that black chokeberry (*Aronia melanocarpa*) and mountain ash (*Sorbus* sp.) have been applied for the Notifier. However, they are not included in the pome fruits group (see Commission Regulation 2018/62).

1.5.2 Further information on representative uses

Volume of diluent (water) per unit of area or volume	Grapes: 1.6 – 4.0 L product/ha in 400 – 1000 litres water. Pome fruits: 2.4 – 4.0 L product/ha in 600 – 1000 litres water.

For preventative control, a maximum of 4 applications can be made between growth stages BBCH 60-89 on grapes and BBCH 75-87 on pome fruits. Applications must be made at least 7 days apart and at the last application should not be made later than 7 days before harvest of grapes, and 1 days before harvest of pome fruits.
For curative control a maximum of 4 applications can be made between growth stages BBCH 60-89 on grapes and BBCH 75-87 on pome fruits. Applications must be made at least 7 days apart and at the last application should not be made later than 7 days before harvest of grapes, there is no pre-harvest interval on pome fruits. Apply weekly when mycelial growth and active sporulation is observed on post veraison fruit.
For preventative control application can be made before signs of infestation.
For curative control apply weekly when mycelial growth and active sporulation is observed on post veraison fruit.
Each application will provide protection for up to 14 days. Mevalone is best applied with multiple applications, as described above. The duration of control will then be up to harvest.

Number and Timings of Applications and Duration of Protection
Not applicable, grape vines and pome fruits are perennial crops not grown in rotation.

Proposed Instructions for Use
The representative formulation is already registered in the EU (please refer to Doc D2). Example of labels for some Member States are available in Doc C.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Summary of additional intended uses , that in addition to the uses above, have also been considered to support the MRL application that accompanies this submission. Regulation (EC) N° 1107/2009 Article 8.1(g))

Important note: efficacy, environmental risk and risk to humans by exposure other than via their diet have not been assessed for these uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	g a.s /hL min-max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
Grape vines	Northern residue zone	3AEY (Mevalone)	F	Botrytis [BOTRCI] Powdery mildew [UNCINE] Grey mould (*Botryotinia fuckeliana, Botrytis cinerea*) [BOTRCI]	CS	eugenol 33 g/L geraniol 66 g/L thymol 66 g/L	Tractor-mounted/ trailed boom or air blast sprayer. Hand-held knapsack sprayer.	BBCH 60-89	1-4	7	Per appl.: 13.2 (E) 26.4 (G) 26.4 (T)	400-1000	1 appl.: 52.8 - 132 (E) 105.6 - 264 (G) 105.6 - 264 (T) 4 appl.: 211.2 - 528 (E) 422.4 - 1056 (G) 422.4 - 1056 (T)	7	The concentration in g a.s./hL is kept constant – the higher application rate is diluted in the higher water volume. Preventative and curative control.

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	g a.s /hL min-max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
Pome Fruit*	Southern residue zone	Mevalone	F	Post-harvest storage diseases* (*Phytophthora* spp. PHYTSP mainly *P. cactorum* PHYTCC or *P. syringae* PHYTSY, ALTESP, *Botrytis cinerea* BOTRCI)	CS	eugenol 33 g/L geraniol 66 g/L thymol 66 g/L	Tractor-mounted/ trailed boom or air blast sprayer. Hand-held knapsack sprayer.	BBCH 75-87	1-4	7	Per appl.: 13.2 (E) 26.4 (G) 26.4 (T)	600-1000	1 appl.: 79.2 - 132 (E) 158 -264 (G) 158 -264 (T) 4 appl.: 317 -528 (E) 634 - 1056 (G) 634 - 1056 (T)	3	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.

* Apple *Malus domestica* MABSD, pear *Pyrus communis* PYUCO, quince *Cydonia oblonga* CYDOB, crab-apple *Malus sylvestris* MABSY, loquat *Eryobotria japonica* EIOJA, medlar *Mespilus germanica* MSPGE, Nashi pear *Pyrus pyrifolia var. culta* PYUPC. **It should be emphasized that black chokeberry (*Aronia melanocarpa*) and mountain ash (*Sorbus* sp.) have been applied for the Notifier. However, they are not included in the pome fruits group (see Commission Regulation 2018/62).**

1.5.4 Overview on authorisations in EU Member States

List of currently authorized uses and extent of use

Country	Trade name	A.S Content	Reg. Number	Registered uses
Albania	Mevalone	66 g/L	650	Grape (wine)
Albania	Mevalone	66 g/L	650	Grape (table)
Albania	Mevalone	66 g/L	650	Aubergine
Albania	Mevalone	66 g/L	650	Pomegranate
Albania	Mevalone	66 g/L	650	Spring onion
Albania	Mevalone	66 g/L	650	Kiwi
Bulgaria	Mevalone	66 g/L	01354 - PPP-1 / 15.02.2016	Grape (wine)
Bulgaria	Mevalone	66 g/L	01354 - PPP-1 / 15.02.2016	Grape (table)
Cyprus	Mevalone	66 g/L	3333	Grape (wine)
Cyprus	Mevalone	66 g/L	3333	Grape (table)
Cyprus	Mevalone	66 g/L	3333	Aubergine
Cyprus	Mevalone	66 g/L	3333	Pomegranate
Cyprus	Mevalone	66 g/L	3333	Spring onion
Cyprus	Mevalone	66 g/L	3333	Kiwi
Cyprus	Mevalone	66 g/L	3333	Tomato
Cyprus	Mevalone	66 g/L	3333	Olives
France	Mevalone	66 g/L	2161080	Grape (wine)
France	Mevalone	66 g/L	2161080	Grape (table)
France	Mevalone	66 g/L	2161080 (conclusions provided, waiting for final decision)	Pome Fruit
Greece	Mevalone	66 g/L	60467	Grape (wine)
Greece	Mevalone	66 g/L	60467	Grape (table)
Greece	Mevalone	66 g/L	60467	Aubergine
Greece	Mevalone	66 g/L	60467	Pomegranate
Greece	Mevalone	66 g/L	60467	Spring onion
Greece	Mevalone	66 g/L	60467	Kiwi
Greece	Mevalone	66 g/L	60467	Tomato
Greece	Mevalone	66 g/L	60467	Olives
Italy	3logy	66 g/L	16480	Grape (wine)
Italy	3logy	66 g/L	16480	Grape (table)
Italy	3logy	66 g/L	16480	Kiwi
Italy	3logy	66 g/L	16480	Strawberry and small fruits
Italy	3logy	66 g/L	16480	Pomegranate
Malta	Mevalone	66 g/L	2015-05-18 P02 (SZ)	Grape (wine)
Malta	Mevalone	66 g/L	2015-05-18 P02 (SZ)	Grape (table)
Portugal	Araw	66 g/L	1012	Grape (wine)
Portugal	Araw	66 g/L	1012	Grape (table)
Romania	Mevalone	66 g/L	684C	Grape (wine)
Spain	Araw	66 g/L	ES-00108	Grape (wine)
Spain	Araw	66 g/L	ES-00108	Grape (table)
Spain	Araw	66 g/L	ES-00108	Veg - numerous
Non EU Countries				
Australia	Novellus	66 g/L	87197/117745	Grape (wine)
Australia	Novellus	66 g/L	87197/117745	Grape (table)
FYROM	Mevalone	66 g/L	25-972/16	Grape (wine)
FYROM	Mevalone	66 g/L	25-972/16	Grape (table)
FYROM	Mevalone	66 g/L	25-972/16	Aubergine
FYROM	Mevalone	66 g/L	25-972/16	Pomegranate
FYROM	Mevalone	66 g/L	25-972/16	Spring onion
FYROM	Mevalone	66 g/L	25-972/16	Kiwi

Country	Trade name	A.S Content	Reg. Number	Registered uses
Kenya	Hawk	66 g/L	PCPB(CR) 1412	Snow Peas
Kenya	Hawk	66 g/L	PCPB(CR) 1412	Squash
Kenya	Hawk	66 g/L	PCPB(CR) 1412	French beans
Kenya	Hawk	66 g/L	PCPB(CR) 1412	Roses
Serbia	Mevalone	66 g/L	321-01-2092 / 2019-11	Grape (wine)
Serbia	Mevalone	66 g/L	321-01-2092 / 2019-11	Grape (table)

Level 2

THYMOL

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

2.1 IDENTITY

2.1.1 Summary or identity

Thymol technical source submitted in the renewal dossier is the same than the source included in DAR 2013.No new sources of TGAI are presented in this RAR. Details of the assessment of the identity is include in the Volume IV of this RAR. Method of manufacturer was described, however notifier is called to clarify whether the manufacturing process of eugenol is a batch to batch process or continuous process as well as to identify the QC critical points for the manufacture of eugenol TGAI, such as temperature, pressure and pH.

Minimum purity has been declared as 990/kg, purity of thymol as manufactured is not relevant to a pilot plant
Thymol technical grade active substance does not contain any additives.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Fine, white, crystalline solid. Strong phenolic/antiseptic, characteristic odour.	White G.A., 2007 Report J16318 *B.2.3/01*	Visual examination Smelling
Melting/freezing point	Melting point: 48.75°C	White G.A., 2007 Report J16318 *B.2.1/01*	Measured
Boiling point	Boiling point: 232.26°C	White G.A., 2007 Report J16318 *B.2.1/02*	Measured
Relative density	D^{20}_4 = 1.012 at 20 ± 0.5°C	White G.A., 2007 Report J16318 *B.2.14/01*	Measured
Vapour pressure	Vapour pressure: 3.4 Pa at 20°C	White G.A., 2007 Report J16318 *B.2.2/01*	Measured
Surface tension	As a 0.54 g/L solution: 52.3 mN/m at 20°C Thymol is surface active.	White G.A., 2007 Report J16318 *B.2.12/01*	Measured
Water solubility	pH 4: 0.597 g/L pH 7: 0.596 g/L pH 9: 0.630 g/L All measured at 20 ± 1°C	White G.A., 2007 Report J16318 *B.2.5/01*	Measured
Partition coefficient n-	pH | 4 | 7 | 9	White G.A., 2007 Report J16318	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
octanol/water	$Log_{10} P_{ow}$ 3.43 3.44 3.41 All measured at 20 to 25 ± 1°C.	*B.2.7/02*	
Henry's law constant	0.86 $Pa.m^3 mol^{-1}$ at 20°C	White G.A., 2007 Report J16318 *B.2.2/02*	Measured
Flash point	Flash point = 105.5°C	White G.A., 2007 Report J16318 *B.2.10/01*	Measured
Flammability	Not flammable. (FP>60°C)	White G.A., 2007 Report J16318 *B.2.9/01*	Measured
Explosive properties	Not explosive	White G.A., 2007 Report J16318 *B.2.11/01*	Measured
Self-ignition temperature	Autoflammability: <400°C	White G.A., 2007 Report J16318 *B.2.9/02*	Measured
Oxidising properties	Not oxidising.	White G.A., 2007 Report J16318 *B.2.13/01*	Measured
Granulometry	Not Applicable	NA	NA
Solubility in organic solvents and identity of relevant degradation products	n-Heptane: 200-250 g/L p-Xylene: >250 g/L 1,2-Dichloroethane: >250 g/L Methanol: >250 g/L Acetone: >250 g/L Ethyl acetate: >250 g/L All measured at 20 ± 1°C	White G.A., 2007 Report J16318 *B.2.6/01*	Measured
Dissociation constant	pKa= 10.77	White G.A., 2007 Report J16318 *B.2.8/01*	Measured
Viscosity	Not applicable	NA	NA
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV/Vis spectrum is typical of Thymol. UV/Vis: no absorption λ > 290 nm.	White G.A., 2007 Report J16318 *B.2.4/01*	Measured
	IR spectrum is typical of Thymol.	White G.A., 2007 Report J16318 *B.2.4/02*	Measured
	NMR spectrum is typical of Thymol.	White G.A., 2007 Report J16318	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
		B.2.4/03	
	MS spectrum is typical of Thymol.	White G.A., 2007 Report J16318 *B.2.4/04*	Measured

Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
ASTM ES37-02 (DSC)	According to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C.	DSC is not a standard technique for assessing explosive properties according to CLP criteria.	White G.A., 2007 Report number J16318

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties
According to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C for thymol. However, it has to be noted that DSC is not a standar technique for assessing explosive properties according to CLP Regulation.

2.2.1.1.1.2 Comparison with the CLP criteria
No explosivity classification is required. According to point 2.1.4.3 of Annex I of CLP Regulation the acceptance procedure for the hazard class ‘explosives’ does not apply for thymol taking into account that thymol molecule does not contain chemical groups associated with explosivity considering the examples given in Table A6.1 of Appendix 6 of the UN RTDF Manual of Tests and Criteria. It has to be noted that thymol contains benzene (as phenol) rather than 1,3,5 cyclohexatriene and hence the molecule does not contain unsaturations.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties
Thymol is not explosive. No explosivity classification required according to CLP criteria.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Not applicable			

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)
Not applicable. The active substance is not a gas.

2.2.1.1.2.2 Comparison with the CLP criteria
Not applicable.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases
Not applicable

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not applicable			

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases
Not applicable. The active substance is not a gas.

2.2.1.1.3.2 Comparison with the CLP criteria
Not applicable

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases
Not applicable

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not applicable			

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure
Not applicable. The active substance is not a gas.

2.2.1.1.4.2 Comparison with the CLP criteria
Not applicable.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure
Not applicable.

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Not applicable			

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids
Not applicable. The active substance is not a liquid..

2.2.1.1.5.2 Comparison with the CLP criteria
Not applicable.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids
Not applicable.

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
ASTM E537 (DSC method)	Melting point: 48.75°C	-	White G.A., 2007, Report number J16318

Method	Results	Remarks	Reference
EEC A.10 - Flammability	Ignition followed by melting of the train; the molten material then ignites	Due to the unclear result, Flash point was determined	White G.A., 2007, Report number J16318
EEC A9 (closed cup)	Not flammable. Flash point: 105.5.5°C	FP>60°C	White G.A., 2007 Report number J16318

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids
Thymol is not a flammable solid.

2.2.1.1.6.2 Comparison with the CLP criteria
No flammability classification required according to CLP criteria. The burning rate of thymol was not determined as its melting point is 48.75°C. However, the flash point of thmol was determined as being 105.5°C which is more than the threshold limit of 60°C. Moreover, the substance is not a gas oil, diesel, or light heating oil with a flash point up to 75°C or a halogenated substance. Therefore thymol should not be classified as flammable .

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids
Thymol is not highly flammable. No flammability classification required according to CLP criteria.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
DSC, ASTM E537-02	According to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C.		White G.A., 2007 Report number J16318
EEC A15	Autoflammability: >400°C	Not autoflammable.	White G.A., 2007 Report number J16318

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances
According to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C.
According to the EEC A15 thymol is not autoinflammable.

2.2.1.1.7.2 Comparison with the CLP criteria
No classification for self-reactivity is required. According to CLP point 2.8.4.2 of Annex I of CLP Criteria, the hazard class does not apply if there are no chemical groups in the molecule associated with explosive or self reactive properties. Thymol molecule does not contain chemical groups associated with explosive or self-reactive considering the examples given in Table A6.1 and Table A6.3 of Appendix 6 of the UN RTDF Manual of Tests and Criteria. It has to be noted that thymol contains benzene (as phenol) rather than 1,3,5 cyclohexatriene and hence the molecule does not contain unsaturations.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances
Thymol is not a self-reactive substance.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not applicable	--	--	--

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids
Not applicable. Thymol is not a liquid.

2.2.1.1.8.2 Comparison with the CLP criteria
Not applicable

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids
Not applicable.

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Not applicable.			

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids
Thymol is unlikely to be pyrophoric and the test for pyrophoricity according to UN Test N.3 described in Part III, Section 33 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria should not be performed.

2.2.1.1.9.2 Comparison with the CLP criteria
Experience in manufacture and handling shows that the substance thymol does not ignite spontaneously on coming into contact with air at normal temperature.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids
Thymol is not a pyrophoric solid.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
DSC, ASTM E537-02	According to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C.	-	White G.A., 2007 Report number J16318
DSC, ASTM E537-02	Melting point: 48.75°C	-	White G.A., 2007 Report number J16318
EEC A15	Autoflammability: >400°C	Not autoflammable.	White G.A., 2007 Report number J16318

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances
Thymol is a liquid at ~49°C and unlikely to be self-heating and the test for self-heating according to UN Test N.4 described in Part III, Section 33 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria should not be performed.

2.2.1.1.10.2 Comparison with the CLP criteria
Thymol is liquid at ~49°C and has an autoflammability temperature of higher than 400°C and according to Differential Scanning Calorimetry (DSC) graphs, no exothermic reaction was observed in the temperature range from 30°C to 400°C. The surface of liquids is not large enough for reaction with air and the test method is not applicable to liquids. Therefore thymol is not classified as self-heating.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Thymol is not a self-heating substance. No auto-flammability classification required according to CLP criteria.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Thymol is a natural substance that is naturally synthesised in plants including high-water content fruits such as grapes. Thymol does not emit flammable gases in contact with water. Moreover, thymol is soluble in waterand the experience in manufacture and handling shows that the substance does not emit flammable gases on coming into contact with water at normal temperature.

2.2.1.1.11.2 Comparison with the CLP criteria

According to CLP Regulation 2.12.4.1, no classification is required if:

a) There are no metals or metalloids in the chemical structure, OR
b) Experience in production or handling shows that the substance does not react with water, e.g. the substance is manufactured with water or washed with water, OR
c) The substance is known to be soluble in water and form stable mixture.

Thymol is a natural substance that is naturally synthesised in plants including high-water content fruits such as grapes. Thymol does not emit flammable gases in contact with water. Moreover, thymol is soluble in waterand the experience in manufacture and handling shows that the substance does not emit flammable gases on coming into contact with water at normal temperature.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Thymol does not emit flammable gases when in contact with water.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Not applicable.			

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not applicable. Thymol is not a liquid.

2.2.1.1.12.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not applicable.

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Not oxidizing	The molecular structure of thymol does not contain any oxidising groups.	White G.A., 2007 Report number J16318

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

Thymol is unlikely to be oxidising according to the method EEC A17.
The required test according to CLP Regulation is not available: UN Test O.1 or O.3 as described in Part III, Section 34 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests.

2.2.1.1.13.2 Comparison with the CLP criteria
Thymol is unlikely to be oxidising according to the method EEC A17.
Moreover, according to criteria included for organic substances in point 2.14.4.1 of Annex I of CLP Regulation, thymol is not an oxydising solid since it does not contain chlorine or fluorine and it contains oxygen but chemically bounded to carbon and hydrogen.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids
Thymol is not an oxidising solid. Not oxidising classification required according to CLP criteria.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
Not applicable			

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides
Not applicable. Thymol is not an organic peroxide.

2.2.1.1.14.2 Comparison with the CLP criteria
Not applicable as thymol does not contain a peroxide group.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides
Not applicable.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
Not applicable.			

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals
Thymol is not expected to be corrosive. Thymol contains no functional groups that are either acidic or alkaline. Thymol does not dissociate at environmental pHs and thymol has very limited water solubility (< 0.6 g/L at pH 4, 7 and 9).
Thymol is unlikely to be corrosive to metal and the test for metal corrosive properties according to UN Test C.1 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria have not been performed.

2.2.1.1.15.2 Comparison with the CLP criteria
Thymol is not expected to be corrosive. Thymol contains no functional groups that are either acidic or alkaline. Thymol does not dissociate at environmental pHs and thymol has very limited water solubility (< 0.6 g/L at pH 4, 7 and 9).

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals
Thymol is not corrosive to metals.

2.2.2 Summary of physical and chemical properties of the plant protection product

Mevalone (3AEY) is a capsule suspension containing geraniol (66 g/L), thymol (66 g/L) and eugenol (33 g/L). Physical characteristics of Mevalone correspond to that of a capsule suspension. Mevalone is a dark cream/beige viscous liquid which is not explosive, not oxidizing and non-flammable up to temperatures of

400°C. It has a pH of around 5.8 when in a 1% suspension. Stability data indicate a shelf-life of at least 2 years at ambient temperature.
The intended concentration of use is 0.4 L Mevalone per 100 L of spray, equivalent to 0.4% v/v.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Terpene compounds such as eugenol, geraniol and thymol generally possess antifungal activity, having effects on spore germination, hyphal penetration, mycelial growth and hyphal growth.

All terpene compounds are reported to have direct effects on cell walls, membranes, which is associated with the capability of the compounds to dissolve lipids and results in leakage of cellular substances leading to cell death. Studies have confirmed that cyclic terpene hydrocarbons accumulate in the cell membrane causing a loss of membrane integrity, with associated changes in composition of fatty acids and phospholipids. This is thought to occur as a result of lesion formation in the cytoplasmic membrane with reductions in ergosterol content due to the disruption of biosynthesis.

Due to these effects on membranes, there is also thought to be an impact on processes involving ATP and active transport of molecules across membranes, leading to depletion of the ATP pool and leakage of cellular substances, with impairment of energy metabolism. Mitochondrial structure disorganization may occur and the effects on membranes have been shown to cause partial dissipation of the pH gradient and electrical potential.

Terpenes have also been observed to cause changes in the hyphal wall. Some effects on enzyme activity have also been reported, including interference with respiratory enzymes and enzymes responsible for cell wall synthesis. There is also evidence to suggest that the synthesis of genetic material is affected.

For the uses grapes/BOTRCI and UNCINE, the representative formulation, MEVALONE, is currently commercially available and supported by efficacy data evaluated under Uniform Principles for national registrations.

Regarding pome fruits/postharvest storage diseases (PHYTSP, ALTESP and BOTRCI), currently, this use is not registered, however, it is considered that the GAP is realistic from an efficacy point of view considering the studies provided by the applicant (studies submitted for new registration in Central Zone in July 2021, for further details, see MCP6 doc). The conclusions on the demonstration of the effectiveness are left to subsequent product dossiers assessed under Uniform Principles, nevertheless, in a first approach, "*in nearly all trials, the efficacy level of all treatment was low to moderate. This can be explain by the fact that no post harvest treatment was performed. However, in most trials, some fungicide programs including MEVALONE performed better than the reference. MEVALONE applied alone performed quite poorly and it is therefore important to include it in a fungicide program (from orchard to post harvest treatments)*."

2.3.2 Summary of information on the development of resistance

Thymol is a contact action fungicide. It prevents the development of fungal mycelium from spores or destroys existing mycelium by a direct action on the cell membranes. Due to the mode of action, no problems with resistance or cross-resistance are expected. Thymol, is a plant extract included in the terpene alcohols chemical group, classified by FRAC into plant oils, FRAC codes F7: cell membrane disruption /46, with resistance not known.

2.3.3 Summary of adverse effects on treated crops

Due to thymol's mode of action, no adverse effects on field crops are expected. Phytotoxic symptoms were regularly checked in all trials provided on grapes and on apples, and MEVALONE demonstrated a high crop safety.
MEVALONE formulation has been applied in various EU member states for many years without reports of adverse effects on treated crops. Available efficacy used to obtain registration of the representative formulation in various countries shows the absence of phytotoxicity when the product is used according to the GAP. Consequently, no negative impact is expected on treated crops when used according to recommendations.

2.3.4 Summary of observations on other undesirable or unintended side-effects

There is no evidence of any undesirable or unintended side-effects.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

The applicant has proposed:

a) Handling:
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Normal measures for preventive fire protection.

b) Storage:
Keep container tightly closed in a dry and well-ventilated place Containers which are opened must be carefully resealed and kept upright to prevent leakage.

c) Transportation:
Not classified as hazardous for transport.

d) Fire:
Not flammable or combustible.
Extinguishing media: Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Fire fighting: Wear self-contained breathing apparatus for firefighting if necessary.
Fire and explosion hazards: Hazardous decomposition products formed under fire conditions. - Carbon oxides.

2.4.2 Summary of procedures for destruction or decontamination

Product: Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.
Contaminated packaging: Dispose of as unused product.

2.4.3 Summary of emergency measures in case of an accident

a) Personal Precautions:
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.
Evacuate personnel to safe areas.

b) Environmental Precautions:
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

c) Methods of Cleaning Up:
Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

- **Analysis of the active substance as manufactured**

Thymol technical material is dissolved in acetone and thymol is quantified by GC-FID using 1-nonanol as internal standard.

- **Formulation analysis**

Total thymol is determined and quantified in 3AEY after dilution in methanol using gas chromatography with a flame-ionisation detector and external standards.

Free thymol is determined and quantified in 3AEY after extraction in hexane using gas chromatography with a flame-ionisation detector and 1-nonanol as internal standard.

Methods for risk assessment

- ***Plants and plant products***

Grapes:
The Limit of Quantification was set at 0.01 mg/kg.

For total thymol, samples are homogenised and mixed with acetone. Homogenised samples are centrifuged and filtrated prior to quantification by GC-MS (monitored ions: 115 m/z, 135 m/z and 150 m/z). Ethyl acetate is also a suitable extraction solvent.
For free thymol, samples are de-stalked but not homogenised prior to extraction using acetone followed by filtration and quantification by GC-MS (monitored ions as above). In the most recent studies, extraction is performed using acetonitrile and solid-phase extraction salts for clean-up followed by evaporation under nitrogen and reconstitution of the extract in acetonitrile prior quantification by GC-MS (monitored ion: 91 m/z) plus LC-MS/MS (monitored mass transition: 149 → 134).

Apples:
The Limit of Quantification was set at 0.01 mg/kg.

Homogenized apple samples are extracted in acetonitrile, cleaned up using solid phase extraction and concentrated under nitrogen prior to quantification by GC-MS (monitored ions: 91 m/z, 150 m/z and 1151 m/z).

- ***Food of animal origin***

There are no methods available for the quantification of thymol in food of animal origin.

- ***Soil***

The DT_{90} of thymol in soil has been found to be < 3 days therefore, a method for the quantification of residues in soil is not required for thymol.

- ***Water***

Medium used in fish toxicity studies:
Samples of fish medium are diluted in acetonitrile and analysed directly by GC-MS. The lowest Limit of Quantification validated was 0.332 mg/L for thymol in fish test medium.
Medium used in daphnia toxicity studies:
Samples of daphnia medium are diluted in acetonitrile and analysed directly by GC-MS. The lowest Limit of Quantification validated was 1.0 mg/L for thymol in daphnia test medium.
Medium used in algal toxicity studies:
Samples of algal medium are diluted in acetonitrile and analysed directly by GC-MS. The lowest Limit of Quantification validated was 0.065 mg/L for thymol in alga test medium.
Water stock solution in ecotoxicological studies:
Thymol is extracted from test medium (sugar feeding solution and water stock solution) with acetonitrile prior to analysis by HPLC-DAD. The Limit of Quantification was 199.9 mg/L in water stock solution.
Water in physicochemical studies:
The contents of thymol in aqueous solutions were determined by HPLC with UV detection.

- ***Air***

Residues of Thymol are extracted from pre-packed XAD-2 cartridges by sonication with ethyl acetate and final determination is performed by GC-MS, monitoring three ions of m/z >100. The limit of quantification was 1.2 $\mu g/m^3$ for thymol content in air.

2.5.2. Methods for post control and monitoring purposes

2.5.2.1. Plants and plant products

Grapes:
The Limit of Quantification was set at 0.01 mg/kg.

For total thymol, samples are homogenised and mixed with acetone. Homogenised samples are centrifuged and filtrated prior to quantification by GC-MS (monitored ions: 115 m/z, 135 m/z and 150 m/z). Ethyl acetate is also a suitable extraction solvent.
For free thymol, samples are de-stalked but not homogenised prior to extraction using acetone followed by filtration and quantification by GC-MS (monitored ions as above). In the most recent studies, extraction is performed using acetonitrile and solid-phase extraction salts for clean-up followed by evaporation under nitrogen and reconstitution of the extract in acetonitrile prior quantification by GC-MS (monitored ion: 91 m/z) plus LC-MS/MS (monitored mass transition: 149 → 134).

Apples:
The Limit of Quantification was set at 0.01 mg/kg.

Homogenized apple samples are extracted in acetonitrile, cleaned up using solid phase extraction and concentrated under nitrogen prior to quantification by GC-MS (monitored ions: 91 m/z, 150 m/z and 115 m/z).

2.5.2.2. Food of animal origin

There are no methods available for the quantification of thymol in food of animal origin.

2.5.2.3. Soil

The DT_{90} of thymol in soil has been found to be < 3 days therefore, a method for the quantification of residues in soil is not required for thymol.

2.5.2.4. Water

Thymol is extracted from surface water via steam distillation and quantified by GC-MS, monitoring three ions of m/z >100. The Limit of Quantification was 0.1 µg/L for thymol in surface water.

2.5.2.5. Air

Residues of Thymol are extracted from pre-packed XAD-2 cartridges by sonication with ethyl acetate and final determination is performed by GC-MS, monitoring three ions of m/z >100. The limit of quantification was 1.2 $\mu g/m^3$ for thymol content in air.

2.5.2.6. Body fluids and tissues

Samples of plasma and urine are extracted in acetonitrile, cleaned up using solid phase extraction followed by primary secondary amine prior to quantification by GC-MS (monitored ions: 115 *m/z* and 150 *m/z*). Confirmation is performed by quantification with LC-MS/MS (monitored transition 149 → 134 m/z). The Limit of Quantification is set at. 0.01 mg/L

Homogenized samples of meat and liver are extracted in acetonitrile, cleaned up using solid phase extraction followed by primary secondary amine (for liver) prior concentration and quantification by GC-MS (monitored ions: 91 *m/z*, 150 *m/z* and 115 *m/z* for meat and 150 *m/z* and 115 *m/z* for liver). Confirmation of liver method is performed by quantification with LC-MS/MS (monitored transition 149 → 134 m/z).
The Limit of Quantification was set at 0.01 mg/kg for both meat and liver.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 17: Summary table of toxicokinetic studies

<table>
<tr><th>Method</th><th>Results / Remarks</th><th>Reference</th></tr>
<tr><td>Absorption, excretion and metabolism in vivo (single dose) rat

No guidance stated

Deviations from OECD TG 417 (2010) include:
-Test material not characterised
-Only one dose tested and no justification on the selection of that dose
-Poorly described method and results.
-No individual data provided

Wistar rats (6♂)

Single oral dose 150 mg/kg (purity >99%)

Urine collected (24h)

GLP: No

Supporting information</td><td><u>Absorption & Excretion</u>:
Relatively rapid and almost complete based on urinary excretion.
Almost completely excreted in urine at 24 h post-dosing.

<u>Metabolic profile</u>:
Thymol (parent) was the major compound detected in urine after 24h post dosing. A total of six metabolites were also identified:

<table><tr><th>Compound</th><th>Relative amount</th></tr><tr><td>Thymol</td><td>Major</td></tr><tr><td>2,5-Dihydroxy-p-cymene (T1)</td><td>+</td></tr><tr><td>2-(2-Hydroxy-4-methylphenyl)propan-1-ol (T2)</td><td>+++</td></tr><tr><td>5-Hydroxymethyl-2-(1-methylethyl)phenol (T3)</td><td>++</td></tr><tr><td>2-(4-Hydroxymethyl-2-hydroxyphenyl)propan-1-ol (T4)</td><td>Trace</td></tr><tr><td>2-(4-Hydroxymethyl-2-hydroxyphenyl)propionic acid (T5)</td><td>++</td></tr><tr><td>3-Hydroxy-4-(1-methylethyl)benzoic acid (T6)</td><td>+</td></tr></table></td><td>Austgulen, L.V. et al. (1987) (CA) B.6.1.1.1</td></tr>
<tr><td>Metabolism and excretion in vivo (single dose) rabbit

Pre-guidance

Deviations from OECD TG 417 (2010) include:
-Test material not characterised
-Species not fully characterised (sex/strain)
-No justification on dose selection
-Poorly described method and results.

Rabbits (3) (strain, sex)

Single oral dose of 500 mg/kg bw

Thymol, purity/batch no not stated

Urine collected for 3 days

Glucuronic and sulphate conjugates analysed by GC and thin layer chromatography</td><td>Urinary excretion of glucuronic acid and ethereal sulphuric acid markedly increased at 24h after dose compared to controls. At 48h only glucuronic acid was slightly increased and both conjugates returned to control levels at 72h.

The extent of conjugation seems to occur via glucuronidation and to a lesser extent sulphation.</td><td>Takada, M. et al. (1979) (CA) B.6.1.1.2</td></tr>
</table>

Method	Results / Remarks	Reference
GLP: No **Supporting information**		
Metabolism and excretion *in vivo* (single dose) human No guideline 2 Male volunteers Single oral dose of 600 mg Thymol, purity/batch no not stated Urine collected for 24h Metabolites extracted and analysed by GC and thin layer chromatography GLP: No **Supporting information**	The main metabolites identified in urine are unconjugated thymol, thymol conjugates (sulphate and glucuronide) and thymolhydroquinone (sulphate conjugate). No quantification given in the study.	Takada, M. *et al.* (1979) (CA) B.6.1.1.3
Summary pharmacokinetics Thymol GLP: No **Supporting information**	Thymol is readily absorbed in the gastrointestinal tract. About 50% is excreted via kidney as glucuronide or sulphate conjugates in 24h. The metabolite thymohydroquinone is also excreted *via* the kidney.	EMEA (1996) (CA) B.6.1.1.4
Summary metabolism and excretion Thymol GLP: No **Supporting information**	Thymol is rapidly absorbed in the gastrointestinal tract. Thymol is reported to be excreted after conjugation with sulphate and glucuronic acid. Thymoquinol has been detected as a minor oxidation product of thymol (ring hydroxylation).	Mattia, A. and Sipes, G.I. (2005) (CA) B.6.1.1.5
Pharmacokinetics Mouse Guideline not stated No deviations from OECD TG 417 (2010) Hsd:ICR (CD.1®) mice (19♂, 19♀) Thymol; Purity: 99.7 % Batch No. 103366 Vehicle: Olive oil Single oral (gavage) dose: Preliminary study: 1250 and 1000 mg/kg bw (2♂, 2♀ each) Main study: 1000 mg/kg bw (15♂, 15♀)	Preliminary study I (1250 mg/kg bw) Clinical observations: ataxia, bradypnoea, prone posture, piloerection, decreased activity and ptosis in all animals up to 4h after dosing. Animals appeared normal on Day 2 post-dosing. Preliminary study II (1000 mg/kg bw) Clinical observations: ataxia, decreased activity, bradypnoea, ptosis and piloerection ranging from 5min to 4h post dose. Prone posture, pallor extremities, loss of righting reflex and ptosis were observed in one male from 2 to 4h after dose. Prone and pallor extremities were also observed in one female from 15min to 4h after doses. Main study (1000 mg/kg bw) Clinical observations: increased activity, piloerection, ataxia and decreased activity ranging from 1 to 8h post-dose in both male and female animals. Additional symptoms included bradypnoea (1 male at 8h), ataxia (4 males up to 2h post dose and 6 females up to 4h post dose) and both hunched posture and lethargy (1 male at 2h post dose, 1 female at 4h post dose). Mean concentration values of thymol at various time points are displayed in the following table: **Time point** | **Mean concentration** | **Standard**	[redacted] (2009) (CA) B.6.1.1.6

<table>
<tr><th>Method</th><th>Results / Remarks</th><th>Reference</th></tr>
<tr><td>Blood samples were taken at 1, 2, 4, 8, 12 and 24 h post dosing.

GLP: Yes

Study acceptable</td><td>
<table>
<tr><th>(h)</th><th>(ng/mL)</th><th>deviation</th></tr>
<tr><td>1</td><td>4891</td><td>2779</td></tr>
<tr><td>2</td><td>5899</td><td>8163</td></tr>
<tr><td>4</td><td>6305</td><td>12704</td></tr>
<tr><td>8</td><td>2798</td><td>5255</td></tr>
<tr><td>12</td><td>227</td><td>120</td></tr>
<tr><td>24</td><td>379</td><td>525</td></tr>
</table>

The calculated pharmacokinetic parameters are the following:

$AUC_{0\text{-}24h}$ = 4891 hours*ng/mL

Cmax = 6305 ng/mL

Tmax = 4 h

$AUC_{0\text{-}24h}$ and half-life not calculated (concentration at 24 h was higher in some animals than at 8 h)</td><td></td></tr>
<tr><td>Pharmacokinetics Human

No guideline

Male human volunteers (12)

Thymol: Purity not stated

Single oral dose of Bronchipret ® TP, equivalent to 1.08 mg thymol

Venous blood samples taken before dosing and at various points up to 72 h after administration.

Urine samples collected at time of dose and at various points up to 72 h after administration.

GLP: No

Study acceptable</td><td><u>Plasma</u>:
No free thymol was detected, only conjugated thymol as thymol sulphate.
Cmax: 93.1 ± 24.5 ng/mL; tmax: 1.97 ± 0.77 hours. Elimination half-life (t1/2) was calculated as 10.2 ± 1.4 hours.

<u>Urine</u>:
No free thymol detected, only phase II conjugates of thymol: sulphate and glucuronide. The ratio of glucuronide vs sulphate was constant over urine sampling times.
Elimination of thymol conjugates was detected for the first 24 h. The combined amount of both thymol conjugates in urine (glucuronide and sulphate) was 16.2 % ± 4.5 % of intake. The renal clearance was calculated to be 0.271 ± 0.7 L/h.</td><td>Kohlert, C. et al. (2002)
(CA)
B.6.1.1.7</td></tr>
<tr><td>Pharmacokinetics Mouse

Guideline not stated

Deviations from OECD TG 417 (2010) include no characterisation of the test item, no justification of the dose, no individual data provided (overall results only)

Male CD-1 mice (16 animals)

Thymol: Purity not stated, batch no. not stated</td><td>C_{max}: 76.6 ± 6.0
t_{max}: 2 min
$t_{1/2}$: 3.43 min
Vd: 136 mL
$AUC_{0\text{-}60min}$: 626.7 (ng/mL*min)
$AUC_{0\text{-}infinity}$: 626.7 (ng/mL*min)
Clt: 27.6 mL/min</td><td>Xie, K. et al.
(2019)
(CA)
B.6.1.2.1</td></tr>
</table>

Method	Results / Remarks	Reference
Single dose: 0.1 mg/mL in saline solution (equivalent to 16 µg or 0.48 mg/kg bw) Volume: 0.16 mL Route: intraperitoneal injection Blood sampling time at baseline, 2, 5, 10, 20, 30 and 60 min post dosing. GLP: No **Supporting information**		
Pharmacokinetics Mouse Guideline not stated Deviations from OECD TG 417 (2010) include no characterisation of the test item, no justification of the dose Male CD-1 mice (16 animals) Thymol: Purity not stated, batch no. not stated Single dose: 0.5% in HFA (equivalent to 15.5 µg or 0.47 mg/kg bw) Route: inhalation Blood sampling time at baseline, 2, 5, 10, 20, 30 and 60 min post dosing. GLP: No **Supporting information**	C_{max}: 76.6 ± 6.0 t_{max}: 2 min $t_{1/2}$: 3.43 min Vd: 136 mL $AUC_{0\text{-}60min}$: 626.7 (ng/mL*min) $AUC_{0\text{-}infinity}$: 626.7 (ng/mL*min) Clt: 27.6 mL/min	Xie, K. *et al.* (2019) (CA) B.6.1.2.2

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

A total of seven studies have been submitted for the renewal of approval of the active substance thymol. Most of these studies were previously evaluated in the original DAR (2011) except for the human pharmacokinetic study and a mouse inhalation/intravenous pharmacokinetic study, which have been submitted for the renewal process. Only one study [redacted]., 2009; B.6.1.1.6) is GLP compliant.

A comparative *in vitro* metabolism study has not been submitted to meet the data requirements of the Commission Regulation (EU) 283/2013. However, based on the available human and animal metabolism data, there is no indication of significant differences between species and therefore, the available toxicological data are relevant for the human safety evaluation.

In a summary report (European Agency for the Evaluation of Medicinal Products 1996; B.6.1.1.4; Mattia, A. and Sipes, G.I., 2005; B.6.1.1.5) thymol is readily absorbed in the gastrointestinal tract. A small amount is oxidised to thymohydroquinone. About 50% of the absorbed substance is excreted *via* the kidney, either unconjugated or as a

glucuronide or sulphate conjugate in 24 h. The metabolite thymohydroquinone is also excreted *via* the kidney.

Thymol is rapidly absorbed and metabolised after oral administration (150 mg/kg bw) to rats (Austgulen, L.T. *et al*, 1987; B.6.1.1.1). Urinary excretion is almost complete within 24 h. Thymol is the major compound identified in urine along with six metabolites. Based on the abundance in urine of these metabolites the primary metabolic route involves an oxidative pathway whereby hydroxylation of the isopropyl group leads to the alcohol (T2) and further oxidised propionic acid derivative (T5) (Figure 2.6.1.1). Minor metabolic routes involve aromatic ring oxidation (T1) and methyl group oxidation to lead to the phenol (T3) and benzoic acid (T6) metabolites.

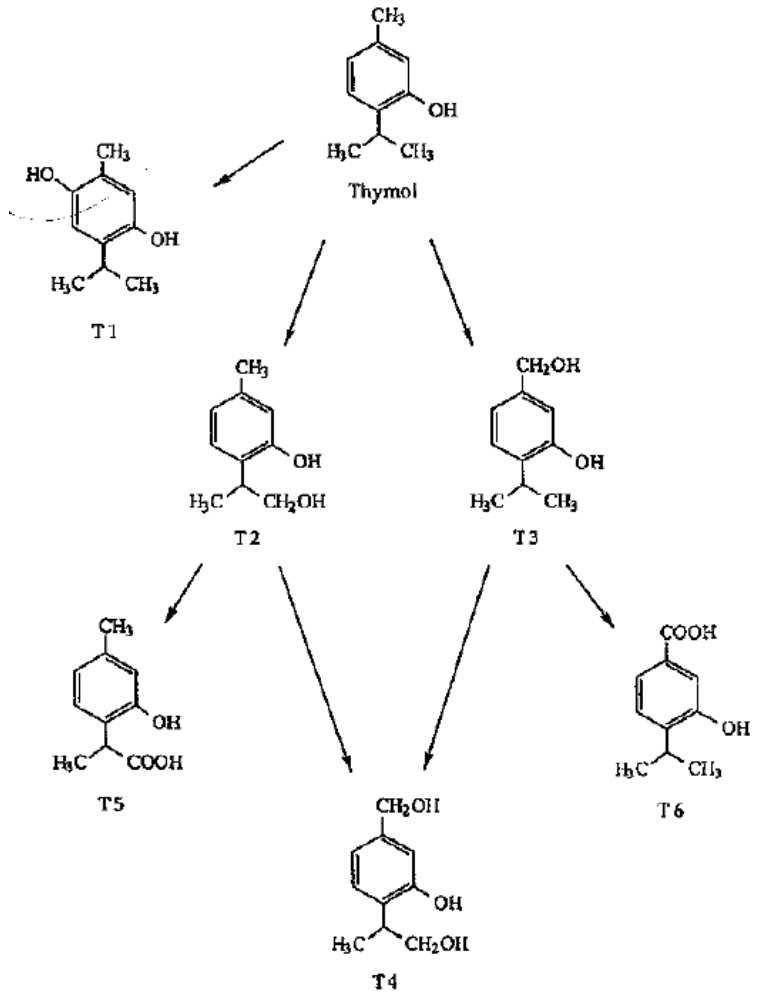


Figure 2.6.1.1: Proposed metabolic pathway of thymol based on urinary metabolites at 24h found in rat following oral administration of thymol (data from study B.6.1.1.1)

In the rabbit, a single oral administration of thymol (500 mg/kg bw) increased the urinary excretion of glucuronic and ethereal sulphuric acid conjugates of thymol (Takada, M. *et al*, 1979; B.6.1.1.2) at 24 h post-dose and to a lesser extent at 48h only the glucuronic acid conjugate. Thymol concentration in various systems was determined in rabbits following a 21-day treatment of thymol in the diet at 148.9 ± 16.7 µg/g dry matter (Bacova, K. *et al*, 2020; B.6.8.2.5). Plasma concentration at 21 days was 0.05 ± 0.02 µg/L, thymol content in the intestinal wall was 0.04 ± 0.03 µg/g of dry matter and a total of 0.89 ± 0.45 µg/g was identified in faeces. After a 7-day withdrawal, no thymol was detected in plasma or intestinal wall but the content in faeces was reduced to 0.08 ± 0.04 µg/g.

In human, urinary metabolites identified following a single oral administration of thymol (600 mg) were thymol (free and conjugated as glucuronide and sulphate) and thymolhydroquinone (Takada, M. *et al*, 1979; B.6.1.1.3). The pharmacokinetic parameters were investigated following a single oral dose of thymol (1.08 mg) in twelve male volunteers (Kohlert, C *et al*, 2002; B.6.1.1.7). The outcome of the study showed that thymol was rapidly absorbed with a maximum concentration in plasma of 93.1 ± 24.5 ng/mL at 1.97 ± 0.77 hours. Free thymol could not be identified in plasma or urine but the sulphate conjugate or the sulphate/glucuronide conjugates, respectively. Urinary excretion of thymol conjugates was detectable only after 24h post dose and thymol phase II conjugates (glucuronide and sulphate) accounted for 16.2 % ± 4.5 %.

The pharmacokinetic parameters in the mouse were investigated following a single oral administration of thymol (1000 mg/kg bw) ([redacted]. 2009; B.6.1.1.6). The mean plasma concentration profile of thymol had a peak at 4 hours after dosing with a mean C_{max} of 6305 ng/mL. The profile in blood included an initial rapid decline followed by a steady decrease afterwards. Clinical signs included increased activity, piloerection, ataxia and decreased activity ranging from 1 to 8h post-dose in both male and female animals. Additional symptoms included bradypnoea (1 male at 8h), ataxia (4 males up to 2h post dose and 6 females up to 4h post dose) and both hunched posture and lethargy (1 male at 2h post dose, 1 female at 4h post dose). In a pharmacokinetic study in mice, intravenous injection of 0.48 mg/kg bw of thymol resulted in a C_{max} of 76.6 ng/mL at 2 min and the data fitted a one compartmental PK model (Xie, K. *et al*, 2019; B.6.1.2.1). Pharmacokinetic parameters were also calculated following inhalation of 0.47 mg/mL thymol, which resulted in C_{max} of 39.2 ng/mL at 2 min (Xie, K. *et al*, 2019; B.6.1.2.2). The data fitted a two-compartmental model for inhalation.

In the opinion of the RMS and the co-RMS the available data on oral absorption are of low quality. In the EFSA conclusion (2012), it was mentioned that excretion was "*Moderately rapid excretion via urine (at least 50% within 24hours) –based on limited studies with thymol and information from structurally-related chemicals"*. The RMS has no information about these data on structurally similar compounds. However, it has been noticed that this value for the percentage of urine excretion was not applied to oral absorption in the DAR. The co-RMS proposes a 50% absorption as a worst-case based on the EFSA conclusion data (2012). In the absence of further details, the RMS disagrees with the setting of a value of 50% as worst-case, considering that it is not well justified, although it recognizes that setting the value at 100%, just based on the study of Austgulen, L.T. *et al* (1987), is also doubtful. Additional justification on thymol oral absorption should be provided.

Residue definition for body fluids and tissues:

Considering the available information, residues in body fluids and tissues could be defined as the active substance and its sulphate and glucuronide conjugates, measured in urine samples.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study in rats and guinea pigs Prior to OECD TG 401 Deviations: Test substance not characterised. Poorly described method and results, no necropsy. GLP: No (prior to GLP enforcement) **Supporting information**	Species: Osborne-Mendel rats 10 rats evenly divided by sex Guinea pig (male, female)	Thymol (20% in propylene glycol) Purity unknown (commercially available material) Oral by intubation Single dose Up to 14-day observation period	Rats: **Thymol LD_{50}: 980 mg/kg bw** Time of death: 4 h – 5 days. Depression, ataxia, coma on high doses Guinea pigs: **Thymol LD_{50}: 880 mg/kg bw** Time of death: 4 h – 5 days. Irritated gastro-intestinal tract, tremors, coma, respiratory failure	Jenner, P.M. *et al.* (1964) (CA) B.6.2.1.1
Acute oral toxicity study in mice Publication on a review of acute toxicity studies performed since 1966. Studies performed according to the requests from the Ministry of health and welfare and other Japanese agencies Deviations: Test	Mice (ddY) Male and females 10 animals/group	Thymol (highest purity available) in squalene (concentration not specified). At least 6 increasing dose levels (not specified) 14-day observation period	**Thymol LD_{50}: 1050 mg/kg bw (f)** LD_{50} = 1200 mg/kg bw (m) Hypoactivity and ataxic gait. Small intestinal congestion	Hasegawa, R. *et al.* (1989) (CA) B.6.2.1.2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
substance not characterised. Poorly described method and results GLP: Not stated (studies from 1966. Data of the study on thymol not provided) **Supporting information**				
Acute oral toxicity review Publication: Review of published literature. No data on the test guidelines followed in each study. Deviations: Test substances not characterised. Poorly described methods and results GLP: Not stated for any study **Supporting information**	Rabbit	Thymol undiluted (500, 750, 2000 and 3000 mg/kg bw) Gelatine capsules	**LD_{50} between 500 and 750 mg/kg bw (undiluted)** **LD_{50} between 750 and 2000 mg/kg bw (50% in olive oil)** Livingston (1921)	Escobar, A. (2006) (CA) B.6.2.1.3
		Thymol 50% in olive oil (250, 500, 750, 1000, 1500, 2000 and 3000 mg/kg bw) Gelatine capsules		
	Mouse	Thymol in cottonseed oil Gavage 620, 940, 1400 and 2100 mg/kg bw	**LD_{50} = 1800 mg/kg bw** All deaths occurred within 24 h. Only depression at lower doses. Depression and prostration at higher doses. McOmie *et al.* (1949) Also in BG Chemie,2000 -B.6.2.1.4	
	Rat (Osborne-Mendel) Guinea pig	Thymol 20% in propylene glycol Intubation	**LD_{50} rat = 980 mg/kg bw** Depression and ataxia in most dose groups, and coma at greater dose. **LD_{50} guinea pig = 880 mg/kg bw** Irritated gastrointestinal tract, tremors, coma and respiratory failure. Jenner *et al.* (1964) (CA) B.6.2.1.1	
	Mouse (ddY)	Thymol in squalene (concentration not specified)	**LD_{50} = 1050 mg/kg bw (f)** LD_{50} = 1200 mg/kg bw (m) Hypoactivity and ataxic gait. Small intestinal congestion. Hasegawa *et al.* (1989) (CA) B.6.2.1.2	
	Mouse Cat Rabbit	Not stated	**LD_{50} mouse = 640 mg/kg bw** **LD_{50} cat = 250 mg/kg bw** **LD_{50} rabbit = 750 mg/kg bw** Instituto Superiore di Sanità (1999)	
	Mouse	Thymol in alcohol/propylene glycol/water Gavage	**LD_{50} = 640 mg/kg bw (alcohol/ propylene glycol/water)** Decrease in spontaneous movements, piloerection and paralysis of anterior limbs within 1h. Death occurred within 5 to 6 h **LD_{50} between 1200 and 1300 mg/kg bw (peanut oil)** **LD_{50} between 600 and 650 mg/kg bw (aqueous emulsion)**	
		Thymol 10% in peanut oil Gavage		
		Thymol 10% in aqueous emulsion Gavage		

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
			RIFM (2001e)	
Acute oral toxicity review Publication: Review of published literature. Deviations: Poorly described method and results **Supporting information**	Mouse (male)	Thymol 1 to 4% aqueous solutions in cottonseed oil Gavage 620 to 2100 mg/kg bw	**LD_{50} = 1800 mg/kg bw** Depressed general condition. Death occurred within 48 h. Gross pathology revealed haemorrhages in the small intestines and severe oedema and congestion of the lungs. McOmie *et al.* (1949) Also in Escobar A, 2006 -B.6.2.1.3	BG Chemie, (2000) (CA) B.6.2.1.4
	Mice (groups of 8)	- thymol as a 10% aqueous emulsion - thymol as a 10% peanut oil solution Oral route. No data on doses	**LD_{50} = 650 mg/kg bw** (aqueous emulsion) **LD_{50} = 1300 mg/kg bw** (peanut oil solution) Drowsiness and paralysis occurred after 2 to 5 minutes. At the later stages: muscle twitching and spasms. Deaths occurred after 6 to 12 hours. The survivors recovered slowly. Oelkers, (1940)	

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Published report: poisoning case	Mouthwash containing thymol (0.064%)	Fatal large-volume mouthwash ingestion in an adult. Ingestion of almost 3 litres of mouthwash by one patient (almost 1.92 g of thymol in the almost 7.74 g of non-alcoholic ingredients)	Cardiovascular collapse, multiorgan system failure and death. The patient presented a profound anion gap metabolic acidosis and a significant osmolar gap. It was concluded that exposure to phenolic ingredients of the mouthwash (i.e. eucalyptol, menthol and thymol) as well as alcohol accounted for the adverse effects.	Soo Hoo. *et al.* (2003) (CA) B.6.9.3.2

Table 20: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
REACH DATA Oral acute study in mice. Reliability 4	Thymol	10 mice/dose received orally 250, 500, 1000, 1500, 2000 or 2500 mg/kg bw thymol. Sex, strain, route of administration and vehicle not available.	LD50 = 1210 mg/kg bw	Bailenger J and Amyot B. (1967) "Etude experimentale du pouvoir anthelminthique de nouveaux derives du diphenyl-methane" (REACH registration dossier data not provided by applicant and not assessed by the RMS)
REACH DATA Oral acute study in rats. Reliability 4	Thymol	3 Female rats/dose received 5, 50, 300 or 2000 mg/kg bw via oral gavage route (dissolved as 20% w/v in methylcellulose). Observation period: 4 days.	No mortality. LD50 = 2000 to 5000 mg/kg bw. Decrease in locomotor activity, recumbent, walking abnormality, muscle relaxation and deep breathing at 2000 mg/kg on administration day.	J-CHECK (2010) "Acute toxicity test using rats of thymol" (REACH registration dossier data not provided by applicant and not assessed by the RMS)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
REACH DATA Oral acute study in rabbits. Reliability 4	Thymol	2-10 rabbits/dose received 250, 500, 750, 1000, 1500, 2000 or 3000 mg/kg bw (solution in olive oil or neat). Route of administration via capsule.	No LD50 was determined.	Livingston, A.E. (1921) "The comparative toxicity of thymol and carvacrol (isothymol)" (REACH registration dossier data not provided by applicant and not assessed by the RMS)
REACH DATA Oral acute study in mice. Reliability 4	Thymol	8 male mice Doses received: unknown. Strain, route of administration and vehicle not available.	LD50 = 640 mg/kg bw in male mice.	Izeki, M. (1956) "Studies on antiseptics - In the toxicity of 3-Methyl-4-isopropylphenol" (REACH registration dossier data not provided by applicant and not assessed by the RMS)

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

All the animal data provided for the evaluation of the acute oral toxicity of thymol is considered supporting information.

In a first study, prior to test guidelines and GLP enforcement (Jenner, P.M. *et al*., 1964), acute oral toxicity of several food flavourings and compounds of related structure was assayed. As reported, no attempt was made to secure chemically pure compounds and, therefore, the commercially available material was used. Once diluted, and administered orally by intubation to rats and guinea pigs, LD_{50} values of 980 mg/kg bw (rats) and 880 mg/kg bw (guinea pigs) were defined. The information provided in the report was very limited. Clinical signs were described in guinea pigs as irritated gastro-intestinal tract, tremors, coma and respiratory failure and, in rats: depression, ataxia and coma on high doses.

A second study (Hasegawa R. *et al.*, 1989) is a publication of a review of acute toxicity studies performed on 113 environmental chemicals since 1966. Very limited information is provided for each chemical. The publication reports hypoactivity and ataxic gait as clinical signs observed after oral administration of thymol, and LD_{50} values of 1050 mg/kg bw for females and 1200 mg/kg bw for males.

The third publication provided as animal data (Escobar A., 2006) is a review of published literature on several related substances (included thymol) used as cosmetic biocides/preservatives and/or fragrance ingredients. The two studies detailed above are included in this review of published literature. Very little information of each study is included. The lowest value of LD_{50} reported in this publication is 250 mg/kg bw in cats. However, for this study not even the concentration, vehicle, mode of administration used or number of treated animals is provided. With such data lacking, RMS deems this result cannot define the LD_{50} value for thymol and rather an overall range of doses should be considered. The results obtained in the rest of the publications reviewed in this paper present LD_{50} values that lie between the threshold values of 300 and 2000 mg/kg bw, established as the CLP criteria for the classification of chemicals as acute oral toxicity (category 4).

In addition, the toxicological evaluation of BG Chemie (2000) includes some details on two studies that were not reported on the abovementioned data, and does not provide new LD_{50} values.

Altogether, none of the animal data provided is considered acceptable, but supportive information. However, from the information gathered in these documents, it can be concluded that the overall LD_{50} value of thymol via oral route lies between the threshold values of 300 and 2000 mg/kg bw, established as the CLP criteria for the classification of chemicals as acute oral toxicity (category 4).

Moreover, provided human data (Soo Hoo *et al.*, 2003) shows thymol contributed to the fatal acute oral toxicity effects provoked by the ingestion of a great amount of mouthwash (containing alcohol, thymol and other non-alcoholic ingredients).

Data from REACH registration dossier were included in the summary table of other studies relevant for acute oral toxicity, since it is an ECHA requirement for the proposal of harmonised classification (CLH) according to Regulation (EC) no. 1272/2008 (CLP). However, it has to be underlined that these data were not available in the applicant submission dossier for the renewal and consequently they have not been evaluated by the RMS and not included in Volume 3 of this RAR.

Altogether, available data confirms the classification of thymol as acute oral toxicity, category 4 (H302), as it is already included in Annex VI of Regulation (EC) No 1272/2008.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

CLP criteria establishes threshold values of 300 and 2000 mg/kg bw for the classification of a substance as Acute (oral) Toxicity 4 (Acute Tox. 4; H302).

Classification of thymol as acute oral toxicity, category 4 (H302), is already included in Annex VI of Regulation (EC) No 1272/2008.

Assessed data confirms this classification since a LD_{50} value of thymol, between 300 and 2000 mg/kg bw (ATE proposed as 500 mg/kg bw), can be derived as an overall conclusion.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available data on the active substance, and according to the criteria under Regulation (EC) No 1272/2008, thymol is classified as **acute (oral) toxicity, category 4, Acute Tox. 4 (H302)**.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity review Publication: data provided to EPA as a waiver of the requirements of a tolerance for residues of thymol Deviations: Only the LD_{50} value was provided GLP: No data **Supporting information**	No data	No data	**LD_{50} > 2000 mg/kg bw** Information collected from a report by the Environmental Risk Management Agency (ERMA, 2005) of New Zealand and Anonymous (2000)	US-EPA (2006) (CA) B.6.2.2.1
Acute dermal toxicity review Publication: Review of published literature. No data on the test guidelines followed in each study. Deviations: Test substance not characterised. Poorly described method and results GLP: Not stated for any study **Supporting information**	Rabbit	Thymol (not characterised) 420 mg/kg bw Application to shaved back 24 h exposition	**LD_{50} > 420 mg/kg bw** No deaths occurred. No apparent systemic effect. Local effects: the skin turned parchment-like within 24 h. Complete necrosis of superficial skin layers occurred after 10 days. McOmie *et al.* (1949)	Escobar, A. (2006) (CA) B.6.2.2.2
	Rabbit New Zealand (10 animals)	Thymol (not characterised) 2000 mg/kg bw Application to the clipped abraded abdominal skin 24 hours exposition 7 d observation Necropsy was performed but no results reported	**LD_{50} > 2000 mg/kg bw** No deaths occurred. Local effects: at 24 h: 10/10 had moderate erythema, 8/10 moderate oedema and 2/10 slight oedema. On day 7: 3/10 showed moderate erythema and 4/10 slight erythema. RIFM (1972b as cited in RIFM 2001e)	
Acute dermal toxicity review EEC/B.3 (1984) Publication: Review of published literature. Deviations: Poorly described method and results **Supporting information**	Rats SPF-Wistar (5 male and 5 female)	Thymol (99.5 to 99.6% pure) mixed into a paste with Cremophor 2000 mg/kg bw Dermal application 24-h occlusive exposure in the shorn dorsal and flank skin (10% of body surface) 14-day observation period	**LD_{50} > 2000 mg/kg bw** No deaths and no signs of toxicity. 3/5 male and 2/5 females showed brownish discoloration at application site which lasted for up to 4 days. No substance-related changes observed at terminal necropsy. Bayer (1986a)	BG Chemie, (2000) (CA) B.6.2.2.3

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Skin sensitization assays (4): - Open Epicutaneous Test (OET) - Draize Test (DT) - Maximization test (GPMT) - Freud's Complete Adjuvant Test (FCAT) Guinea pigs, Himalayan 6 – 8 male and female/group. Prior to OECD TG 406 Deviations: -Male instead of female mice used -No positive control -Limited level of reporting (clinical signs, body weights) **Supportive information**	Thymol up to 10%	Higher concentrations of thymol (*i.e.* >10%) were not tested due to systemic toxicity.	The mentioned systemic toxicity was not described. Negative results up to 10% in all four techniques *See table 36 for more details.*	Klecak *et al.* (1977) (CA) B.6.2.6.1

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Provided data on acute dermal toxicity of thymol is very limited and is considered as supportive only. On a first document (US-EPA, 2006), an exemption for an US-EPA requirement of a tolerance was provided for thymol, based on collected data from a report by the Environmental Risk Management Agency of New Zealand (ERMA, 2005) and Anonymous (2000) which found dermal LD_{50} value of >2000 mg/kg bw. No more data was provided and, therefore, it is considered as supportive only.

The other document provided by the applicant as animal data (Escobar A., 2006) is a review of published literature on several related substances (included thymol) used as cosmetic biocides/preservatives and/or fragrance ingredients. Very little information of each study is reported. No deaths occurred in either of the two acute dermal toxicity studies reported in the review, showing LD_{50} values greater than 420 and 2000 mg/kg bw, respectively (*i.e.* the tested doses).

In another review of published literature (BG Chemie, 2000) that was provided by the applicant to support the Medical Data point, a brief summary of an acute dermal toxicity study in rats was included. Thymol was tested mixed into a paste with cremophor, resulting in a LD_{50} value greater than 2000 mg/kg bw.

The only document referring an acute dermal toxicity potential of thymol is a sensitization study, provided to assess the cutaneous sensitization potential of thymol (Klecak *et al.,* 1977). The study report indicates that the tested concentration did not exceed the 10% of thymol due to systemic toxicity. However, neither description nor any other data on the mentioned toxicity is reported.

The information on acute dermal toxicity of thymol is, therefore, very limited. However, since thymol is currently classified in Annex VI of Regulation (EC) No 1272/2008 as corrosive, category 1B (Skin Corr. 1B; H314), no acute dermal toxicity test should be performed, according to the test method OECD TG 402.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

According to the classification criteria under Regulation (EC) No 1272/2008 the threshold value for triggering acute dermal toxicity classification is 2000 mg/kg bw.

The information on acute dermal toxicity of thymol is very limited. In addition, thymol is classified in Annex VI of Regulation (EC) No 1272/2008 as corrosive, category 1B (Skin Corr. 1B; H314). According to the test method OECD TG 402, acute dermal toxicity tests should not be carried out with corrosive substances and, therefore, no classification can be derived for this hazard class.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification on acute dermal toxicity can be derived for thymol, due to the corrosive properties of this substance.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute toxicity review Publication: data provided to EPA as a waiver of the requirements of a tolerance for residues of thymol Deviations: Only a justification for no need of a study is provided GLP: Not applicable **Supporting information**	Dogs, cats and other animals	No data	Thymol is added to the anaesthetic halothane as a preservative (0.01%), and may be used for periods exceeding 4 hours using concentrations typically around 5% (calculated concentration of thymol ≈5 mg/L)	**LC_{50} > 5 mg/L (calculated)** Used in the mentioned conditions without adverse effects	US-EPA (2006) (CA) B.6.2.3

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Publication: Review of published literature	Halotane (inhalation anaesthetic) containing 0.01% thymol	Postoperative "halothane hepatitis"	It's been suggested that thymol might play a causative role in very rarely occurring instances of postoperative "halothane hepatitis" Hutter and Laing (1993)	BG Chemie, (2000) (CA) B.6.9.2
	Thymol in water	Olfactory threshold test in 9 to 12 subjects	The olfactory threshold of thymol in water was found to be 500 µg/L Dietz and Traud (1978)	
Publication: Review of published literature	Cold remedy that contained thymol, in addition to menthol and other volatile oils.	A 3 week-old infant inhaling the cold remedy.	The infant suffered a respiratory collapse. The authors considered it very unlikely that the collapse was attributable to the remedy. No further details Davis and Livingstone (1986).	BG Chemie, (2000) (CA) B.6.9.3.1

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
REACH DATA Inhalation acute study in mouse. Reliability 2	Cumene	Dose: 10000 mg/m³.	LC50: 10000 mg/m³ when mice were exposed for 7 h.	U.S. National Library of Medicine (2017) "Acute inhalation toxicity in mouse". (REACH registration dossier data not provided by applicant and not assessed by the RMS)

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Provided data on acute inhalation toxicity of thymol is limited to a US-EPA paper, which provides reasoning for the non-submission of an acute inhalation study.

According to this publication, thymol is added to the anaesthetic halothane as a preservative (0.01%), and may be

used for periods exceeding 4 hours in cats, dogs and other animals. Concentrations used are typically around 5% (calculated concentration of thymol ≈5 mg/L), showing no adverse effects under the mentioned conditions.

Applicant also mentions that thymol is used in non-prescription medicines acting by vapour inhalation for the treatment of colds, coughs and associated respiratory infections. However, the concentration at which thymol enters the respiratory system is not referred. Only one case on human data was reported regarding the inhalation of one of these cold remedies, a 3-week-old infant (BG Chemie, 2000) who suffered a respiratory collapse, but it could not be attributable to the remedy.

Additionally, among the human data provided in the published report of BG Chemie (2000), a concern on the possible causative role of thymol in the postoperative "halotane hepatitis" was suggested. However, this suggestion was published in 1993 and is referred to a very rarely occurring effect, so it is not considered relevant in the assessment of the acute inhalation toxicity of thymol. Moreover, subsequent papers indicate that, although the exact mechanism of halothane-induced hepatotoxicity is unknown, there is strong evidence that the immune system mediates hepatitis-related mortality. This immune system mediation is related with the extremely reactive molecule trifluoroacetylchloride (TFA), which is generated by the reaction between lysine and halothane metabolites, yielding neo-antigens by binding to the hepatocyte macromolecules. These neo-antigens can provoke immune responses against hepatocytes and inducing fulminant hepatitis (Habibollahi P, *et al.,* 2011)[1].

Data from REACH registration dossier were included in the summary table of other studies relevant for acute inhalation toxicity, since it is an ECHA requirement for the proposal of harmonised classification (CLH) according to Regulation (EC) no. 1272/2008 (CLP). However, it has to be underlined that these data were not available in the applicant submission dossier for the renewal and consequently they have not been evaluated by the RMS and not included in Volume 3 of this RAR. The only remaining acute inhalation toxicity in the REACH dossier was performed with cumene instead of thymol.

Altogether, considering the abovementioned use of thymol by inhalation in anaesthesia procedures, low acute toxicity is to be expected via the inhalation route.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

According to the classification criteria under Regulation (EC) No 1272/2008 the threshold for no classification for acute inhalation toxicity is an LC_{50} > 5 mg/L for dusts or mists.

No study on acute inhalation toxicity is provided. However, data on the use of thymol as a preservative in anaesthetic procedures in cats, dogs and other animals shows no adverse effects at calculated concentrations of 5 mg/L and, therefore, low toxicity is to be expected via the inhalation route and no classification is proposed.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Data available indicates that thymol does not require classification for acute inhalation toxicity.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Primary skin irritancy OECD 404 Publication: Review of published literature. Deviations: Poorly described	6 albino rabbits (both sexes)	Thymol (99.5% pure) Made to a paste with water	500 mg of test substance (paste) Applied to the mechanically depilated flank skin for a 4-hour semi-occlusive exposure. Findings were scored 1, 24, 48	Thymol proved corrosive in all of the animals. The necrotic changes were irreversible in all of the animals up to the end of the observation period Bayer (1986 b)	BG Chemie (2000) (CA) B.6.2.4

[1] Habibollahi P, Mahboobi N, Esmaeili S, Safari S, Dabbagh A, Alavian SM. Halothane-induced hepatitis: A forgotten issue in developing countries: Halothane-induced hepatitis. *Hepat Mon*. 2011 Jan;11(1):3-6.

method and results **Supporting information**			and 72 h, and after 7 and 14 days		

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute dermal toxicity review Publication: Review of published literature. No data on the test guidelines followed in each study. Deviations: Test substances not characterised. Poorly described methods and results GLP: Not stated for any study **Supporting information**	Thymol (not characterised)	Rabbit 2000 mg/kg bw Application to shaved back 24 h exposition	The skin turned parchment-like within 24 h. Complete necrosis of superficial skin layers occurred after 10 days. McOmie *et al.* (1949) *See table 21 for more details.*	Escobar, A. (2006) (CA) B.6.2.2.2
	Thymol (not characterised)	10 New Zealand Rabbits 2000 mg/kg bw Application to the clipped abraded abdominal skin 24 hours exposition 7 d observation Necropsy was performed but no results were reported	At 24 h: 10/10 had moderate erythema, 8/10 moderate oedema and 2/10 slight oedema. On day 7: 3/10 showed moderate erythema and 4/10 slight erythema. RIFM 1972b, as cited in RIFM 2001e *See table 21 for more details.*	

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Classification of thymol as corrosive (Skin Corr. 1B, H314) is included in Annex VI of Regulation (EC) No 1272/2008. Therefore, further testing is not required and has not been performed.

A review of published literature provided by the applicant to support the Medical Data point (BG Chemie, 2000), included a brief summary of a primary skin irritation study in rabbits where all animals presented necrotic changes up to the end of the observation period.

Additionally, the applicant provided a review of published literature on several related substances (included thymol) used as cosmetic biocides/preservatives and/or fragrance ingredients (Escobar A., 2006), where local clinical signs were reported for two acute dermal toxicity studies in rabbits. The findings included changes in the skin, which turned parchment-like within 24 h and necrosis of superficial skin layers after 10 days in one study, and moderate oedema and erythema in the other study. This data is in line with the actual classification of the active substance in Annex VI.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

According to the current EU Criteria (Regulation (EC) No 1272/2008), classification as skin corrosive is required if at least one animal shows a corrosive response (such as scars) at the end of the observation period. When data are sufficient substances shall be classified in one of the three sub-categories 1A, 1B, or 1C; otherwise, corrosive substances shall be classified in Category 1.

Available animal data on thymol described necrotic effects after the dermal application, both in a skin irritation and an acute dermal toxicity studies performed in rabbits. Although data is very limited, it confirms the actual classification of thymol in Annex VI of Regulation (EC) No 1272/2008 as skin corrosive. However data is regarded not sufficient for subcategorization and consequently a modification to category 1 is proposesd.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Classification of thymol as **skin corrosion, category 1 (Skin Corr. 1, H314)** is proposed.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>Eye irritation study in rabbits
Prior to OECD TG 405
Deviations: Poorly described method and results.
GLP: No (prior to GLP enforcement)
Supporting information</td><td>Rabbits (no further detail provided)</td><td>Thymol (less than 1% impurities)
0.03 ml of 40% thymol in ethylene glycol on the cornea of rabbits</td><td>Draize scores (1944) used:
<table>
<tr><th>Observation time</th><th>1 h</th><th>24 h</th><th>72 h</th><th>Fluorescein test at 24 h</th></tr>
<tr><td>Score</td><td>32</td><td>80*</td><td>80*</td><td>100</td></tr>
</table>
* Treated eye was closed so completely that eye injury could not be evaluated by the scoring method
Thymol was found to be extremely irritant to the eyes of rabbits</td><td>McOmie W.A. et al. (1949) (CA) B.6.2.5.1</td></tr>
<tr><td>Eye irritancy OECD 405
Publication: Review of published literature.
Deviations: Poorly described method and results
Supporting information</td><td>Albino rabbits
3 females</td><td>Thymol (99.5% pure)
100 µl (bulk weight approx. 60 mg) instilled into the conjunctival sac of one eye.
Rinsed out with physiological saline solution after 24h.
Findings were scored according with Draize: 1h after rinsing, and again 24, 48 and 72 hours, and 7, 14 and 21 days.</td><td>Irritation scores:
- Conjunctivae: 2 to 2.3
- Iris: 1.0
- Cornea: 1.3 to 2.7.
Up to day 21 the animals had positive fluorescein test results and, as of the day 14 of the observation period, 2 rabbits developed pannus while the third showed swelling in the lower part of the eyeball.

Thymol was evaluated as strong irritant on the basis of these irreversible findings.

Bayer (1986 b)</td><td>BG Chemie (2000) (CA) B.6.2.5.2</td></tr>
</table>

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Classification of thymol as corrosive (Skin Corr. 1B, H314) is included in Annex VI of Regulation (EC) No 1272/2008. Therefore, further testing on eye damage/eye irritation is not required and has not been performed.

An older published study was provided (McOmie W.A. *et al.,* 1949), which confirms that thymol is extremely irritant to the eyes of rabbits.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Considering thymol is classified as skin corrosive (Skin Corr. 1B; H314), classification as serious eye damage, category 1 (Eye Dam.1; H318) is also required. Since the hazard statement H318 is already included in the hazard statement H314 for skin corrosion (Causes severe skin burns and eye damage), H318 is considered in this section, but it is not included in the End Points for labelling purposes to avoid redundancy (according to the Guidance on the Application of CLP Criteria, July 2017).

This classification is supported by the available data on eye irritation provided by the applicant: an old publication on eye irritation study in rabbits (McOmie W.A. *et al.,* 1949), concluding the extremely irritant effects of thymol in contact with the eyes of rabbits.

Additionally, to support the Medical Data point, the applicant provided a review of published literature (BG Chemie, 2000) which also included a brief summary of an eye irritation study in rabbits. The animals presented pannus in two of the three animals and swelling of the lower part of the eyeball from day 14, and at the end of the observation period of 21 days, the three rabbits showed corneal lesions (positive fluorescein test results).

The irreversibility of the ocular lesions after 21 days fulfils the CLP criteria for classification as serious eye damage, category 1 (Eye Dam. 1).

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

According to the criteria under Regulation (EC) No 1272/2008, thymol is classified as **serious eye damage, category 1 (Eye Dam. 1, H318).**

Since the hazard statement H318 is already included in the hazard statement H314 for skin corrosion (Causes severe skin burns and eye damage), H318 is considered in this section, but it is not included in the End Points for labelling purposes to avoid redundancy (according to the Guidance on the Application of CLP Criteria, July 2017).

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data available.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

No data available.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

In the absence of any data this hazard class is not assessed for harmonisation of classification and labelling.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance Dose levels, duration of exposure	Results	Reference
4 Skin sensitization assays (screening for allergenicity of 32 chemicals): - Open Epicutaneous Test (OET) - Draize Test (DT) - Maximization test (GPMT) - Freud's Complete Adjuvant Test (FCAT) Prior to OECD TG 406 Deviations: -Limited level of reporting (dermal responses after application in preliminary and main study, clinical signs, body weights) -No positive control (test substances were considered positive to humans) **Supportive information**	Guinea pigs, Himalayan 6 – 8 male and female/group	Thymol (purity not stated) Concentrations above 10% of thymol were not tested, due to systemic toxicity. **Open Epicutaneous Test (OET):** *Induction*: 0.1 ml containing 4 to 6 concentrations between 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03%, were applied daily for 21 days (open applications). In the case of thymol concentrations used, apart of 3 and 10%, are unknown. *Challenge*: 0.025 ml of a solution containing thymol at the minimal irritating concentration (3%) were applied on days 21 and 35 to the contralateral flank. Reactions were evaluated at 24, 48 and 72 h **Draize Test (DT):** *Induction*: intradermal injection on day 0 of 0.05 ml of a 0.1% solution of the compound in isotonic saline. Further doses of 0.1 ml were injected on 9 alternate days. *Challenge*: 0.05 ml of the 0.1% solution intradermally on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions. **Maximization test (GPMT):** *Induction*: on day 0, 3 paired intradermal injections: two injections of 0.1 ml of a 5% compound solution (one with FCA and one without); and 0.1 mL FCA alone. On day 8, 250 mg of 25% test substance in petrolatum were applied under occlusion for 48 h. *Challenge*: On day 21 an occlusive patch test with the test substance at a sub-irritant concentration in petrolatum was applied to the flank for 24 h. Reactions were read 24 and 48 h after patch removal. **Freud's Complete Adjuvant Test (FCAT):** *Induction*: Doses of 0.05 mL undiluted compound mixed with 0.05 of FCA were injected intradermally into the neck on days 0, 2, 4, 7, and 9. Controls were similarly treated with FCA alone. *Challenge*: Days 21 and 35, a 24-h patch test using thymol in petrolatum just like in the GPMT.	Negative results up to 10% in all four techniques (10% was the highest concentration tested)	Klecak *et al.* (1977) (CA) B.6.2.6.1
Skin sensitization review Publication: Review of published literature. No data on the test guidelines followed in each study. Deviations: Test	Guinea pig 20 females GPMT	*Induction:* Intradermal injections of FCA plus distilled water, 10% thymol in FCA and 10% thymol in FCA plus distilled water. 7d later, after pretreatment with 10% sodium lauryl sulfate, 0.2 ml of 10% thymol in FCA was applied topically for 48 h under occlusion. *Challenge*: 2w after topical application, 0.02	Weak sensitization effects with 20% thymol were observed (mean response was 0.4 using 20% thymol) Thymol was	Escobar, A. (2006) (CA) B.6.2.6.2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance Dose levels, duration of exposure	Results	Reference
substance not characterised. Poorly described method and results GLP: Not stated for any study **Supporting information**		ml of 5, 10 or 20% thymol in acetone was applied topically. Reactions were graded per Draize.	considered negative at 10% concentration under the conditions of this test. (mean response was 0.2 at 10% thymol 48 h later) CTFA (1997b)	
	Himalayan guinea pigs 6 – 8 male and female/group -Open Epicutaneous Test (OET) -Draize Test (DT) -Maximization test (GPMT) -Freud's Complete Adjuvant Test (FCAT)	Thymol (purity not stated) OET: *Induction*: 3% by 21 daily open applications. *Challenge*: days 21 and 35 by open application to the contralateral flank. Reactions evaluated at 24, 48 and 72 h.	Negative results reported for all four techniques Klecak *et al.* (1977) (CA) B.6.2.6.1	
		Thymol (purity not stated) DT: *Induction*: intradermal injection (day 0) of 0.05 ml thymol in a 0.1% solution (in saline). On 9 alternate days, 0.1 ml of thymol was injected. *Challenge*: intradermal on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.		
		Thymol (purity not stated) GPMT: *Induction*: on day 0, 2 intradermal injections (0.1 ml at 5% concentration) with and without FCA. On day 8, 250 mg of 25% thymol in petrolatum was applied under occlusion for 48 h. *Challenge*: 24-h patch test using thymol in petrolatum on day 21. Reactions were read at 24 and 48 h after removing the patch.		
		Thymol (purity not stated) FCAT: *Induction*: injection of 0.1 ml of a 50:50 mixture of thymol and FCA on days 0, 2, 3, 7 and 9. Control animals were treated similarly with 0.05 ml of FCA alone. *Challenge*: Days 21 and 35, a 24-h patch test using thymol in petrolatum.		
	Albino Dunkin-Hartley guinea pigs Cross-reactivity	Guinea pigs sensitized to *p*-methoxyphenol were tested for cross-reactivity to thymol. Vehicle was methylethyl ketone and arachis oil.	No effects reported Van Der Walle *et al.* (1982)	

Table 37: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Medical monitoring of workers	Thymol	Regular monitoring of workers involved in the production, packaging or handling of thymol	There have been no reports of reactions or ill health in any workers.	Volpe (2021) (CA) B.6.9.1
Publication: Review of published literature	Thymol	No more data provided	Thymol reportedly caused dermatitis in dentists Schwartz *et al.* (1957)	BG Chemie, (2000) (CA) B.6.9.2
	Thymol 4% in petrolatum	Maximisation test in 25 volunteers. 48-h occlusive exposure	No primary skin irritation nor allergenic reactions Kligman (1972)	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	Thymol 1% in petrolatum	Patch-test in patients with contact dermatitis (38 dentist, 18 dental nurses and 28 dental technicians)	Only 1 dental nurse (1.2% of patients) developed a positive reaction to thymol Berova *et al.* (1990)	
	Ethanolic antiseptic solution ("Listerine") containing 0.6% thymol Patch-test: 1% thymol in petrolatum	43-year-old patient with chronic paronychia 3-week occlusive application of "Listerine" (0.6% thymol, 0.9% eucalyptus oil, 0.6% methyl salicylate, 0.4% menthol and traces of benzoic acid) Also 48-h patch test with the antiseptic and with the ingredients was performed to the patient and 3 controls	The 3-w application resulted in spreading pruritic dermatitis in the patient. 48-h patch test with the antiseptic produced a positive reaction in the patients and none in the 3 controls. 48-h patch test with thymol (1% in petrolatum) led to a reaction (2+). 48-h patch test with eucalyptus oil (1% in alcohol), methyl salicylate (2% in olive oil), menthol (1% in petrolatum) and benzoic acid (5% in petrolatum) proved negative in the patient and in the 3 controls. However, the patient used "Listerine" as mouthwash several times without developing any noticeable reactions. According to the author, thymol appears to be a weak sensitiser which needs prolonged contact, occlusive application and inflamed skin to produce sensitisation and dermatitis Fisher (1989)	
	Thymol 1% formulation (composition not specified)	Patch test in 365 patients at a dermatology clinic (1981 to 1986)	2 cases (0.5%) of positive reactions Itoh *et al*. (1988)	
	Thymol 1% formulation (composition not specified)	Test in 131 patients at a dermatology clinic (1979 - 1982) This publication is previous to Itoh *et al.* (1988), but same investigators and similar group of patients	None of the individual reacted Nishimura *et al*. (1984)	
	"spir. Dilute" solution of thymol (only data available)	Patch test in 221 patients treated at a dermatology department	One female patient (0.45%) showed a positive reaction Dohn (1980)	
	Thymol (no more data)	31-year-old woman with skin allergy to menthol	It was possible to exclude a simultaneous allergy to thymol Papa and Shelley (1964)	
	Thymol (5% in glycerine)	Patch-test in 300 workers (217 women and 83 men, 20-27 years) of stomatology offices 12 different substances used in dental practice were patch-tested	39 individuals reacted to thymol, in a total of 213 who tested positive. 87 were negative Djerassi and Berowa (1966)	
	Thymol (1% in petrolatum)	Patch-test in 79 patients with eyelid dermatitis	All results were negative Nethercott *et al*. (1989)	
	Thymol (1% in petrolatum)	Patch-tests in 290 patients Thymol was assayed in the context of a study on allergising effect of topical medicaments	None of the 290 patients developed a positive reaction to thymol Meneghini *et al.* (1971)	
	Thymol (1% in petrolatum)	Patch-test in 100 eczematous patients of a dermatology clinic	None of the patients showed a positive reaction to thymol Rantuccio and Meneghini (1970)	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Numerous substances were patch-tested		
	Thymol (0.01 to 0.015 ml, equivalent to 0.1 to 0.15 mg)	Patch-test in a 51-year-old sawmill worker Within 2 years working, he developed itching vesicular dermatitis on the face, dorsa of his hands and the flexures of his forearms. Patch-test was performed with various extracts of wood and pure constituents.	Negative results for thymol in the patch-test. Strongly positive reactions to western red cedar, in particular Bleumink *et al*. (1973)	
	Hirudoid cream, containing 0.1% of thymol Patch-test: thymol (not specified)	Patch-test in 23 patients with contact dermatitis following the use of Hirudoid cream These patients developed allergic contact dermatitis over a period of 10 years. Individual components of the cream were patch-tested.	The individual components of the cream proved negative in the patch-test However, the cause of the allergies was identified as a reaction product of thymol, ethanolamine and formaldehyde. The last two substances are degradation products of 1,3,5-trihydroxyethylexahydrotriazine, of which Hirudoid cream contains 0.15% Smeenk *et al*. (1987)	

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Two published papers were provided as animal data on skin sensitization, and both were considered as supportive information. The first publication (Klecak *et al.,* 1977) reported the screening of 32 fragrance materials (including thymol) for allergenicity in the guinea pig. According to the report, these test substances had previously been described in the literature as being allergenic for man. The study reported negative results using four techniques. However, the highest concentration of thymol tested was 10% due to systemic toxicity at higher concentrations (such toxicity was not described).

The other publication provided (Escobar A., 2006) is a review of published literature on several related substances (included thymol) used as cosmetic biocides/preservatives and/or fragrance ingredients. The abovementioned study (Klecac *et al*., 1977), that gave negative results tested up to 10%, was included in this review together with other two publications. Positive results (weak sensitization effects) were observed in animals treated with 20% thymol CTFA (1997b), and the last publication (Van Der Walle *et al.* 1982) reported negative results for the cross-reactivity of thymol with *p*-methoxyphenol in animals sensitized with this last one (so this result is not valid for the assessment of the sensitization potential of thymol).

Regarding the human data, the medical monitoring of workers involved in the production, packaging or handling of thymol reported no reactions or ill health (Volpe, 2021). Although no information is given on the conditions of the handling of thymol, it is expected that workers at the manufacturing plant wear personal protective equipment (PPE), preventing any contact with the substance and, therefore, the absence of reactions reported in this specific group does not grant the absence of skin sensitization potential of thymol.

Additionally, the applicant submitted a publication on the toxicological evaluation of thymol (BG Chemie, 2000) which reviews the experience in humans, including publications with information on skin sensitization.

Some of the publications were addressed to dental practices: an old publication (Swartz *et al*., 1957) reported that thymol caused dermatitis in dentists (no more data is provided). In a patch-test performed with thymol (1%) in 84 dental professionals with contact dermatitis, 1 nurse developed a positive reaction to thymol (Berova *et al*., 1990). Another patch-test was performed in 300 workers of stomatology offices using thymol (5%), and 39 individuals reacted to thymol (Djerassi and Berowa, 1966).

In a different context, dermatology patients were also tested with thymol. According to Nishimura *et al*. (1984) thymol (1%) was tested in 131 patients at a dermatology clinic between 1979 and 1982, and none of the individual reacted. However, a later publication (Itoh *et al.*, 1988) reviewed patch-tests performed with thymol (1%) in 365 patients at a dermatology clinic, from 1981 to 1986, and reported 2 cases of positive reactions. In another patch-test performed in 221 patients treated at a dermatology department with a solution of thymol, 1 female patient showed positive reaction (Dohn, 1980). Negative results with thymol (1%) were also reported in several publications about dermatology patients: none of the 290 patients patch-tested in the context of a study on allergising effect of topical medicaments showed positive reactions to thymol (Meneghini *et al.*, 1971); 79 patients with eyelid dermatitis also gave negative results in a patch-test with thymol (Nethercott *et al*., 1989); none of the 100 eczematous patients of a dermatology clinic showed positive reaction to thymol (Rantuccio and Meneghini, 1970). Finally, 23 patients with contact dermatitis following the use of Hirudoid cream (containing 0.1% of thymol), where patch-tested, providing negative responses to the individual components of the cream (Smeenk *et al*., 1987).

Additionally, neither allergenic reactions nor skin irritation was observed following a 48-hour occlusive exposure of thymol (4%) in a maximisation test performed in 25 volunteers (Kligman, 1972).

Lastly, BG Chemie (2000) also includes publications on individual tests. One 43-year-old patient with chronic paronychia was treated for 3 weeks with occlusive application of an ethanolic antiseptic containing 0.6% thymol, finalising with a 48-h patch test performed to the patient and 3 controls, with the antiseptic and its ingredients. The patient showed positive reactions with the antiseptic and with thymol, and no reactions were observed with the other ingredients. The 3 controls showed no reaction to any of the ingredients or the antiseptic (Fisher, 1989).

A simultaneous allergy to menthol and thymol was excluded in a 31-year-old woman with skin allergy to menthol (Papa and Shelley, 1964). In addition, a patch-test with 0.1 to 0.15 mg of thymol was performed in a 51-year-old sawmill worker who had developed itching vesicular dermatitis; strongly positive reactions to western red cedar were observed, but for thymol the results were negative (Bleumink et *al.*, 1973).

In summary, although negative results were reported in human and animal data, RMS deems thymol should be considered a weak sensitizer, based on the positive results presented in human data, together with the (weak) positive effects in one animal study.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

According to Regulation (EC) No 1272/2008, positive effects seen in either humans or animals will normally justify the classification of a substance as a skin sensitiser.

In this case, although the available information is considered supportive since it comes mainly from a review of published data, positive reactions to thymol from patch-testing in humans are reported in several publications, and also positive results were reported in one animal study.

The Guidance on the Application of the CLP Criteria establishes that the relatively high or low frequency of occurrence of skin sensitisation can be determined attending the following considerations:

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

1.2% patients with contact dermatitis developed a positive reaction to thymol in Berova *et al.* (1990). This was considered as a study with selected dermatitis patients, being all of them dental professionals with contact dermatitis, and therefore the frequency of occurrence was regarded low/moderate, as it was below the limit of 2%. 0.5% patients were positive among 365 patients tested at a dermatology clinic between 1981 and 1986 in Itoh *et al*. (1988). It was not possible to determine if this should be regarded as general population study or a study in dermatitis patients, taking into account that they were patients from a dermatology clinic but no dermatitis was specifically mentioned. In any case, the composition of the formulate was not clarified. A similar situation was observed in the study from Dohn (1980), with a 0.45% of positive cases from a dermatology department.

No positive cases were observed in a monitoring report in workers involved in the production, packaging or handling of thymol. In a patch-test carried out in 300 workers of stomatology offices, 39 individuals (13%) reacted to thymol

(Djerassi and Berowa, 1966). It was not specified whether they were selected with known exposure or not, but in any case the positive cases were above the limit set in the table from CLP criteria to establish that it occurred with high frequency in workers.

In Fisher (1989), one case was reported in which thymol was only one of the components of the mixture to which the patient was exposed. In any case, it was far from the level of 100 cases established in the CLP criteria to consider it as high frequency.

Following the CLP criteria, substances shall be classified as skin sensitisers (Category 1) when no sufficient data are available for classification into subcategories. Available information on thymol does not include information about the grade of exposure to the test substance and, therefore, no subcategorization can be performed.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the available data on the active substance, and according to the criteria under Regulation (EC) No 1272/2008, RMS proposes the classification of thymol as **skin sensitizer, category 1, Skin Sens. (H317)**.

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations if any	Test substance	Dose levels duration of exposure	Results	Reference
No data available				

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Observations	Reference
UV/Visible spectroscopy	thymol (99.7% pure)	**No absorption > 290 nm** Neutral: Extinction Coef. = 49925 at λ = 210 nm Acid: Extinction Coef. = 37121 at λ = 198 nm Alkaline: Extinction Coef. = 18696 at λ = 210 nm	White G.A., (2007) (CA) (B.2.4)

According to Regulation (EU) No 283/2013, the *in vitro* study is not required since the results of UV/Visible spectroscopy performed with the active substance thymol (99.7% pure) showed no absorption > 290 nm (White G.A., 2007).

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No evidence of aspiration hazard of thymol was found in the provided data.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Although the definition of aspiration in section 3.10.1.2 of Regulation (EC) No 1272/2008 includes the entry of solids into the respiratory system, classification criteria for this hazard is established for liquid, aerosol and mist

forms of a substance or a mixture.

Thymol is presented in a solid form and, therefore, no aspiration toxicity hazard is expected.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Data available indicates that thymol does not require classification for aspiration hazard.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Pharmacokinetics Mouse No deviations from OECD TG 417 (2010) GLP: Yes **Study acceptable** Hsd:ICR (CD.1®) mice (19♂, 19♀)	Thymol (99.7% purity) in olive oil Single oral (gavage) dose: Preliminary study: 1250 and 1000 mg/kg bw (2♂, 2♀ each) Main study: 1000 mg/kg bw (15♂, 15♀) Blood samples were taken at 1, 2, 4, 8, 12 and 24 h post dosing.	<u>Preliminary study I</u> (1250 mg/kg bw) Clinical observations: ataxia, bradypnoea, prone posture, piloerection, decreased activity and ptosis in all animals up to 4 h after dosing. Animals appeared normal on Day 2 post-dosing. <u>Preliminary study II</u> (1000 mg/kg bw) Clinical observations : ataxia, decreased activity, bradypnoea, ptosis and piloerection ranging from 5min to 4h post dose. Prone posture, pallor extremities, loss of righting reflex and ptosis were observed in one male from 2 to 4h after dose. Prone and pallor extremities were also observed in one female from 15min to 4h after doses. <u>Main study</u> (1000 mg/kg bw) Clinical observations : increased activity, piloerection, ataxia and decreased activity ranging from 1 to 8h post-dose in both male and female animals. Additional symptoms included bradypnoea (1 male at 8h), ataxia (4 males up to 2h post dose and 6 females up to 4h post dose) and both hunched posture and lethargy (1 male at 2h post dose, 1 female at 4h post dose).	[redacted] (2009) (CA) B.6.1.1.6
Acute oral toxicity study in rats and guinea pigs Prior to OECD TG 401 Deviations: Test substance not characterised. Poorly described method and results, no necropsy. GLP: No **Supporting information** 10 Osborne-Mendel rats evenly divided by sex Guinea pig (male, female)	Thymol (20% in propylene glycol) Purity unknown (commercially available material) Oral by intubation Single dose Up to 14-day observation period	Rats: **Thymol LD_{50}: 980 mg/kg bw** Time of death: 4 h – 5 days. Depression, ataxia, coma on high doses Guinea pigs: **Thymol LD_{50}: 880 mg/kg bw** Time of death: 4 h – 5 days. Irritated gastro-intestinal tract, tremors, coma, respiratory failure *See table 18 for more details.*	Jenner, P.M. *et al.* (1964) (CA) B.6.2.1.1
Acute oral toxicity study in mice Publication on a review of acute toxicity studies. Deviations: Test	Thymol (highest purity available) in squalene (concentration not specified). At least 6 increasing dose	**Thymol LD_{50}: 1050 mg/kg bw (f)** LD_{50} = 1200 mg/kg bw (m) Hypoactivity and ataxic gait. Small intestinal congestion *See table 18 for more details.*	Hasegawa, R. *et al.* (1989) (CA) B.6.2.1.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
substance not characterised. Poorly described method and results GLP: Not stated **Supporting information** 10 Mice (ddY) /group Males and females	levels (not specified) 14-day observation period		
Acute oral toxicity review Publications: Review of published literature. Poorly described method and results **Supporting information** Mouse (male)	Thymol 1 to 4% aqueous solutions in cottonseed oil Gavage 620 to 2100 mg/kg bw	**LD_{50} = 1800 mg/kg bw** Depressed general condition. Death occurred within 48 h. Only depression at lower doses. Depression and prostration at higher doses. Gross pathology revealed haemorrhages in the small intestines and severe oedema and congestion of the lungs. McOmie *et al.* (1949) *See table 18 for more details.*	Escobar, A. (2006) (CA) B.6.2.1.3 BG Chemie, (2000) (CA) B.6.2.1.4
Acute oral toxicity review Publication: Review of published literature. Deviations: Test substances not characterised. Poorly described methods and results GLP: Not stated **Supporting information** Mouse	- Thymol in alcohol/propylene glycol/water - Thymol 10% in peanut oil - Thymol 10% in aqueous emulsion Gavage	- **LD_{50} = 640 mg/kg bw (alcohol/ propylene glycol/water)** Decrease in spontaneous movements, piloerection and paralysis of anterior limbs within 1h. Death occurred within 5 to 6 h - **LD_{50} between 1200 and 1300 mg/kg bw (peanut oil)** - **LD_{50} between 600 and 650 mg/kg bw (aqueous emulsion)** RIFM (2001e) *See table 18 for more details.*	Escobar, A. (2006) (CA) B.6.2.1.3
Skin sensitization assays (4): - Open Epicutaneous Test (OET) - Draize Test (DT) - Maximization test (GPMT) - Freud's Complete Adjuvant Test (FCAT) Prior to OECD **Supportive information** Guinea pigs, Himalayan 6 – 8 male and female/group.	Thymol up to 10%	Higher concentrations of thymol (*i.e.* >10%) were not tested due to **systemic toxicity**. The mentioned systemic toxicity was not described Negative results up to 10% in all four techniques *See table 36 for more details.*	Klecak *et al.* (1977) (CA) B.6.2.6.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Acute oral toxicity review Publication: Review of published literature. Deviations: Poorly described method and results **Supporting information** Mice (groups of 8)	- Thymol as a 10% aqueous emulsion - Thymol as a 10% peanut oil solution Oral route. No data on doses	- **LD_{50} = 650 mg/kg bw** (aqueous emulsion) - **LD_{50} = 1300 mg/kg bw** (peanut oil solution) Drowsiness and paralysis occurred after 2 to 5 minutes. At the later stages: muscle twitching and spasms. Deaths occurred after 6 to 12 hours. The survivors recovered slowly. Oelkers, (1940) *See table 18 for more details.*	BG Chemie, (2000) (CA) B.6.2.1.4
Acute (inhalation) toxicity review Publication: data provided to EPA as a waiver GLP: Not applicable **Supporting information** Dogs, cats and other animals	0.01% Thymol, added to the anaesthetic halothane (as a preservative) Used for periods that may exceed 4 h Concentrations typically around 5% anaesthetic	**LC_{50} > 5 mg/L (calculated)** Used in the mentioned conditions (calculated concentration of thymol ≈5 mg/L) without adverse effects *See table 18 for more details.*	US-EPA (2006) (CA) B.6.2.3
Mammalian CA (MN test in mouse PCEs of bone marrow) Not guideline specified. Some deviations from OECD TG 474 (2016). **Only supportive** Crj:BDF1 mice (♂, ♀)	Thymol (purity >98%, batch No. CAN1119). Vehicle: Pharmacopeia olive oil. Dosage: 156.3 – 2000 mg/kg bw (single oral dose by gavage).	Mortality: from 1500 mg/kg bw (♂, ♀). Clinical signs: reduced spontaneous activity from 500 mg/kg bw (♂, ♀). Staggering, abdominal position and respiratory stress "with increases in dose" (no doses detailed).	Shibuya, T. *et al.* (1996) CA B.6.4.2.1-01
Mammalian CA (CA test in bone marrow cells of rats) OECD TG 475 (1997). Some deviations from OECD TG 475 (2016). **Acceptable** Sprague-Dawley Crl:CD ® rats (♂, ♀).	Thymol (purity 99.7%, batch No. 103366). Vehicle: 1% methylcellulose. Range finding test: 1000 - 2000 mg/kg bw. Dosage (single oral dose by gavage) and sampling: 500 - 2000 mg/kg bw and sampling after 16 h, and 2000 mg/kg bw and sampling after 42 h.	Toxicity: Mortality at 2000 mg/kg bw: 1♀ at 42 h and 2♂, at 16 and 42 h. Ataxia and decreased activity in ♂/♀ from 1000 mg/kg bw. Piloerection and lethargy in ♂/♀ at 2000 mg/kg bw. Most of the effects were reversible.	[redacted] (2009) (CA) B.6.4.2.1-03
Acute intraperitoneal toxicity test in mice Publication: pharmacological effect of Essential oil of *Lippia grata*. Thymol was used for pharmacological	Commercial Thymol (not characterized) Intraperitoneal (i.p.) injection Doses: 33.3, 50.0, 73.3, 110.0, 166.6	Mortality and clinical sings: **Dose** mg/kg bw / **No. deaths/No. mice per group** / **Signs of toxicity** 33.3 / 0/3 / No effect 50.0 / 0/3 / Slight ataxia 73.3 / 1/3 / Ataxia, decreased spontaneous motor activity	Viana *et al.* (1981) (CA) B.6.8.2.1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>comparisons with Lippia-derived compounds (the actual material tested).
Supporting information
Swiss mice:
6 groups of
3 adults each
(sex at random)</td><td>and 233.3 mg/kg bw

3-d observation period</td><td><table>
<tr><td>110.0</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>166.6</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>233.3</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
</table>
-LOAEL (i.p.) = 50 mg/kg bw
-NOAEL (i.p.) = 33.3 mg/kg bw
-Target organs/tissues: CNS - Narcotic effects.
-Critical effect at the LOAEL: Ataxia.</td><td></td></tr>
<tr><td rowspan="3">Acute subcutaneous, intravenous and intraperitoneal toxicity review
Publication: Review of published literature. No data on the test guidelines followed in each study.
Deviations: Test substances not characterised. Poorly described methods and results
GLP: Not stated for any study

Supporting information</td><td>6 dogs
Thymol in oil
Doses: not indicated.
Perfusion (i.v.) for 20 to 25 min

Monitoring: blood pressure, respiration and survival</td><td>Clinical signs at the dose of 60 mg/kg bw, were convulsions and respiratory arrest.
Thymol LD_{50} in dog (i.v.) = 150 mg/kg bw

Caujolle and Franck (1944)</td><td rowspan="3">Escobar, A. (2006) (CA) B.6.8.2.2</td></tr>
<tr><td>Rats
Thymol in ethylene glycol
1 to 3 ml/100 g bw
Single subcutaneous (s.c.) injection</td><td>No convulsions were caused by the administration of thymol.
No additional information available

Matsumoto et al. (1963)</td></tr>
<tr><td>Mice (Swiss)
3 mice/test group
Thymol
Doses from 33.3 to 233.3 mg/kg bw
Intraperitoneal (i.p.) injections
Observation up to 3 days post-adm.</td><td>Effects:
33.3 mg/kg bw. No effects.
50 mg/kg bw: nonspecific effects and slight ataxia.
73.3 mg/kg bw: 1/3 died; ataxia, decreased spontaneous motor activity.
110 mg/kg bw: 3/3 died; ataxia, decreased spontaneous motor activity and somnolence. The same was observed in the 166.6 and 233.3 mg/kg bw groups.

Calculated Thymol LD_{50} in mice (i.p.) = 110 mg/kg bw. Actual testing of this dose resulted in the death of 3/3 animals in the first 3 days.
Viana et al. (1981)
(CA) B.6.8.2.1</td></tr>
</table>

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Published report: poisoning case	Mouthwash containing thymol (0.064%)	Fatal large-volume mouthwash ingestion in an adult. Ingestion of almost 3 litres of mouthwash by one patient (almost 1.92 g of thymol in the almost 7.74 g of non-alcoholic ingredients)	Cardiovascular collapse, multiorgan system failure and death. The patient presented a profound anion gap metabolic acidosis and a significant osmolar gap.	Soo Hoo. *et al.* (2003) (CA) B.6.9.3.2

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
			It was concluded that exposure to phenolic ingredients of the mouthwash (i.e. eucalyptol, menthol and thymol) as well as alcohol accounted for the adverse effects.	
Publication: Review of published literature	Halotane (inhalation anaesthetic) containing 0.01% thymol	Inhalation Postoperative "halothane hepatitis"	It's been suggested that thymol might play a causative role in very rarely occurring instances of postoperative "halothane hepatitis" Hutter and Laing (1993)	BG Chemie, (2000) (CA) B.6.9.2
Publication: Review of published literature	Cold remedy that contained thymol, in addition to menthol and other volatile oils.	Inhalation A 3 week-old infant inhaling the cold remedy.	The infant suffered a respiratory collapse. The authors considered it very unlikely that the collapse was attributable to the remedy. No further details Davis and Livingstone (1986).	BG Chemie, (2000) (CA) B.6.9.3.1

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Repeated dose and reproductive/ developmental study OECD TG (1990) SD rats (Crj:CD, SPF) 10/sex/group	Thymol (purity 99.6%) Oral - Gastric tube Preliminary test: 30, 100 and 300 mg/kg bw/day Main test: 0, 8, 40 and 200 mg/kg bw/day Males: 43 days Females: 14 days prior to mating and also during mating, gestation and lactation (females)	Preliminary (range-finding) study: 300 mg/kg bw/day: **reduction in spontaneous motor activity, ambulatory ataxia,** abdominal recumbency, bradypnea, ptosis and hypersalivation, as well as reduction in body-weight. Main study: *Clinical signs:* only noted in the 200 mg/kg bw/day groups: Females: 9/10: **Decrease in spontaneous motor activity from day 1**, just after administration and was observed for 10 days in 2 females, 3 days in 2 animals and 1 day in 5 females. 6/10: **Ataxic gait from day 1** (accompanying the ↓spontaneous motor activity)	*SYSTEMIC effects:* -LOAEL = 200 mg/kg bw/day -NOAEL = 40 mg/kg bw/day -Target organs/tissues: CNS/muscle and thymus. -Critical effect at the LOAEL: reduced motor activity and ataxia in females (attributed to pharmacological effects of thymol suppressing CNS or muscular contraction), and increased thymus weight (absolute and relative). *LOCAL effects:* -LOAEL = 40 mg/kg bw/day -NOAEL = 8 mg/kg bw/day -Target organs/tissues: Gastric effects (gavage), considered consistent with the irritant properties of thymol. -Critical effect at the LOAEL: histopathological changes in the forestomach of male and female rats. *See table 46 for more details.*	[redacted] (1996) (CA) B.6.3.1.1

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Most of the available data is considered as supportive information due to methodological and/or reporting deficiencies. However, effects of thymol after single exposure were described in several studies performed in different species and routes of administration.

In a pharmacokinetic study in mice ([redacted]., 2009; B.6.1.1.6), in the preliminary tests (single oral doses of 1250 and 1000 mg/kg bw, respectively) several effects were observed in all animals, which included ataxia, bradypnoea, prone posture, piloerection, decreased activity and ptosis. One male in the preliminary study at 1000 mg/kg bw dose also displayed loss of righting reflex and ptosis from 2 to 4 h after dose and one female displayed prone and pallor extremities from 15 min to 4h after dose. In the main study (single oral 1000 mg/kg bw dose), similar clinical

observations were reported: increased activity, piloerection, ataxia and decreased activity ranging from 1 to 8 h post-dose in both male and female animals. Additional symptoms included bradypnoea (1 male at 8 h), ataxia (4 males up to 2 h post dose and 6 females up to 4 h post dose) and both hunched posture and lethargy (1 male at 2 h post dose, 1 female at 4 h post dose).

Five acute oral toxicity studies described motor effects after single administration by oral route: ataxia, hipoactivity, depression, prostration, drowsiness, paralysis, muscle twitching, spams and decrease in spontaneous movements (Jenner, P.M. *et al.*, 1964; Hasegawa R. *et al.*, 1989; McOmie *et al.*, 1949; RIFM, 2001e and Oelkers, 1940). The available documents for these acute oral toxicity studies did not report the doses at which these effects were observed.

In two *in vivo* genotoxicity studies, the effects of thymol were observed after single oral administration by gavage. One of them was an *in vivo* micronucleus test in mice (Shibuya, T. et al., 1996) which showed reduced spontaneous activity from 500 mg/kg bw in both sexes, and staggering, abdominal position and respiratory stress "with increases in dose" (although this last data was not detailed). The other genotoxicity study was an *in vivo* chromosomal aberration test performed in rats [redacted]., 2009), fully acceptable, in which ataxia and decreased activity were found from 1000 mg/kg bw, and piloerection and lethargy from 2000 mg/kg bw. Most of the effects reported in rats were reversible and all of them were observed in both sexes, within 1 h after administration.

In a repeated-dose toxicity test by oral route in rats ([redacted], 1996) decrease in spontaneous motor activity and ataxic gait were also observed from day 1 of administration (at 200 mg/kg bw/day). However, these effects were transient.

Human data also includes information on acute oral toxicity of thymol: a poisoning case after ingestion of a large-volume of mouthwash. The exposure to the components eucalyptol, menthol and thymol, besides the alcohol, accounted for the adverse effects: cardiovascular collapse, multiorgan system failure, metabolic acidosis, profound anion gap and a significant osmolar gap (Soo Hoo. *et al.*, 2003).

Additionally, data on acute toxicity of thymol through other routes has been provided.

Available dermal toxicity data does not describe systemic adverse effects of thymol through this route, with the exception of a sensitization study (Klecak *et al.*, 1977) which indicated that the tested concentration did not exceed the 10% of thymol due to systemic toxicity. However, neither description nor any other data on the mentioned toxicity is reported.

Regarding the inhalation route in animal data, no adverse effects have been detected after the use of the anaesthetic halothane (containing 0.01% of thymol, as a preservative) in dogs, cats and other animals, during periods exceeding 4 hours at concentrations typically around 5% (calculated concentration of thymol ≈5 mg/L).

Two excerpts from inhalation toxicity cases in humans were reported in BG Chemie (2000): A concern on the possible causative role of thymol in the postoperative "halotane hepatitis" was suggested. However, this suggestion was published in 1993 and subsequent papers indicate that, although the exact mechanism of halothane-induced hepatotoxicity is unknown, there is strong evidence that the immune system mediates hepatitis-related mortality. This immune system mediation is related with the extremely reactive molecule trifluoroacetylchloride (TFA), which is generated by the reaction between lysine and halothane metabolites, yielding neo-antigens by binding to the hepatocyte macromolecules. These neo-antigens can provoke immune responses against hepatocytes and inducing fulminant hepatitis (Habibollahi P, *et al.,* 2011)[2].

BG Chemie (2000) also reported the respiratory collapse suffered by a 3-week-old infant after the inhalation of a cold remedy that contained thymol, in addition to menthol and other volatile oils. However, it could not be attributable to the remedy.

Additionally, intraperitoneal injections of thymol in mice, caused ataxia (50 mg/kg bw and above), decrease in spontaneous motor activity (73.3 mg/kg bw and above) and somnolence (110 mg/kg bw and above) (Viana *et al.,* 1981).

Lastly, intravenous perfusion of thymol in oil caused convulsions and respiratory arrest in dogs (Caujolle and Franck, 1944), but subcutaneous injection in rats did not cause convulsions (Matsumoto *et al*., 1963).

Altogether, two effects are considered relevant in the assessment of the STOT SE hazard class of thymol. On one hand, the narcotic effects observed, like drowsiness, ataxia, reduction of the motor activity or somnolence, were observed in several oral studies in rats and mice, and one intraperitoneal study in mice. In the oral repeated-dose toxicity study ataxia and reduction of the motor activity started from the first day of administration and were described as transient. Therefore, these narcotic effects should be assessed as category 3.

[2] Habibollahi P, Mahboobi N, Esmaeili S, Safari S, Dabbagh A, Alavian SM. Halothane-induced hepatitis: A forgotten issue in developing countries: Halothane-induced hepatitis. *Hepat Mon*. 2011 Jan;11(1):3-6.

On the other hand, available inhalation information (human and animal data) is derived from mixtures containing thymol (0.01% in halothane and a cold remedy containing thymol, in a concentration not specified). Since thymol is classified as skin corrosive (Skin Corr. 1B; H314), and no acceptable data is available on the inhalation of the technical substance, it is considered necessary to assess also the potential irritative effects of thymol in the respiratory tract.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

According to the current EU Criteria (Regulation (EC) No 1272/2008), the criteria for classifying substances as Category 3 for narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

Available animal data on thymol described clinical signs like drowsiness, ataxia, reduction of the motor activity or somnolence, and were considered transient. Therefore, classification of thymol as Category 3 for narcotic effects (STOT SE 3; H336) is considered justified.

Regarding the potential irritative effect in the respiratory tract, no inhalation toxicity study with thymol (technical grade) is available, and no human data on possible respiratory tract irritation effects has been reported. Therefore, the criteria for the classification as STOT SE 3 (H335) is not fulfilled, and no classification is proposed for this hazard class.

However, thymol is classified in Annex VI of Regulation (EC) No 1272/200 as skin corrosive (Skin Corr. 1B; H314), and the supplementary labelling of thymol as **EUH071 "corrosive to the respiratory tract"** is proposed: in addition to its classification for skin corrosivity, and since no inhalation toxicity study is available.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Based on the available data on the active substance, and according to the criteria under Regulation (EC) No 1272/2008, thymol is classified as **STOT SE (specific target organ toxicity-single exposure), as category 3 for narcotic effects, STOT SE 3 (H336).**

Data available indicates that thymol does not require classification for STOT SE 3; H335. However, RMS deems the supplementary labelling of thymol as **EUH071 "corrosive to the respiratory tract"** is justified.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any/Acceptability, Species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Repeated dose and reproductive/ developmental study GLP: Yes OECD TG (1990) Deviations: Historical Control Data not provided.	Thymol (purity 99.6%) Oral -Gastric tube Preliminary test: 30, 100 and 300 mg/kg bw/day Main test: 0, 8, 40 and 200 mg/kg bw/day	<u>Preliminary (range-finding) study:</u> 10 mg/kg bw/day: no effects observed. 100 mg/kg bw/day: only salivation was noted. 300 mg/kg bw/day: reduction in spontaneous motor activity, ambulatory ataxia, abdominal recumbency, bradypnea, ptosis and hypersalivation, as well as reduction in body-weight. <u>Main study</u>: *For results on fertility toxicity, see Table 57* *For results on developmental toxicity, see Table 60* *For results on effects on or via lactation, see Table 63*	[redacted] (1996) (CA) B.6.3.1.1

Method, guideline, deviations if any/Acceptability, Species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
SD rats (Crj:CD, SPF) 10/sex/group **Supporting information**	Males: 43 days Females: 14 days prior to mating and also during mating, gestation and lactation (females)	*Mortality:* One male of the 200 mg/kg bw/day died the last day of the study. Also one female of the high dose died on gestation day 18 (day 33 of administration) due to an error during administration. *Clinical signs:* only noted in the 200 mg/kg bw/day groups: Males: All males showed transient salivation following dose administration, from approximately 13 – 15 days after the start of the study. Females: 5/10 females showed transient salivation following dose administration, from gestation day 0. 9/10: Decrease in spontaneous motor activity from day 1, just after administration and was observed for 10 days in 2 females, 3 days in 2 animals and 1 day in 5 females. 6/10: Ataxic gait from day 1 (accompanying the ↓spontaneous motor activity) *Body weight*: Tendency for the suppression of BWG was observed in males of the 200 mg/kg bw/day from day 14 of administration. Differences were not statistically significant. *Haematology (males only)*: ↑lymphocytes within the differential leukocyte counts, statistically significant in all treated groups as percentage values, but not statistically significant as absolute values. ↓monocytes (slight) within the differential leukocyte counts, statistically significant in the 40 and 200 mg/kg bw/day dose groups as percentage values but only statistically significant, as absolute values, in the 40 mg/kg bw/day dose group. Differences described in the study report as being within the laboratory background data (not provided). *Clinical chemistry (males only)*: ↓triglycerides statistically significant in the 8 mg/kg bw/day group. No significant change in the 40 and 200 mg/kg bw/day dose groups. *Organ weights*: No significant differences between the control group and any of the test substance groups for either absolute or relative organ weights of both males and females. However, a trend could be seen in the absolute thymus weight of males, reaching a ↑ 9.8% and ↑15.3% for the absolute and relative weights, respectively (compared to controls), in the 200 mg/kg bw/day dose group. *Gross necropsy and histopathology*: Thickening of the forestomach wall (proventricular wall) was observed in 9 males and 1 female in the 200 mg/kg bw/day group. This finding was confirmed at histopathological examination as hyperplasia of the mucosal epithelium (hyperplasia of the stratified squamous epithelium and accelerated keratinisation), which was observed also in males and females in the 40 mg/kg bw/day group. In some cases it was accompanied by infiltration of inflammatory cells and oedema. Reduction of the size of the thymus was observed in 1 female of the 400 mg/kg bw/day and 1 female of the 200 mg/kg bw/day dose group. This finding was confirmed in both females, showing slight thymic involution in the histopathological examination. Whitening of the adrenal was observed in 2 females in the 200 mg/kg bw/day group, confirmed histopathologically in one of the two females, by the presence of an increase of fatty droplets in the fascicular zone of the adrenal. Findings in other organs and tissues were considered incidental (also observed in control animals).	

Method, guideline, deviations if any/Acceptability, Species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		LOCAL effects: -**LOAEL**(oral) = 40 mg/kg bw/day -**NOAEL**(oral) = 8 mg/kg bw/day -**Target organs/tissues**: Gastric effects (gavage), considered consistent with the irritant properties of thymol. -**Critical effect at the LOAEL**: histopathological changes in the forestomach of male and female rats. ***SYSTEMIC effects:*** -**LOAEL**(oral) = 200 mg/kg bw/day -**NOAEL**(oral) = 40 mg/kg bw/day -**Target organs/tissues**: CNS/muscle and thymus. -**Critical effect at the LOAEL**: reduced motor activity and ataxia in females (attributed to pharmacological effects of thymol suppressing CNS or muscular contraction), and increased thymus weight (absolute and relative).	
19-week feed study Publication: prior to OECD Test Guidelines Deviations: Test substance not characterised. Poorly described method and results. GLP: No (prior to GLP enforcement) **Supporting information** Weanling Osborne-Mendel rats 10/sex/group	Commercially available thymol used as food additive (purity: unknown) Dietary, 0, 1000 and 10000 ppm, for 19 weeks (equivalent to 0, 90 and 900 mg/kg bw/day).	Very limited report, only the effects believed to be due to the administration of the compound were listed in a single entry of a summary table. No effects on growth or haematology and no macroscopic nor microscopic changes were observed in the high dose group. -**NOAEL** (oral) = 10000 ppm (~900 mg/kg bw/day)	Hagan E.C., *et al*. (1967) (CA) B.6.3.2.1
Repeated-dose subcutaneous and intravenous toxicity review Publication: Review of published literature. No data on the test guidelines followed in each study. Deviations: Test substances not characterised. Poorly described methods and results GLP: Not stated for any study **Supporting information**	Guinea pigs 4 male Thymol in olive oil Doses: 106 or 233 mg/kg bw/day (20 or 40 mg/day, respectively) Subcutaneous (s.c.) dosing for 8 to 9 d	Clear thyroid activation was seen in 2 animals and weakly in a third animal No effect was seen in the oxygen consumption in any animal. - Insufficient information to establish NOAEL or LOAEL. -**Target organs/tissues**: Thyroid gland. -**Critical effect at the LOAEL**: thyroid activation. Möller (1939) Also in BG Chemie,2000 -B.6.8.2.3	Escobar, A. (2006) (CA) B.6.8.2.2
	Guinea pigs 4 males Thymol in olive oil Dose: 243, 313 and 375 mg/kg bw/day (60, 80 and 100 mg/day, respectively) 9 injections (s.c.) over 16 d	Thyroid activation: after 2 injections at 243 mg/kg bw/day; after 3 injections at 313 mg/kg bw/day and after 4 injections at 375 mg/kg bw/day No effect was seen in the oxygen consumption in any animal. -**LOAEL**(s.c) = 243 mg/kg bw/day -Not NOAEL can be established -**Target organs/tissues**: Thyroid gland. -**Critical effect at the LOAEL**: thyroid activation. Möller (1939) Also in BG Chemie,2000 -B.6.8.2.3	
	Mice (A/He5)	Maximum tolerated dose (MTD) of all five mice = 0.25 g/kg	

Method, guideline, deviations if any/Acceptability, Species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
	Males and females Thymol Serial two-fold dilutions 6 injections over 2 weeks Via and vehicle not specified	Stoner *et al.* (1973)	
	Mice (SPF) 5 males Thymol Vehicle not specified 6 to 8 doses intravenously (i.v.)	Thymol LD_{50} in mice (i.v.) = 100 mg/kg bw James and Glen (1980)	
Repeated-dose subcutaneous toxicity review Publication: Review of published literature. Deviations: Poorly described method and results **Supporting information**	Guinea pigs (males) Thymol Doses: 20 to 100 mg/animal/day Subcutaneous (s.c.) dosing for 8 to 9 d	Animals showed histological marked "activation" of the thyroid gland without any increase in oxygen consumption. According to the investigator, signs of activation included proliferation of the interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes. - Insufficient information to establish NOAEL or LOAEL. -**Target organs/tissues**: Thyroid gland. -**Critical effect at the LOAEL**: activation of the thyroid gland (proliferation of interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes). Möller (1939) Also in Escobar A, 2006 -B.6.8.2.2	BG Chemie, (2000) (CA) B.6.8.2.3

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Publication: Review of published literature	Mouthwash containing thymol	Three male patients following the use of the mouthwash for periods of 6 months – 3 years	Thyroid intoxication: weight loss, tremors, restlessness, sleeplessness and diarrhoea. Upon cessation of the use of the mouthwash the patients showed recovery and weight gain. Edens (1937)	BG Chemie, (2000) (CA) B.6.9.3.1

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.3.2 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Available animal data on thymol is considered as supportive information due to methodological and/or reporting deficiencies. However, effects of thymol after repeated exposure were described in several studies performed in different species and routes of administration.

In a repeated-dose toxicity test by oral route (gastric tube) in rats ([redacted] 1996), salivation was noted in the

preliminary study with 300 mg/kg bw/day and in the main study with the high dose (200 mg/kg bw/day). The onset of this clinical sign was 13 to 15 days after the start of the study in males, and from the beginning of gestation in females. Salivation was considered related with the irritant effect of thymol in the upper gastrointestinal tract, which was confirmed at necropsy (thickening of forestomach wall) and with histopathological changes in the forestomach of male and female rats (from 40 mg/kg bw/day). Therefore, this local effect is covered by the current classification of thymol as corrosive (Skin. Corr. 1B; H314).

Systemic effects have also been described in this study of [redacted] 1996. Transitional decrease in spontaneous motor activity and ataxic gait were observed from the first day of administration and, therefore, these effects have been addressed in the previous point (2.6.2.10 for STOT SE). Additionally, an increase in thymus absolute and relative weights of males was also described and, although not significant, the trend reached a 9.8% increment for the absolute weight and a 15.3% increase for the relative thymus weight in the 200 mg/kg bw/day male dose group.

In the case of females, reduction of the size of the thymus was observed in two animals (dosed with 40 or 200 mg/kg bw/day, each), which was confirmed in the histopathological examination with slight thymic involution. However, these effects were not considered statistically significant nor increased with the dose.

The other available study with oral administration of thymol in rats consisted in a 19-week feed toxicity study (Hagan E.C., *et al*., 1967), where no effects on growth or haematology, and no macroscopic nor microscopic changes were observed after administration of 10000 ppm (~900 mg/kg bw/day) of thymol. It should be noted that this study specifies that the studied material was commercially available thymol (used as food additive), "rather than pure chemicals, since the purpose of these studies was to evaluate the toxicity of these materials in relation to their use as food additives".

Additionally, human data reports thyroid intoxication of three male patients following the use of the mouthwash containing thymol for periods of 6 months to 3 years, (with weight loss, tremors, restlessness, sleeplessness and diarrhoea), showing recovery and weight gain upon cessation of the use of the mouthwash.

Regarding other routes relevant to humans (i.e. principally oral, dermal or inhalation, according to Regulation CLP), no more data is available. However, since thyroid effects were observed in human cases, it should be noted that thyroid effects were also observed after repeated subcutaneous administration of thymol, as described in the study by Möller, 1939 (included in both publications: Escobar A, 2006 and BG Chemie, 2000). Putting together the available data, thymol was subcutaneously administered for 8 to 9 days at doses ranging from 20 to 100 mg/animal day (equivalent to 106 to 375 mg/kg bw/day), resulting in thyroid activation without an increase in oxygen consumption. According to BG Chemie (2000), Möller's report described the thyroid activation as a proliferation of interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes. In Escobar (2006) report it was also reported another experiment with thymol after subcutaneous administration with 9 injections at 16 days with thyroid activation at doses greater than 243 mg/kg bw/day.

These effects on thyroid were described in an old study (Möller 1939) or in human cases after the use of mouthwash containing thymol (no clear relationship with thymol can be derived). Since thyroid effects were not addressed in the repeated dose and reproductive toxicity study of [redacted], 1996 (which is the available report that gives more data on thymol after repeated administration), the information available does not allow confirming or ruling out the possible effect of thymol in the thyroid gland. Therefore, a concern on this field should be raised up.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
[redacted] (1996) (CA) (B.6.3.1.1) (B.6.6.1.1)	200 (oral) Systemic effect (thymus weight)	43 d	95 mg/kg bw/day	None

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Möller (1939) (CA) (In B.6.8.2.2: Escobar A, 2006 and B.6.8.2.3: BG Chemie, 2000)	233 (subcutaneous administration)	9 d	Not applicable	-
Möller (1939) (CA) (In B.6.8.2.2: Escobar A, 2006)	243 (subcutaneous administration)	16d	Not applicable	-

2.6.3.3 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Available data on thymol does not provide substantial evidence of specific target organ toxicity after repeated exposure.

Systemic effects produced after 43-day repeated dose of thymol consisted in an increase of thymus weight in males (dose-related but not significant) and decrease in spontaneous motor activity and ataxic gait in females, as described in [redacted] 1996. These effects were observed in the high dose group (200 mg/kg bw/day). Since motor effects were observed from the first day of administration, these effects have been addressed in the previous point (2.6.2.10 for STOT SE). When extrapolated to 90-day exposure (see table 49), the effective dose for the observed thymic effect (approximately 95 mg/kg bw/day) is below the guidance dose of 100 mg/kg bw/day, established in Regulation CLP for the classification as STOT RE category 2 for the oral route. However, this value is very closed to the limit and it should be noted that the weight changes found in thymus of the highest dose males cannot be confirmed by histopathology, since it was only examined in female groups.

As commented above, thyroid effects were observed in a very old study where thymol was injected subcutaneously for 9 days in guinea pigs (Möller, 1939). In another reference to this study thyroid activation was seen at doses greater than 243 mg/kg bw/day after 9 injections administered in a period of 16 days. This is not a relevant route for humans, but is the only available animal data on thyroid effects of thymol. Since a human report described three cases of thyroid effects after the use of a mouthwash containing thymol, the single data available in animals has been included in the weight of evidence. The thyroid effect was observed at a dose of 233 mg/kg bw/day after subcuteanous administrarion. Considering the subcutaneous route of administration, a comparison to STOT RE cut-off criteria cannot be performed.

2.6.3.4 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Data available indicates that thymol does not require classification for STOT RE (specific target organ toxicity-repeated exposure).

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation (Ames test, plate incorporation) GLP: No Not guideline specified. Some deviations from OECD TG 471 (2020). **Only supportive**	**Thymol** (purity >99%, unknown batch No.)	Test system: *S. typhimurium* TA98 and TA100. Metabolic activation: Rat liver S9 induced by phenobarbitone. Dosage: 6.25, 12.50 and 25 µL of 0.1 M thymol/plate (eq. to 94, 188 and 375 µg/plate) (±S9). Solvent: ethanol (85%) Dose selection: Cytotoxicity pre-test.	Mutagenicity: Negative. Cytotoxicity: Not observed.	Stammati, A., *et al.* (1999) **B.6.4.1.1-01 (AS)**

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation (Ames test, pre-incubation method) GLP: No Not guideline specified. Some deviations from OECD TG 471 (2020). **Not acceptable**	**Thymol** (unknown batch No. and purity)	Test system: *S. typhimurium* TA97, TA98 and TA100. Metabolic activation: S9 (no further information). Dosage: not reported but indicated to be non-toxic. Solvent: DMSO.	Mutagenicity: Negative.	Azizan, A. and Blevins, R.D. (1995) **B.6.4.1.1-02 (AS)**
Bacterial gene mutation (Ames test) GLP: No Not guideline specified. Some deviations from OECD TG 471 (2020). **Only supportive**	**Thymol** (purity ≥97%, unknown batch No.)	Test system: *S. typhimurium* TA98, TA100, TA1535 and TA1537. Metabolic activation: Rat liver S9 induced by Aroclor 1254 or 3-methylcholanthrene. Dosage: 450 µg/plate (±S9). Solvent: Ethanol	Mutagenicity: Negative. Cytotoxicity: Not observed.	Florin, I., *et al.* (1980) **B.6.4.1.1-03 (AS)**
Bacterial gene mutation (Ames test, plate incorporation) GLP: No Not guideline specified. Some deviations from OECD TG 471 (2020). **Acceptable**	**Thymol** (purity >98%, batch No. CAN1119)	Test system: *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA.* Metabolic activation: Rat liver S9 induced by phenobarbital and 5,6-benzoflavone. Dosage: 15.6-500 µg/plate (for all strains, -S9), 62.5-2000 µg/plate (for TA98, TA100 and WP2 *uvrA*, +S9) and 31.3-1000 µg/plate (for TA1535 and TA1537, +S9). The same dosage in two experiments. Solvent: DMSO. Dose selection: Cytotoxicity pre-test at a range of 50-5000 µg/plate.	Mutagenicity: Negative. Cytotoxicity: -S9: All strains from 500 µg/plate, but TA1537 in exp. I from 250 µg/plate. +S9: TA98, TA100 and WP2 *uvrA* from 1000 µg/plate, and TA1535 and TA1537 from 500 µg/plate in exp. I. WP2 *uvrA* from 2000 µg/plate, TA98 and TA100 from 1000 µg/plate, and TA1535 and TA1537 from 500 µg/plate in exp. II.	Shibuya, T. *et al.* (1996) **B.6.4.1.1-04 (AS)**
Bacterial gene mutation (Ames test, plate incorporation) GLP: No OECD TG 471 (1997). Some deviations from OECD TG 471 (2020). **Only supportive**	**Thymol** (purity >99.5%, unknown batch No.)	Test system: *S. typhimurium* TA97A, TA98, TA100, TA102 and TA104. Metabolic activation: Rat liver S9 induced by Aroclor 1254. Dosage: 15.6, 31.3, 62.5, 125 and 250 µM (±S9). Solvent: DMSO. Dose selection: Cytotoxicity pre-test with human cell line.	Mutagenicity: Negative. Cytotoxicity: No observed.	Llana-Ruiz-Cabello, M. *et al.* (2014) **B.6.4.1.1-05 (AS)**
Mammalian cell gene mutation (HPRT test) GLP: Yes OECD TG 476 (1997) Some minor deviations from OECD TG 476 (2016). **Acceptable**	**Thymol** (purity 99.7%, batch No. 103366)	Test system: L5178Y $tk^{+/-}$ (3.7.2C) mouse lymphoma cells. Metabolic activation: Rat liver S9 induced by Aroclor 1254. Dosage: *Exp.1:* -S9: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 µg/mL. +S9: 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140 µg/mL. *Exp. 2:* -S9: 10, 20, 40, 50, 60, 70, 80, 90, 100, 125 µg/mL. +S9: 10, 20, 40, 50, 60, 70, 80, 90, 100, 110, 125, 140 µg/mL. Solvent: DMSO.	Mutagenicity: Negative. Cytotoxicity: *Exp.1:* -S9: from 80 µg/mL (RS = 24% compared to control) +S9: from 105 µg/mL (RS = 15% compared to control) *Exp. 2:* -S9: from 90µg/mL (RS = 16% compared to control) +S9: from 110 µg/mL	Lloyd, M. (2011) **B.6.4.1.2-01 (AS)**

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
		Dose selection: A preliminary cytotoxicity test was performed using 1600 µg/mL as the highest concentration.	(RS = 20% compared to control)	
Mammalian cell gene mutation (TK test) GLP: No Not guideline specified. Some deviations from OECD TG 490 (2016). **Only supportive**	**Thymol** (purity >99.5%, unknown batch No.)	Test system: L5178Y $tk^{+/-}$ mouse lymphoma cells. Dosage: 7.8, 15.65, 31.25, 62.5, 125 and 250 µM (-S9) in exp. 1 and 2. Solvent: DMSO. Dose selection: Cytotoxicity pre-test.	Mutagenicity: Negative. Cytotoxicity: Based on RTG: not observed at the doses tested.	Maisanaba, S., *et al.* (2015) **B.6.4.1.2-02 (AS)**
Mammalian cell CA (cytogenetic test in SHE cells) GLP: No Not guideline specified. Some deviations from OECD TG 473 (2016). **Only supportive**	**Thymol** (purity >98%, unknown batch No.)	Test system: Syrian hamster embryo (SHE) cells. Metabolic activation: Rat liver post-mitochondrial supernatant. Dosage: 130, 260, 390 and 520 µM (-S9), and 130, 260 and 390 µM (+S9). Solvent: DMSO.	Mutagenicity: Negative (-S9) Positive (+S9) Cytotoxicity: Based on relative colony forming efficiency: from 520 µM (-S9). Not tested +S9.	Hikiba, H., *et al.* (2005) **B.6.4.1.3-01 (AS)**
Mammalian cell CA (cytogenetic test in Chinese hamster cells) GLP: No OECD TG 473 Some deviations from OECD TG 473 (2016). **Only supportive**	**Thymol** (purity >98%, unknown batch No.)	Test system: CHL/IU cells of Chinese hamster. Metabolic activation: Rat liver S9 induced by phenobarbital and benzoflavone. Dosage: 0.02, 0.04 and 0.08 mg/mL (24 and 48 h, -S9; 6 h, ±S9). Solvent: DMSO. Dose selection: Cytotoxicity pre-test.	Mutagenicity: Negative (only positive in the 6 h +S9 test with gaps included at 0.08 mg/mL). Cytotoxicity: No parameters. Reported at 0.08 mg/mL (in 24 and 48 h –S9 and 6 h –S9).	Tanaka, N., *et al.* (1996) **B.6.4.1.3-02 (AS)**
Mammalian cell CA (cytogenetic test in human lymphocytes) GLP: No IPCS guidelines. Some deviations from OECD TG 487 (2016). **Acceptable**	**Thymol** (purity >99.6%, unknown batch No.)	Test system: Human peripheral lymphocytes from whole blood cells. Dosage: 25, 50, 75 and 100 µg/mL (24 and 48 h, -S9). Solvent: DMSO.	Mutagenicity: Positive Cytotoxicity: Reported at 100 µg/mL for both treatments.	Buyukleyla, M. and Rencuzogullari, E. (2009) **B.6.4.1.3-03 (AS)**
Mammalian cell CA (cytogenetic test in human dental pulp cells) GLP: No OECD TG 473. Some deviations from OECD TG 473 (2016). **Only supportive**	**Thymol** (purity >98%, unknown batch No.)	Test system: Human dental pulp cells (D824 cells). Dosage: 3 or 30 h at 30, 100 and 300 µM. Solvent: DMSO. Dose selection: Cytotoxicity pre-test.	Mutagenicity: Equivocal. Cytotoxicity: Reported ≥ 50% of control in all CA experiments (no parameter specified).	Someya, H., *et al.* (2008) **B.6.4.1.3-04 (AS)**
Mammalian cell CA (MN test) GLP: No IPCS guidelines. Some deviations from OECD TG 487 (2016). **Acceptable**	**Thymol** (purity >99.6%, unknown batch No.)	Test system: Human peripheral lymphocytes from whole blood cells. Dosage: 25, 50, 75 and 100 µg/mL (24 and 48 h, -S9). Solvent: DMSO.	Mutagenicity: Positive (in the 24 and 48 h, -S9 test). Cytotoxicity: Not observed at the tested doses.	Buyukleyla, M. and Rencuzogullari, E. (2009) **B.6.4.1.3-05 (AS)**

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Mammalian cell CA (MN test) GLP: No OECD TG 487 (2010). Some deviations from OECD TG 487 (2016). **Only supportive**	**Thymol** (purity >99.5%, unknown batch No.)	Test system: L5178Y *tk*$^{+/-}$ mouse lymphoma cells. Metabolic activation: S9. Dosage: 15.62, 31.25, 62.5, 125 and 250 µM (24 h, -S9; 4 h, +S9). Solvent: DMSO.	Mutagenicity: Negative. Cytotoxicity: Not observed at the tested doses.	Maisanaba, S., *et al.* (2015) **B.6.4.1.3-06 (AS)**
Mammalian DNA damage (SCE test in human peripheral lymphocytes) GLP: No IPCS guidelines. Some deviations from OECD TG 479 (1986), deleted in 2014. **Supportive only**	**Thymol** (purity >99.6%, unknown batch No.)	Test system: Human peripheral lymphocytes from whole blood cells. Dosage: 25, 50, 75 and 100 µg/mL (24 and 48 h, -S9). Solvent: DMSO.	Genotoxicity: Positive (24 and 48 h, -S9). Cytotoxicity: At 100 µg/mL in the 48- h test, there was a reduction in the RI.	Buyukleyla, M. and Rencuzogullari, E. (2009) **B.6.4.1.4-01 (AS)**
Mammalian DNA damage (Comet assay in human colon intestinal cells) GLP: No Not guideline specified. **Supportive only**	**Thymol** (purity >99.5%, unknown batch No.)	Test system: Human colon intestinal cell line Caco-2. Dosage: 62.5, 125 and 250 µM (24 and 48h, -S9). Solvent: DMSO.	Genotoxicity: Negative	Llana-Ruiz-Cabello, M. *et al.* (2014) **B.6.4.1.4-02 (AS)**
Mammalian DNA damage (Comet assay in human lymphocytes) GLP: No Not guideline specified. **Supportive only**	**Thymol** (unknown batch No. and purity)	Test system: Human lymphocytes. Dosage: 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, 1 and 2 mM (0.5 h, -S9). Solvent: DMSO.	Genotoxicity: Positive (increased DNA damage after treatment with thymol from 0.2 mM).	Aydin, S. *et al.* (2005) **B.6.4.1.4-03 (AS)**

CA Chromosomal Aberrations; MN Micronucleus; RS relative survival; SCE sister chromatid exchange MI mitotic index; RI replication index, RTG relative total growth.

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Mammalian CA (MN test in mouse bone marrow) GLP: No Not guideline specified. Some deviations from OECD TG 474 (2016). **Acceptable**	**Thymol** (purity >98%, batch No. CAN1119)	Test system: Crj:BDF1 mice (♂, ♀). Dosage: 156.3, 312.5, 625 and 1250 mg/kg bw (single oral dose by gavage). Vehicle: Pharmacopeia olive oil. Sampling: 24 h after administration. Preliminary toxicity test: performed using 2000 mg/kg bw as the highest concentration. Preliminary MN test: performed using 1250 mg/kg bw with sample times of 24, 48 and 72 h.	Mutagenicity: Negative. Toxicity: Mortality from 1500 mg/kg bw (♂, ♀). Clinical signs: reduced spontaneous activity from 500 mg/kg bw (♂, ♀). Staggering, abdominal position and respiratory stress "with increases in dose" (no doses detailed). Cytotoxicity: Not observed.	Shibuya, T. *et al.* (1996) **B.6.4.2.1-01 (AS)**
Mammalian CA (CA test in bone marrow cells of rats)	**Thymol** (purity >99.6%,	Test system: Sprague-Dawley rats (♂, ♀).	Mutagenicity:	Azirak, S. and Rencuzogullari, E. (2008)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
GLP: No OECD TG 475 (1997). Some deviations from OECD TG 475 (2016). **Only supportive**	unknown batch No.)	Dosage: 40, 60, 80 and 100 mg/kg bw (a single i.p. dose). Vehicle: DMSO. Sampling: 6, 12 and 24 h after administration.	Positive in all tested doses and all sampling times. Toxicity: Not reported. Cytotoxicity: Decreased MI in all tested doses and all sampling times.	**B.6.4.2.1-02 (AS)**
Mammalian CA (CA test in bone marrow cells of rats) GLP: Yes OECD TG 475 (1997). Some deviations from OECD TG 475 (2016). **Acceptable**	**Thymol** (purity 99.7%, batch No. 103366)	Test system: Sprague-Dawley Crl:CD ® (SD) rats (♂, ♀). Dosage and sampling: single oral dose by gavage with 500, 1000 and 2000 mg/kg bw and sampling after 16 h, or 2000 mg/kg bw and sampling after 42 h. Vehicle: 1% methylcellulose. Preliminary toxicity test: performed with 1000, 14000 and 2000 mg/kg bw. Bioanalysis: of thymol in plasma at 2, 4 and 24 h in ♂, ♀ at 2000 mg/kg bw.	Mutagenicity: Negative. Toxicity: Mortality at 2000 mg/kg bw: 1♀ at 42 h and 2♂, at 16 and 42 h. Ataxia and decreased activity in ♂/♀ from 1000 mg/kg bw. Piloerection and lethargy at 2000 mg/kg bw. Most of the effects were reversible. Cytotoxicity: Decreased MI in ♀, 42 h. Bone marrow exposure: Thymol detected in plasma in ♂, ♀ at all sampling times. Peak at 8 h/2 h (♂/♀).	[redacted] (2009) **B.6.4.2.1-03 (AS)**

CA Chromosomal Aberrations; MN Micronucleus; MI mitotic index;

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data relevant for genotoxicity were available				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

- Genotoxicity potential of thymol has been assessed by *in vitro* (gene mutations in bacteria and mammalian cells, chromosomal aberrations in mammalian cells and DNA damage in mammalian cells) and *in vivo* studies in somatic cells (chromosomal aberrations). Thirteen studies were already considered in the previous DAR (2011) and were provided by the applicant for the renewal, and six studies were newly provided for this renewal.

- Five bacterial gene mutation studies were presented, none of them were GLP compliant and one of them was considered as not acceptable due to the relevant deficiencies. In all of them, thymol was negative in the Ames test (Vol.3 B.6.4.1.1-01, B.6.4.1.1-02, B.6.4.1.1-03, B.6.4.1.1-04 and B.6.4.1.1-05).

- Two *in vitro* mammalian cell gene mutation tests with thymol were included, one of them GLP compliant (Vol.3 B.6.4.1.2-01 and B.6.4.1.2-02). Both tests yielded a negative result.

- Regarding chromosomal aberrations *in vitro*, six tests were assessed, four chromosomal aberration tests (Vol.3 B.6.4.1.3-01, B.6.4.1.3-02, B.6.4.1.3-03 and B.6.4.1.3-04) and two micronucleus tests (Vol.3 B.6.4.1.3-05 and B.6.4.1.3-06). None of them were GLP compliant. Contradictory results were observed in these tests both with and without metabolic activation. In one of the *in vitro* chromosomal aberration tests, thymol was positive in the absence of metabolic activation, in one was equivocal in the absence of metabolic activation, in one was negative and in other one was positive in the presence of metabolic activation. In one of the *in vitro* micronucleus tests a positive result was obtained in the absence of metabolic activation and a negative result was observed in the other one.

- Three *in vitro* DNA damage studies with human cells were presented, none of them GLP compliant. Thymol

was positive in a SCE test performed without metabolic activation (Vol.3 B.6.4.1.4-01). Moreover, two *in vitro* comet assays were provided: one of them was negative (Vol.3 B.6.4.1.4-02) and the other one was positive (Vol.3 B.6.4.1.4-03).

- *In vivo* studies were submitted about chromosomal aberrations with thymol. A non-GLP *in vivo* micronucleus test in bone marrow (Vol.3 B.6.4.2.1-01) showed a negative response. A non-GLP *in vivo* chromosomal aberrations test was presented, in which a positive result was obtained in bone marrow, after intraperitoneal dosing of thymol (Vol.3 B.6.4.2.1-02). However, a negative result was observed in a GLP compliant *in vivo* chromosomal aberration test in bone marrow, in which thymol was dosed orally by gavage and was found in plasma as a proof of target organ exposure (Vol.3 B.6.4.2.1-03).

Based on the results of the available studies evaluated in this RAR:

- No indications of *in vitro* gene mutation were observed in bacteria and mammalian cells. Overall, these data showed that thymol was not likely to induce gene mutations. This opinion was based on the acceptable bacterial gene mutation test available that was negative (B.6.4.1.1-04) and supported by three additional supportive bacterial gene mutation tests, also with negative outcomes.

- The induction of structural chromosomal aberrations by thymol was suggested in several *in vitro* studies. The acceptable *in vivo* chromosomal aberration study was considered by the RMS as enough to follow up these *in vitro* concerns. Overall, thymol was not likely to be clastogenic.

- To analyse aneugenicity in thymol *in vitro* supportive data about numerical chromosome aberrations showed contradictory results but an *in vivo* acceptable micronucleus study was negative. Therefore, the RMS considered that thymol was not likely to be aneugenic.

In summary, with the available dataset, thymol is not of genotoxic concern.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

No human data are available for thymol, therefore a classification as Muta. 1A is not supported. There are no data from in vivo heritable germ cell mutagenicity tests showing mutagenic effects in germ cells of humans therefore a classification as Muta. 1B is precluded. The weight of evidence of the available genotoxicity data from *in vitro* and *in vivo* assays indicates that thymol is of no genotoxic concern. Therefore, with the data currently available, no classification for mutagenicity under the CLP regulation is required.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

No classification for genotoxicity/germ cell mutagenicity is proposed by the RMS with the available information.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
No animal studies on long-term toxicity and carcinogenicity were available			

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on long-term toxicity and carcinogenicity were available				

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
REACH DATA Carcinogenicity in mouse Reliability 2	Thymol	Mice received ip injections of 50 or 250 mg/kg bw, for 8 weeks, 3 times weekly and were killed at 24 weeks after the 1st injection.	Thymol was negative for lung tumour induction in A mice.	Stoner GD, (1973) "Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice". (REACH registration dossier data not provided by applicant and not assessed by the RMS)

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

No long-term toxicity or carcinogenicity data are available for thymol.

The RMS agrees with the applicant in that a waiving for this data requirement could be applied for thymol due to the following reasons.

Humans have been regularly exposed to thymol via physical contact and in their diet without known reports of adverse effects on the long-term; the available data and information do not show any concern regarding the long-term exposure of humans. Thymol occurs naturally in a variety of fruits (including grapes), vegetables, herbs and spices. It is used in cosmetics as a fragrance, as a flavouring agent in food, in dentistry and dental preparations for its anti-microbial properties, as a medical disinfectant, in human medicine for the treatment of worms and parasites, topical treatments and respiratory disorders and is also a constituent of oils used in aromatherapy.

Data from REACH registration dossier were included in the summary table of other studies relevant for long-term toxicity and carcinogenicity, since it is an ECHA requirement for the proposal of harmonised classification (CLH) according to Regulation (EC) no. 1272/2008 (CLP). However, it has to be underlined that these data were not available in the applicant submission dossier for the renewal and consequently they have not been evaluated by the RMS and not included in Volume 3 of this RAR. Only one study was found in the REACH dossier in which the route of administration (ip) and the duration of the treatment (8 weeks, 3 times weekly) were deemed not appropriate. Taking all these reasons into account, and also to preserve animal welfare, the RMS considers that long-term studies should not be required for thymol.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 56: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
No data to compare								

No comparison was possible in the absence of data regarding carcinogenicity.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Hazard class not assessed for harmonisation of classification and labelling according to CLP criteria due to data lacking.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
Repeated dose and reproductive/ developmental study GLP: Yes OECD TG (1990) Deviations: Historical Control Data not provided. SD rats (Crj:CD, SPF) 10/sex/group **Supporting information**	Thymol (purity 99.6%) Oral -Gastric tube Preliminary test: 30, 100 and 300 mg/kg bw/day Main test: 0, 8, 40 and 200 mg/kg bw/day Males: 43 days Females: 14 days prior to mating and also during mating, gestation and lactation (females)	*For results on repeated dose toxicity, see Table 46* *For results on developmental toxicity, see Table 60* *For results on effects on or via lactation, see Table 63* Main study: *Oestrus cycle:* No effect in any group. *Reproductive performance:* ↑ pre-coital time in treated groups (dose-related trend not statistically significant): ↑17%, compared to controls, at 8 mg/kg bw/day, ↑30% at 40 mg/kg bw/day and ↑56% at 200 mg/kg bw/day. This increase in the pairing days until mating was associated with the suppressive effect of thymol on activity levels at high dose levels. *Delivery and lactation:* Effects were only observed at 200 mg/kg bw/day dose level: - One female at 200 mg/kg bw/day failed to deliver. Black mucous was discharged from the vagina on day 24 of pregnancy; 3 dead foetuses and 3 implantation sites were found in this female's uterus at necropsy. - Pups of one dam's litter showed small quantity of milk in the stomach on day 1, and 5 out of 17 pups of this litter died. This was considered secondary to the emaciation showed by the female around delivery (leading to a temporary deterioration of its general condition) rather than any effect of the test substance on lactation. ***FERTILITY:*** -**LOAEL**(oral) = - -**NOAEL**(oral) = 200 mg/kg bw/day -**Target organs/tissues**: - -**Critical effect at the LOAEL**: - *PARENTAL NOAEL (see table 46):* - *LOCAL effects:* NOAEL(oral) = 8 mg/kg bw/day - *SYSTEMIC effects:* NOAEL(oral) = 40 mg/kg bw/day	[redacted], (1996) (CA) (B.6.6.1.1)
Reproductive/ developmental toxicity review Publication: data provided to EPA as a waiver of the requirements of a tolerance for residues of thymol. Deviations: Not applicable GLP: Not applicable **Supporting information**	Thymol via drinking water Rats Test material preparation not given Estimated doses: 5.6 and 11.3 mg thymol/kg bw/day (estimation based in the composition of the oil of *Satureja khuzestanica,* which is expected to be more concentrated than other extracts)	After the administration of an estimated dose of 5.6 and 11.3 mg thymol/kg bw/day to rats in drinking water, no difference in external abnormalities between treated and control groups were found. Very limited data on the method followed (no data available on the duration or phases (mating, gestation, lactation…) of the dosing, group size, strain, etc., Mortazavi *et al*., 2003.	US-EPA (2006) (CA) B.6.6.1.2
Fertility test in rats Publication No test guideline.	*Satureja khuzestanica* essential oil (No reference to thymol) 0, 75, 150 and 225 mg/kg bw/day	Potency, fecundity, fertility index, litter size and FSH and testosterone concentrations were all significantly increased. Weights of testes, seminal vesicles and prostate were increased at the high dose and histopathological effects were recorded at the mid and high dose (increased numbers of spermatogonium, spermatid cords, Leydig cells and spermatozoids, plus	Haeri *et al.* (2006) (CA) B.6.6.1.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
No GLP statement **Supporting information**	45 days exposure via drinking water	hypertrophic Sertoli cells). No adverse effects on reproductive performance are identified in this limited study.	

Table 58: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
REACH DATA Reproductive toxicity in rabbit. Reliability 4	Thymol	3 Female rabbits treated with 294-299 mg/kg bw orally in gelatin capsule for 7 days.	1 spontaneous abortion on the fifth day of treatment. NOAEL: 294-299 mg/kg bw for P and F1 generation.	Flavor and Extract Manufacturers Association (1985). "Scientific Literature Review of Phenols (C12)" (REACH registration dossier data not provided by applicant and not assessed by the RMS)
No data available				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The main study provided by the applicant for the assessment of the reproductive toxicity of thymol is a repeated dose and reproductive/developmental toxicity study in rats ([redacted]., 1996), provided in Japanese and accompained by its English translation (two word-documents). This is the only GLP compliant study provided for this section, but methodological deficits have been detected and, therefore, RMS deems it as supportive information only.

Pre-coital time showed a dose-related increasing trend (not statistically significant) in treated groups compared to controls. However, this increase in the pairing days until mating was associated with the suppressive effect of thymol on activity at high dose levels. All the other fertility indices evaluated in this study were similar between the groups. Thus, no adverse effects on fertility were related to thymol, leading to a NOAEL of 200 mg/kg bw/day for fertility.

In this study two parental NOAEL were set: NOAEL for systemic toxicity (40 mg/kg bw/day) and NOAEL for local effects (8 mg/kg bw/day) based on the corrosive properties of thymol. As it has been noted in the prevous paragraph, no reproductive effects were seen in parental animals at non-corrosive doses (8 mg/kg bw/day) or corrosive doses (≥ (40 mg/kg bw/day).

Additionally, two published non-guideline studies with *Satureja khuzestanica* in rats have been presented. The first publication cited a study performed in rats, via drinking water, with an herbal infusion of the plant *Satureja khuzestanica* (Mortazavi *et al*., 2003). The only reported results were that no difference in external abnormalities between treated and control groups were found. No data on sexual function or fertility was included in this study.

In the second publication (Haeri *et al.*, 2006), no adverse effects on reproductive performance were identified, although several parameters were reported as statistically increased (potency, fecundity, fertility index, litter size and FSH and testosterone concentrations).

The reported data of these two studies is very limited. Although no adverse effects on reproductive fertility were reported, the estimated content of thymol in the test material assayed was 0.6%, making it impossible to differentiate if the effects were due to the lack of reproductive toxicity of its components, or to the low concentrations of thymol administered.

Data from REACH registration dossier were included in the summary table of other studies relevant for toxicity on sexual function and fertility, since it is an ECHA requirement for the proposal of harmonised classification (CLH) according to Regulation (EC) no. 1272/2008 (CLP). However, it has to be underlined that these data were not available in the applicant submission dossier for the renewal and consequently they have not been evaluated by the RMS and not included in Volume 3 of this RAR. Only one study was found in the REACH dossier with low reliability, low number of tested animals and only one tested dose level.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

No adverse effects on fertility or sexual function were related to thymol in the available data. Although the main study available ([redacted], 1996) is considered as supportive, and no appropriated studies were performed with thymol to assess the reproductive toxicity, the results of this preliminary test reported no adverse effect on these functions.

Thus, the available data does not suggest any effect of thymol on sexual function and fertility.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 60: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group.	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL**	**Reference**
Repeated dose and reproductive/ developmental study GLP: Yes OECD TG (1990) Deviations: Historical Control Data not provided. SD rats (Crj:CD, SPF) 10/sex/group **Supporting information**	Thymol (purity 99.6%) Oral - Gastric tube Preliminary test: 30, 100 and 300 mg/kg bw/day Main test: 0, 8, 40 and 200 mg/kg bw/day Males: 43 days Females: 14 days prior to mating and also during mating, gestation and lactation (females)	*For results on repeated dose toxicity, see Table 46* *For results on fertility toxicity, see Table 57* *For results on effects on or via lactation, see Table 63* <u>Main study</u>: <u>*Delivery and lactation*</u>: Effects were only observed at 200 mg/kg bw/day dose level: - One female at 200 mg/kg bw/day failed to deliver. Black mucous was discharged from the vagina on day 24 of pregnancy; 3 dead foetuses and 3 implantation sites were found in this female's uterus at necropsy. - Pups of one dam's litter showed small quantity of milk in the stomach on day 1, and 5 out of 17 pups of this litter died. This was considered secondary to the emaciation showed by the female around delivery (leading to a temporary deterioration of its general condition) rather than any effect of the test substance on lactation. <u>*Effects on neonates*</u>: - Survival: 5 out of 17 pups of one litter died at 200 mg/kg bw/day, and small quantity of milk was found in the stomach on Day 1. No other abnormalities were seen in any other group. - Pup weights at birth and on Day 4: ↓ 10.2% males and ↓8.8% females at 200 mg/kg bw/day (not statistically significant). - Pup body weight gains: ↓14.6% in males and ↓10.5% in females at 200 mg/kg bw/day (not statistically significant). - External observations: missing tail and swelling of the umbilical region were noted each in 1 animal of the 40 mg/kg bw/day. No other abnormalities or alteration of general condition were observed in any animal in any group. - Necropsy: in surviving neonates: 2 cases of thymic remnant in the neck at the 8 mg/kg bw/day dose and 1 animal with umbilical hernia at the 40 mg/kg bw/day dose (the one that showed swelling of the region). Dead newborns showed persistent *foramen ovale* in 1, 2 and 1 pups of the 8, 40 and 200 mg/kg bw/day	[redacted] (1996) (CA) (B.6.6.1.1)

Method, guideline, deviations if any, species, strain, sex, no/group.	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
		groups, respectively. No abnormalities were noted in the mortality cases after day 1 of lactation. ***DEVELOPMENTAL:*** - **LOAEL**(oral) = 200 mg/kg bw/day - **NOAEL**(oral) = 40 mg/kg bw/day - **Target organs/tissues**: no specific - **Critical effect at the LOAEL**: reduction in pup weight and weight gain. *PARENTAL NOAEL (see table 46):* - *LOCAL effects:* NOAEL(oral) = 8 mg/kg bw/day - *SYSTEMIC effects:* NOAEL(oral) = 40 mg/kg bw/day	
Reproductive/ developmental toxicity review Publication: data provided to EPA as a waiver of the requirements of a tolerance for residues of thymol. Deviations: Not applicable GLP: Not applicable Rats **Supporting information**	Thymol via drinking water Test material preparation not given Estimated doses: 5.6 and 11.3 mg thymol/kg bw/day (estimation based in the composition of the oil of *Satureja khuzestanica,* which is expected to be more concentrated than other extracts)	After the administration of an estimated dose of 5.6 and 11.3 mg thymol/kg bw/day to rats in drinking water, no difference in external abnormalities between treated and control groups were found. Very limited data on the method followed (no data available on the duration or phases (mating, gestation, lactation…) of the dosing, group size, strain, etc., Mortazavi *et al*., 2003	US-EPA (2006) (CA) B.6.6.1.2

Table 61: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 62: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

The main data on developmental toxicity provided by the applicant is a combined repeated-dose reproductive/developmental toxicity-screening test ([redacted]., 1996), summarised also in points 2.6.3 and 2.6.6.3. RMS deems this study as supportive information due to methodological deficits.

Effects in offspring were addressed only up to post-natal day 4, where externally visible abnormalities in neonates showed low incidences and the abnormalities identified at necropsy did not suggest any treatment-related effect. Additionally, reduction in pup weight and weight gain was observed at 200 mg/kg bw/day, leading to the establishment of a developmental toxicity NOAEL of 40 mg/kg bw/day.

In this study two parental NOAEL were set: NOAEL for systemic toxicity (40 mg/kg bw/day) and NOAEL for local effects (8 mg/kg bw/day) based on the corrosive properties of thymol. It has to be noted that developmental effects

based on the reduction in pup weight and weight gain at 200 mg/kg bw/day were concomitant to corrosive effects seen in parental animals and with systemic toxicity. No developmental effects were seen at lower doses, even at 40 mg/kg bw/day at which corrosion was observed in parental animals.

Additionally, a published non-guideline study with *Satureja khuzestanica* in rats has been presented. This publication cited a study performed in rats, via drinking water, with an herbal infusion of the plant *Satureja khuzestanica* (Mortazavi *et al*., 2003, summarised also in point 2.6.6.1). The only reported results were that no difference in external abnormalities between treated and control groups were found. The reported data of this study is very limited. Although no adverse effects on development were reported, the estimated content of thymol in the test material assayed (herbal infusion of the plant *Satureja khuzestanica*) was up to 0.6%, making it impossible to differentiate if the effects were due to the lack of reproductive toxicity of its components (included thymol), or to the low concentrations of thymol administered.

The RMS has been informed by ECHA that, under REACH, there is an ongoing testing proposal for developmental toxicity following the OECD TG 414.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

A reduction in pup weight and bodyweight gain was the only adverse effect observed in offspring (since the abnormalities found in neonates did not suggest any treatment-related effect), in the main study available.

According to Regulation (EC) No 1272/2008: "The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency".

Although a growth alteration was observed in the available study, it cannot be directly attributable to a developmental toxicity of thymol. However, given the limited data available for the assessment of this hazard class, it cannot be discarded either.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 63: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Repeated dose and reproductive/ developmental study GLP: Yes OECD TG (1990) Deviations: Historical Control Data not provided. SD rats (Crj:CD, SPF) 10/sex/group **Supporting information**	Thymol (purity 99.6%) Oral -Gastric tube Preliminary test: 30, 100 and 300 mg/kg bw/day Main test: 0, 8, 40 and 200 mg/kg bw/day Males: 43 days Females: 14 days prior to mating and also during mating, gestation and lactation (females)	*For results on repeated dose toxicity, see Table 46* *For results on fertility toxicity, see Table 57* *For results on developmental toxicity, see Table 60* <u>Main study</u>: <u>*Delivery and lactation*</u>*:* Effects were only observed at 200 mg/kg bw/day dose level: - One female at 200 mg/kg bw/day failed to deliver. Black mucous was discharged from the vagina on day 24 of pregnancy; 3 dead foetuses and 3 implantation sites were found in this female's uterus at necropsy. - Pups of one dam's litter showed small quantity of milk in the stomach on day 1, and 5 out of 17 pups of this litter died. This was considered secondary to the emaciation showed by the female around delivery (leading to a temporary deterioration of its general condition) rather than any effect of the test substance on lactation. <u>*Effects on neonates*</u>*:* - Survival: 5 out of 17 pups of one litter died at 200 mg/kg bw/day, and small quantity of milk was found in the	[redacted], (1996) (CA) (B.6.6.1.1)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		stomach on Day 1. No other abnormalities were seen in any other group. - Pup weights at birth and on Day 4: ↓ 10.2% males and ↓8.8% females at 200 mg/kg bw/day. (not statistically significant). - Pup body weight gains: ↓14.6% in males and ↓10.5% in females at 200 mg/kg bw/day (not statistically significant). - External observations: missing tail and swelling of the umbilical region were noted each in 1 animal of the 40 mg/kg bw/day. No other abnormalities or alteration of general condition were observed in any animal in any group. - Necropsy: in surviving neonates: 2 cases of thymic remnant in the neck at the 8 mg/kg bw/day dose and 1 animal with umbilical hernia at the 40 mg/kg bw/day dose (the one that showed swelling of the region). Dead newborns showed persistent *foramen ovale* in 1, 2 and 1 pups of the 8, 40 and 200 mg/kg bw/day groups, respectively. No abnormalities were noted in the mortality cases after day 1 of lactation. ***DEVELOPMENTAL:*** - **LOAEL**(oral) = 200 mg/kg bw/day - **NOAEL**(oral) = 40 mg/kg bw/day - **Target organs/tissues**: no specific - **Critical effect at the LOAEL**: reduction in pup weight and weight gain. *PARENTAL NOAEL (see table 46):* - *LOCAL effects:* NOAEL(oral) = 8 mg/kg bw/day - *SYSTEMIC effects:* NOAEL(oral) = 40 mg/kg bw/day	

Table 64: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 65: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

The only study on reproduction and developmental toxicity provided by the applicant with data after birth is a combined repeated-dose reproductive/developmental toxicity-screening test ([redacted]., 1996), summarised also in points 2.6.3 and 2.6.6.2. RMS deems this study as supportive information due to methodological deficits.

Effects in offspring were addressed only up to post-natal day 4. Due to the reduction in pup weight and weight gain observed at 200 mg/kg bw/day, a NOAEL of 40 mg/kg bw/day was established for developmental toxicity (see point 2.6.6.2 for more details).

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

Given the limited data available for the assessment of this hazard class, and considering that the reduction in pup weight and bodyweight gain was the only adverse effect observed in offspring, a potential effect of thymol on or via lactation cannot be discarded.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Given the limited data available for the assessment of the reproductive toxicity on thymol, a conclusion on classification for fertility, development or effects on or via lactation cannot be reached. Results don't grant the classification, but data is considered insufficient to discard a possible effect.

Consequently, this hazard class is not assessed for harmonisation of classification and labelling according to CLP criteria due to insufficient data.

2.6.7 Summary of neurotoxicity

Table 66: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Neurotoxicity data			
Repeated dose neurotoxicity in mice No method followed No GLP **Supporting information** Swiss male mice 6 animals/group	Thymol (>98.5% pure) in water Oral route 0, 10, 20 and 40 mg/kg bw/day for 30 days	Statistically significant (with dose-related trend) differences, compared with control group, were observed for the following doses and parameters: - Behaviour test: at 20 and 40 mg/kg bw/day: ↓latency time - ↑Blood-brain barrier permeability at 20 and 40 mg/kg bw/day - ↑AChE activity at 40 mg/kg bw/day - ↑ROS levels and ↑XO (cerebral xantine oxidase) levels at 20 and 40 mg/kg bw/day - ↓Na+, K+-ATPase activity at 20 and 40 mg/kg bw/day - ↑Thymol levels in brain tissue at 20 and 40 mg/kg bw/day No effects were exerted by thymol on open-field test.	Baldissera *et al*., (2018) (CA) B.6.7.1
ADME			
Pharmacokinetics Mouse No deviations from OECD TG 417 (2010) GLP: Yes **Study acceptable** Hsd:ICR (CD.1®) mice (19♂, 19♀)	Thymol (99.7% purity) in olive oil Single oral (gavage) dose: Preliminary study: 1250 and 1000 mg/kg bw (2♂, 2♀ each) Main study: 1000 mg/kg bw (15♂, 15♀) Blood samples were taken at 1, 2, 4, 8, 12 and 24 h post dosing.	Preliminary study I (1250 mg/kg bw) Clinical observations: ataxia, bradypnoea, prone posture, piloerection, decreased activity and ptosis in all animals up to 4 h after dosing. Animals appeared normal on Day 2 post-dosing. Preliminary study II (1000 mg/kg bw) Clinical observations: ataxia, decreased activity, bradypnoea, ptosis and piloerection ranging from 5 min to 4 h post dose. Prone posture, pallor extremities, loss of righting reflex and ptosis were observed in one male from 2 to 4 h after dose. Prone and pallor extremities were also observed in one female from 15 min to 4 h after doses. Main study (1000 mg/kg bw) Clinical observations: increased activity, piloerection, ataxia and decreased activity ranging from 1 to 8 h post-dose in both male and female animals. Additional symptoms included bradypnoea (1 male at 8 h), ataxia (4 males up to 2 h post dose and 6 females up to 4 h post dose) and both hunched posture and lethargy (1 male at 2 h post dose, 1 female at 4 h post dose).	[redacted] (2009) (CA) B.6.1.1.6
Acute toxicity data			
Acute oral toxicity study in rats and guinea pigs	Thymol (20% in propylene glycol) Purity unknown	Rats: Thymol LD_{50}: 980 mg/kg bw Depression, ataxia, coma on high doses	Jenner, P.M. *et al.* (1964)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Prior to OECD TG 401 Deviations: Test substance not characterised. Poorly described method and results, no necropsy. GLP: No **Supporting information** 10 Osborne-Mendel rats evenly divided by sex Guinea pig (male, female)	(commercially available material) Oral by intubation Single dose Up to 14-day observation period	Guinea pigs: Thymol LD_{50}: 880 mg/kg bw Irritated gastro-intestinal tract, tremors, coma, respiratory failure *See table 18 for more details.*	(CA) B.6.2.1.1
Acute oral toxicity study in mice Publication on a review of acute toxicity studies. Deviations: Test substance not characterised. Poorly described method and results GLP: Not stated **Supporting information** 10 Mice (ddY) /group Males and females	Thymol (highest purity available) in squalene (concentration not specified). At least 6 increasing dose levels (not specified) 14-day observation period	Thymol LD_{50}: 1050 mg/kg bw (f); 1200 mg/kg bw (m) Hypoactivity and ataxic gait. Small intestinal congestion *See table 18 for more details.*	Hasegawa, R. *et al.* (1989) (CA) B.6.2.1.2
Acute oral toxicity review Publications: Review of published literature. Poorly described method and results **Supporting information** Mouse (male)	Thymol 1 to 4% aqueous solutions in cottonseed oil Gavage 620 to 2100 mg/kg bw	**LD_{50} = 1800 mg/kg bw** Depressed general condition. Death occurred within 48 h. Only depression at lower doses. Depression and prostration at higher doses. McOmie *et al.* (1949) *See table 18 for more details.*	Escobar, A. (2006) (CA) B.6.2.1.3 --- BG Chemie, (2000) (CA) B.6.2.1.4
Acute oral toxicity review Publication: Review of published literature. Deviations: Test substances not characterised. Poorly described methods and results GLP: Not stated **Supporting information**	- Thymol in alcohol/propylene glycol/water Gavage	**- LD_{50} = 640 mg/kg bw (alcohol/ propylene glycol/water)** Decrease in spontaneous movemets, piloerection and paralysis of anterior limbs within 1h. Death occurred within 5 to 6 h RIFM (2001e) *See table 18 for more details.*	Escobar, A. (2006) (CA) B.6.2.1.3

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>Mouse</td><td></td><td></td><td></td></tr>
<tr><td>Acute oral toxicity review
Publication: Review of published literature.
Deviations: Poorly described method and results
Supporting information
Mice (groups of 8)</td><td>- Thymol as a 10% aqueous emulsion
- Thymol as a 10% peanut oil solution
Oral route. No data on doses</td><td>- LD₅₀ = 650 mg/kg bw (aqueous emulsion)
- LD₅₀ = 1300 mg/kg bw (peanut oil solution)
Drowsiness and paralysis occurred after 2 to 5 minutes. At the later stages: muscle twitching and spasms.
Deaths occurred after 6 to 12 hours. The survivors recovered slowly.
Oelkers, (1940)
See table 18 for more details.</td><td>BG Chemie, (2000) (CA) B.6.2.1.4</td></tr>
<tr><td>Acute intraperitoneal toxicity test in mice
Publication: pharmacological effect of Essential oil of Lippia grata. Thymol was used for pharmacological comparisons with Lippia-derived compounds (the actual material tested).
Supporting information
Swiss mice:
6 groups of
3 adults each
(sex at random)</td><td>Commercial Thymol (not characterized)
Intraperitoneal (i.p.) injection
Doses: 33.3, 50.0, 73.3, 110.0, 166.6 and 233.3 mg/kg bw
3-d observation period</td><td>Mortality and clinical sings:
<table>
<tr><th>Dose
mg/kg bw</th><th>No. deaths/No. mice per group</th><th>Signs of toxicity</th></tr>
<tr><td>33.3</td><td>0/3</td><td>No effect</td></tr>
<tr><td>50.0</td><td>0/3</td><td>Slight ataxia</td></tr>
<tr><td>73.3</td><td>1/3</td><td>Ataxia, decreased spontaneous motor activity</td></tr>
<tr><td>110.0</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>166.6</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>233.3</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
</table>
-LOAEL (i.p.) = 50 mg/kg bw
-NOAEL (i.p.) = 33.3 mg/kg bw
-Target organs/tissues: CNS - Narcotic effects.
-Critical effect at the LOAEL: Ataxia.
See table 43 for more details.</td><td>Viana et al. (1981) (CA) B.6.8.2.1</td></tr>
<tr><td colspan="4">Repeated dose toxicity data</td></tr>
<tr><td>Repeated dose and reproductive/ developmental study
OECD TG (1990)
Supporting information
SD rats (Crj:CD, SPF) 10/sex/group</td><td>Thymol (purity 99.6%)
Oral - Gastric tube
Preliminary test: 30, 100 and 300 mg/kg bw/day
Main test: 0, 8, 40 and 200 mg/kg bw/day
Males: 43 days</td><td><u>Preliminary (range-finding) study:</u>
300 mg/kg bw/day: reduction in spontaneous motor activity, ambulatory ataxia.
<u>Main study</u>:
<u>Clinical signs</u>: only noted in the 200 mg/kg bw/day groups:
Females: 9/10: Decrease in spontaneous motor activity from day 1, just after administration and was observed for 10 days in 2 females, 3 days in 2 animals and 1 day in 5 females.
6/10: Ataxic gait from day 1 (accompanying the ↓spontaneous motor activity)</td><td>[redacted] (1996) (CA) (B.6.3.1.1) 2.6.3.1</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
	Females: 14 days prior to mating and also during mating, gestation and lactation (females)	*SYSTEMIC effects:* -LOAEL = 200 mg/kg bw/day -NOAEL = 40 mg/kg bw/day -Target organs/tissues: CNS/muscle and thymus. -Critical effect at the LOAEL: reduced motor activity and ataxia in females (attributed to pharmacological effects of thymol suppressing CNS or muscular contraction), and increased thymus weight (absolute and relative). *See table 46 for more details.*	

No specific data on neurotoxicity was included by the applicant for the assessment of the active substance in this review process of thymol. However, the first publication included in the table above (Baldissera *et al*., 2018) was mentioned by the applicant, and discarded due to methodological deficits. Nevertheless, RMS deems this data, although supportive, should not be overlooked.

The results reported in this publication show dose-related trend and statistically significant differences, compared with the control group, from the mid dose group (20 mg/kg bw/day) in the following parameters: inhibitory avoidance task (reduced latency time), BBB permeability (increase), ROS levels (increase), cerebral xanthine oxidase activity (increase) and Na+, K+-ATPase activity (reduced). Additionally, AChE activity was increased (statistically significant and with a dose-related trend) at the high dose (40 mg/kg bw/day). RMS deems these results suggest that thymol might elicit neurotoxicity at certain concentrations.

Additionally, the other eight studies included in the table above reported different neurological effects (mainly ataxic gait, lethargy and/or decreased spontaneous activity), after the administration of thymol by oral or intraperitoneal routes. These neurological effects have already been assessed and included in point 2.6.2.10 (STOT SE) of this document, since these effects were considered transient, and it has been proposed the classification of thymol as STOT SE 3 (H336) due to the narcotic effects observed.

As highlighted in the publication of Baldissera *et al.* (2018), several evidences have related the biochemical and pharmacological properties of thymol, but the possible neurotoxic effects of this compound remain unknown and not evaluated. Moreover, thymol, as other monoterpenes referred as “natural”, are commonly considered safe and without side effects, boosting their consumption in animal and human medicine, but little research has been performed to study their possible toxic effects.

Based on the available data, the RMS is of the opinion that there is sufficient evidence to suggest the active substance thymol might elicit neurotoxic effects. For this reason, the RMS concludes that neurotoxicity cannot be discarded for thymol.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

There were not available toxicity studies on metabolites or relevant impurities.

2.6.8.2 Supplementary studies on the active substance

Supplementary studies on the active substance

Table 2.6.8.2/01. Summary table of supplementary studies

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
Acute intraperitoneal toxicity test in mice	Commercial Thymol (not characterized)	Mortality and clinical sings: **Dose** | **No. deaths/No. mice per group** | **Signs of toxicity**	Viana *et al.* (1981) (CA)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels, route of exposure duration of exposure</th><th>Observations</th><th>Reference</th></tr>
<tr><td>Publication: pharmacological effect of Essential oil of Lippia grata.
Thymol was used for pharmacological comparisons with Lippia-derived compounds (the actual material tested).

GLP. No
Supportive only
Swiss mice:
6 groups of
3 adults each
(sex at random)</td><td>Intraperitoneal (i.p.) injection

Doses: 33.3, 50.0, 73.3, 110.0, 166.6 and 233.3 mg/kg bw

3-d observation period</td><td>
<table>
<tr><th>mg/kg bw</th><th></th><th></th></tr>
<tr><td>33.3</td><td>0/3</td><td>No effect</td></tr>
<tr><td>50.0</td><td>0/3</td><td>Slight ataxia</td></tr>
<tr><td>73.3</td><td>1/3</td><td>Ataxia, decreased spontaneous motor activity</td></tr>
<tr><td>110.0</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>166.6</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
<tr><td>233.3</td><td>3/3</td><td>Ataxia, decreased spontaneous motor activity, somnolence</td></tr>
</table>
-LOAEL (i.p.) = 50 mg/kg bw
-NOAEL (i.p.) = 33.3 mg/kg bw
-Target organs/tissues: CNS - Narcotic effects.
-Critical effect at the LOAEL: Ataxia.</td><td>B.6.8.2.1</td></tr>
<tr><td rowspan="6">Subcutaneous, intravenous and intraperitoneal toxicity review
Publication: Review of published literature. No data on the test guidelines followed in each study.
Deviations: Test substances not characterised. Poorly described methods and results
GLP: Not stated for any study

Supporting information</td><td>Guinea pigs
4 males
Thymol in olive oil
Doses: 106 or 233 mg/kg bw/day (20 or 40 mg/day, respectively)
Subcutaneous (s.c.) dosing for 8 to 9 d</td><td>Clear thyroid activation was seen in 2 animals and weakly in a third animal.

No effect was seen in the oxygen consumption in any animal.

- Insufficient information to establish NOAEL or LOAEL.
-Target organs/tissues: Thyroid gland.
-Critical effect at the LOAEL: thyroid activation.

Möller (1939)
Also in BG Chemie,2000 -B.6.8.2.3</td><td rowspan="6">Escobar, A. (2006) (CA) B.6.8.2.2</td></tr>
<tr><td>6 dogs
Thymol in oil
Doses: not indicated. Perfusion (i.v.) for 20 to 25 min

Monitoring: blood pressure, respiration and survival</td><td>Clinical signs at the dose of 60 mg/kg bw, were convulsions and respiratory arrest.
Thymol LD_{50} in dog (i.v.) = 150 mg/kg bw

Caujolle and Franck (1944)</td></tr>
<tr><td>Rats
Thymol in ethylene glycol
1 to 3 mL/100 g bw
Single subcutaneous (s.c.) injection</td><td>No convulsions were caused by the administration of thymol. No additional information available

Matsumoto et al. (1963)</td></tr>
<tr><td>Mice (A/He5)
Males and females
Thymol
Serial 2-fold dilutions
6 injections over 2 weeks
Via and vehicle not specified</td><td>Maximum tolerated dose (MTD) of all five mice = 0.25 g/kg

Stoner et al. (1973)</td></tr>
<tr><td>Mice (SPF)
5 males
Thymol
Vehicle not specified
6 to 8 doses of thymol intravenously (i.v.)</td><td>Thymol LD50 in mice (i.v.) = 100 mg/kg bw

James and Glen (1980)</td></tr>
<tr><td>Mice (Swiss)
3 mice/test group
Thymol</td><td>Effects:
33.3 mg/kg bw. No effects.
50 mg/kg bw: nonspecific effects and slight ataxia.</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
	Doses from 33.3 to 233.3 mg/kg bw Intraperitoneal (i.p.) injections Observation up to 3 days post-adm.	73.3 mg/kg bw: 1/3 died; ataxia, decreased spontaneous motor activity. 110 mg/kg bw: 3/3 died; ataxia, decreased spontaneous motor activity and somnolence. The same was observed in the 166.6 and 233.3 mg/kg bw groups. Calculated Thymol LD_{50} in mice (i.p.) = 110 mg/kg bw. Actual testing of this dose resulted in the death of 3/3 animals in the first 3 days. Viana *et al*. (1981) (CA) B.6.8.2.1	
	Mice Rats Rabbits Dogs Thymol Vehicle not specified Subcutaneous (s.c.) or intravenous (i.v.) injections	Thymol LD_{50} in mouse (s.c.) = 800 mg/kg bw Thymol LD_{50} in rat (s.c.) =1600 mg/kg bw Thymol LD_{50} in rabbit (i.v.) = 60 mg/kg bw Thymol LD_{50} in mice (i.v.) = 100 mg/kg bw Thymol LD_{50} in dog (i.v.) = 150 mg/kg bw Instituto Superiore di Sanità (1999)	
	Mice (male) Thymol in an alcohol/propylene glycol/water vehicle Doses not reported Subcutaneous (s.c.)	Calculated Thymol LD_{50} in male mice (s.c.) = 243 mg/kg bw. RIFM (2001e)	
Repeated-dose subcutaneous toxicity review Publication: Review of published literature. Deviations: Poorly described method and results **Supporting information**	Guinea pigs (males) Thymol Doses: 20 to 100 mg/animal/day Subcutaneous (s.c.) dosing for 8 to 9 d	Animals showed histological marked "activation" of the thyroid gland without any increase in oxygen consumption. According to the investigator, signs of activation included proliferation of the interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes. - Insufficient information to establish NOAEL or LOAEL. -**Target organs/tissues**: Thyroid gland. -**Critical effect at the LOAEL**: activation of the thyroid gland (proliferation of interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes). Möller (1939) Also in Escobar A, 2006 -B.6.8.2.2	BG Chemie, (2000) (CA) B.6.8.2.3
***In vitro* effect of thymol on human sperm motility and function** Not guidelined GLP: No **Supporting information**	Thymol Unknown batch No. and purity. Solvent: ethanol. Human semen samples Doses: 100, 200, 300, 400 and 500 µg/mL of thymol.	Total progressive spermatozoa decreased from 200 µg/mL of thymol. Abnormalities of the acrosome occurred at 500 µg/mL of thymol.	Chikhoune, A., *et al.* (2015) (CA) B.6.8.2.4
Oral 21-day study in rabbits Not guidelined GLP: No **Supporting information**	Thymol Unknown batch No. Purity ≥ 99.9%. M9 rabbits. Dose: 148.9 ± 16.7 µg/g dry matter in diet. 21-day exposure 7-day withdrawal	Thymol concentrations: 0.05 ± 0.02 µg/L in plasma 0.04 ± 0.03 µg/g of dry matter in intestinal wall, after 21-day exposure. Not detected in intestinal wall, after 7-day withdrawal. 0.89 ± 0.45 µg/g in faeces, after 21-day exposure. 0.08 ± 0.04 µg/g in faeces, after 7-day withdrawal.	Bacova, K., *et al.* (2020) (CA) B.6.8.2.5.

Immunotoxicity

No supplementary studies on the active substance regarding immunotoxicity were provided.

With the aim of analysing the effect of thymol on the normal function of the immune system, a detailed review of the existing studies from other human and animal health sections has been carried out. Immune-related parameters were reviewed from short-term studies, chronic studies, and reproductive studies in order to evaluate the immunotoxic potential of thymol. Furthermore, ADME studies and medical data were also evaluated for this purpose.

The distribution of the active substance and its metabolites in tissues may be indicative of its immunotoxic potential, whilst medical data can provide useful information about effects related to inability to fight infection or excessive, poorly controlled responses (as anaphylaxis or autoimmunity). None of the ADME studies investigated the distribution and accumulation of thymol in tissues.

Therefore, the effects of thymol on each endpoint were collected from the available studies and evaluation was performed according to the respective OECD guidelines.

The most relevant endpoints for this task were selected based on the *Retrospective analysis of the immunotoxic effects of plant protection products as reported in the Draft Assessment Reports for their peer review at EU level* (Dewhurst, I. *et al.* 2015, EFSA supporting publication 2015: EN-782) and *Guidance for Immunotoxicity risk assessment for chemicals* (World Health Organization & International Programme on Chemical Safety (2012). Guidance for immunotoxicity risk assessment for chemicals. World Health Organization. IPCS harmonization project document; no. 10).

These endpoints were the following:
- Survival and infections, as indicators of potential immunotoxicity, considering that laboratory animals do not use to be exposed to infections.
- From the haematology parameters, total white blood cell counts (WBC) and/or differential counts, as key cell types involved in immune functioning/response.
- Globulin levels in serum, as an indicator of antibody synthesis. If globulin levels were not presented, A:G ratio or serum protein changes were used as surrogates.
- Lymph nodes, as integral parts of the immune system. At least one site of lymph nodes should be included for histopathological examination in most study protocols, but also the finding of lymph nodes with increased size from the clinical observations was included in the analysis.
- Gut associated lymphoid tissue or Peyer's patches, as important tissue in antigen presentation.
- Spleen (weight and histopathology), as organ involved in the maturation of lymphocytes. Altered weights can indicate atrophy or abnormal stimulation. Pathology can indicate altered immune function but can be secondary to other functions of the spleen.
- Thymus (weight and histopathology), as primary organ for T-lymphocyte maturation. Changes in young animals are reported to be more likely to indicate immunotoxicity, as thymus weight varies with the age of the animal, due to its normal age-associated shrinking or involution.
- Bone marrow smear, as reduced cellularity could indicate reduced potential to produce WBC (& RBC).
- Adrenal glands are an organ target of cytokines, and indeed, ACTH and adrenal steroids regulates the cytokine synthesis. Besides, deposits of immunoglobulins have been observed in adrenal glands. Some autoimmune syndromes as Addison´s disease are characterised by adrenal cortex damage.

Table 2.6.8.2/02: Analysis of immune parameters in studies with thymol

Study	Immune parameters analysed	Not analysed*
ADME studies		
Substance: Thymol -ADME studies (all)	-Relatively rapid and almost complete based on urinary excretion in rats and humans. - None of the ADME studies investigated the distribution and accumulation of thymol in tissues.	-Not applicable.
Short-term studies		
Substance: Thymol - [redacted] (1996)	- Mortality: One male and one female died in the top dose group (200 mg/kg bw/day).	- Haematology (females) - Biochemistry

Study	Immune parameters analysed	Not analysed*
- Combined repeat dose and reproductive/developmental studies of thymol in SD rats - Species: rat (♀,♂) - 43-day study - Route: oral (gavage) (AS) B.6.3.1 Supportive study	- Haematology (males only): Slight increase in lymphocytes from 8 mg/kg bw/day (this effect is concluded to be not treatment-related). Slight reduction in monocytes observed in all treatment groups although statistically significant only at 40 mg/kg bw/day (absolute value). - Thymus: increase absolute and relative weight in 200 mg/kg bw/day males. - Thymus gross pathology: small thymus in 1/10 female at 40 mg/kg bw/day and 1/9 female at 200 mg/kg bw/day dose groups. - Thymus histopathology: involution of thymus in 1/10 female at 40 mg/kg bw/day and 1/9 female at 200 mg/kg bw/day dose groups. - Spleen histopathology: extramedullary haematopoiesis in 1/9 female at 200 mg/kg bw/day dose group. - Adrenal: reduction in absolute and relative weight in all dose groups (not statistically significant) - Adrenals gross pathology: whitish adrenal in 2/9 females at the 200 mg/kg bw/day dose group. - Adrenal histopathology: increase in fatty droplets, fascicular zone in 1/9 females in the 200 mg/kg bw/day dose group. - Infections: not reported.	- Organ weights (spleen) - Histopathology (males) - Globulin levels
Substance: Thymol - Hagan, E.C. *et al*, (1967) - Subacute and chronic toxicity (feed study) - Species: rat (♀,♂) - 19-week study (AS) B.6.3.2 Supportive study	- No effects on haematology. - No macroscopic and microscopic changes in spleen - Infections: not reported.	- Mortality - Biochemistry - Globulin levels
Medical Data		
Substance: Thymol -Volpe, M.J. (2021) (AS) B.6.9.1 Document acceptable	The manufacturing plant conducts regular medical monitoring of workers involved in the production, packaging or handling of thymol. There have been no reports of reactions or ill health in any workers.	-n/a
Substance: Thymol - BG Chemie (2000) -Thymol (review on skin sensitisation) (AS) B.6.9.2 Supporting information	Thymol reportedly caused dermatitis in dentists No primary skin irritation or allergenic reactions in 25 volunteers tested in a Maximisation test (48h occlusive exposure) Patch test run in patients with contact dermatitis in which one positive reaction reported for one dental nurse test (1.2 % patients) Spreading of pruritic dermatitis following a 3-week application of "Listerine" (0.6% thymol) was reported on a patient with chronic paronychia. A 48-h patch test with the antiseptic produced a positive reaction on the patient. Patch test run in 364 patients with thymol 1% formulation reported 2 cases (0.5 %) of positive reactions No reactions observed in any of 131 individuals tested with thymol 1% formulation	-n/a

Study	Immune parameters analysed	Not analysed*
	Patch test run in 221 patients with a solution of thymol (no further details) reported one female patient with a positive reaction (0.45% patients) A total of 39 out of 300 reacted to thymol (5% in glycerine) following patch-test Negative results obtained in patch test in 79 patients with eyelid dermatitis treated with 1% thymol in petrolatum Negative results obtained in allergy test in 290 patients treated with 1% thymol in petrolatum No positive results obtained in patch test in 100 eczematous patients treated with thymol 1% in petrolatum Negative result in patch test in one individual treated with thymol (0.01 to 0.015 mL) The outcome of a patch test in 23 patients with contact dermatitis treated with thymol was negative	
Substance: Thymol - BG Chemie (2000) - Thymol (review) (AS) B.6.9.3.1 Supporting information	This is a clinical case report of thyroid intoxication in three male patients following the use of a mouthwash containing thymol for periods of 6 months to 3 years. Symptoms included weight loss, tremors, restlessness, sleeplessness and diarrhoea. Recovery was observed upon cessation in the use of the mouthwash. A 3 week-old infant suffered a respiratory collapse when inhaling a cold remedy, which in addition to menthol and other volatile oils also contained thymol. The authors considered it very unlikely that the collapse was attributable to the remedy.	-n/a

** Parameters not analysed, despite they should be reported according to the respective OECD protocol.*

The collected data permit to build an overview on the immunotoxic potential of thymol, considering the following groups of parameters:

General health condition

Within the limitations of the experiments performed under laboratory conditions, no particular concern about the immune system functioning arises from the analysis of mortality and infections in this dataset.

Haematology parameters

In a 43-day oral study in rats, slight increase in lymphocytes from 8 mg/kg bw/day was observed, although this effect is concluded to be not treatment-related. Slight reduction in monocytes was observed in all treatment groups although it was statistically significant only at 40 mg/kg bw/day in the absolute value).

Biochemical parameters

Globulins levels and other immunological-related parameters were not measured in the studies provided.

Organs and tissues

Spleen: Spleen weight was not reported in the rat 43-day repeat dose study. Histopathological assessment of the spleen revealed extramedullary haematopoiesis in 1/9 female rat at the top dose group (200 mg/kg bw/d).

Thymus: Increase in absolute (9.8 %) and relative weight (15.3 %) in males in the 200 mg/kg bw/day dose group was observed in a rat 43-day repeat dose study. Gross pathology revealed small thymus in 1/10 female at 40 mg/kg bw/day and 1/9 female at 200 mg/kg bw/day dose groups. Thymus histopathology showed involution of thymus in 1/10 female at 40 mg/kg bw/day and 1/9 female at 200 mg/kg bw/day dose groups.

Bone marrow: No data reported.

Adrenal gland: In a rat 43-day repeat dose toxicity study, adrenal gland weight was slightly reduced in all dose groups (6.4 % reduction in absolute and 2.7% reduction in relative organ weight in 200 mg/kg bw/day dose group,

not statistically significant). Gross pathology of the adrenal gland revealed a whitish adrenal in 2/9 female rats at the top dose group. Histopathologically, increase in fatty droplets (fascicular zone) occurred in 1/9 female rat in the 200 mg/kg bw/d dose group.

Human data

Regarding to the available medical data, one manufacturing plant involved in the production, packaging or handling of thymol, no reported reactions or ill health in any workers.

Data collected on humans involved skin sensitisation tests and accidental oral ingestion of variable quantities of mouthwash containing thymol. Regarding skin sensitisation, tests performed on human volunteers or individuals who usually used thymol-containing products displayed diverse results. Wide-battery tests carried out in human volunteers did not show relevant results regarding skin sensitisation for thymol.

In conclusion, taking into consideration all available medical data and the overall assessment from studies conducted in animals, the active substance thymol must be considered as a skin sensitiser and the classification of **Skin Sensitisation Category 1, H317,** "May cause an allergic skin reaction" is proposed.

Conclusion on immunotoxicity

Based on the available toxicology data, no treatment related changes in the immunotoxic sensitive parameters were observed. In addition, thymol does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. Within the scope of this brief analysis, **it can be concluded that thymol is devoid of immunotoxic potential**.

2.6.9 Summary of medical data and information

There have been no reports of reactions or ill health in any workers involved in the production, packaging or handling of thymol (Volpe, 2021) following the regular medical monitoring of workers conducted by the manufacturing plant.

The applicant also submitted a publication on the toxicological evaluation of thymol (BG Chemie, 2000) which reviews the experience in humans, including publications for the assessment of the toxicity of thymol. Regarding human data these publications reported information on skin sensitization (with positive and negative results, discussed in point 2.6.2.7), but also reported data of oral metabolsim studies (discussed in point 2.6.1), cases via inhalation exposure (discussed in points 2.6.2.3 and 2.6.2.10) or due to the repeated use of a mouthwash containing thymol (discussed in point 2.6.3.1).

Finally, the applicant included the information of the fatal large-volume mouthwash ingestion in an adult (Soo Hoo. et al., 2003) as part of the previous publication (BG Chemie, 2000), that has been discussed in points 2.6.2.1 and 2.6.2.10).

No data on epidemiological studies is available.

Table 2.6.9: Summary table of medical data and information

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Medical surveillance on manufacturing plant personnel				
Medical monitoring of workers	Thymol	Regular monitoring of workers involved in the production, packaging of handling of thymol	There have been no reports of reactions or ill health in any workers.	Volpe (2021) (CA) B.6.9.1
Data collected on humans				
Publication : Review of published literature	**Oral route**			BG Chemie, (2000) (CA) B.6.9.2
	Thymol 0.6 g/person	Studies on the metabolism of thymol in 2 volunteers. Single oral administration of thymol and the 24-h urine was analysed (thin-layer chromatography)	Thymol glucuronide, thymol sulfate were detected, to a lesser extent thymolhydroquinone sulfate and small amounts of unchanged thymol. No furder quantitative details. Takada *et al*. (1979)	
	Dermal route (skin sensitization)			
	Thymol	No more data provided	Thymol reportedly caused dermatitis in dentists Schwartz *et al.* (1957)	
	Thymol 4% in petrolatum	Maximisation test in 25 volunteers.	No primary skin irritation nor allergenic reactions	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		48-h occlusive exposure	Kligman (1972)	
	Thymol 1% in petrolatum	Patch-test in patients with contact dermatitis (38 dentist, 18 dental nurses and 28 dental technicians)	Only 1 dental nurse (1.2% of patients) developed a positive reaction to thymol Berova *et al.* (1990)	
	Ethanolic antiseptic solution ("Listerine") containing 0.6% thymol Patch-test: 1% thymol in petrolatum	43-year-old patient with chronic paronychia 3-week occlusive application of "Listerine" (0.6% thymol, 0.9% eucalyptus oil, 0.6% methyl salicylate, 0.4% menthol and traces of benzoic acid) Also 48-h patch test with the antiseptic and with the ingredients was performed to the patient and 3 controls	The 3-w application resulted in spreading pruritic dermatitis in the patient. 48-h patch test with the antiseptic produced a positive reaction in the patients and none in the 3 controls. 48-h patch test with thymol (1% in petrolatum) led to a reaction (2+). 48-h patch test with eucalyptus oil (1% in alcohol), methyl salicylate (2% in olive oil), menthol (1% in petrolatum) and benzoic acid (5% in petrolatum) proved negative in the patient and in the 3 controls. However, the patient used "Listerine" as mouthwash several times without developing any noticeable reactions. According to the author, thymol appears to be a weak sensitiser which needs prolonged contact, occlusive application and inflamed skin to produce sensitisation and dermatitis Fisher (1989)	
	Thymol 1% formulation (composition not specified)	Patch test in 365 patients at a dermatology clinic (1981 to 1986)	2 cases (0.5%) of positive reactions Itoh *et al*. (1988)	
	Thymol 1% formulation (composition not specified)	Test in 131 patients at a dermatology clinic (1979 - 1982) This publication is previous to Itoh *et al.* (1988), but same investigators and similar group of patients	None of the individual reacted Nishimura *et al*. (1984)	
	"spir. Dilute" solution of thymol (only data available)	Patch test in 221 patients treated at a dermatology department	One female patient (0.45%) showed a positive reaction Dohn (1980)	
	Thymol (no more data)	31-year-old woman with skin allergy to menthol	It was possible to exclude a simultaneous allergy to thymol Papa and Shelley (1964)	
	Thymol (5% in glycerine)	Patch-test in 300 workers (217 women and 83 men, 20-27 years) of stomatology offices 12 different substances used in dental practice were patch-tested	39 individuals reacted to thymol, in a total of 213 who tested positive. 87 were negative Djerassi and Berowa (1966)	
	Thymol (1% in petrolatum)	Patch-test in 79 patients with eyelid dermatitis	All results were negative Nethercott *et al*. (1989)	
	Thymol (1% in petrolatum)	Patch-tests in 290 patients Thymol was assayed in the context of a study on allergising effect of topical medicaments	None of the 290 patients developed a positive reaction to thymol Meneghini *et al.* (1971)	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	Thymol (1% in petrolatum)	Patch-test in 100 eczematous patients of a dermatology clinic Numerous substances were patch-tested	None of the patients showed a positive reaction to thymol Rantuccio and Meneghini (1970)	
	Thymol (0.01 to 0.015 ml, equivalent to 0.1 to 0.15 mg)	Patch-test in a 51-year-old sawmill worker Within 2 years working, he developed itching vesicular dermatitis on the face, dorsa of his hands and the flexures of his forearms. Pathc-test was performed with various extracts of wood and pure constituents.	Negative results for thymol in the patch-test. Strongly positive reactions to western red cedar, in particular Bleumink *et al*. (1973)	
	Hirudoid cream, containin 0.1% of thymol Patch-test: thymol (not specified)	Patch-test in 23 patients with contact dermatitis following the use of Hirudoid cream These patients developed allergic contact dermatitis over a period of 10 years. Individual components of the cream were patch-tested.	The individual components of the cream proved negative in the patch-test However, the cause of the allergies was identified as a reaction product of thymol, ethanolamine and formaldehyde. The last two substances are degradation products of 1,3,5-trihydroxyethylexahydrotriazine, of which Hirudoid cream contains 0.15% Smeenk *et al*. (1987)	
	Other routes			
	Halotane (inhalation anaesthetic) containing 0.01% thymol	Inhalation Postoperative "halothane hepatitis"	It's been suggested that thymol might play a causative role in very rarely occurring instances of postoperative "halothane hepatitis" Hutter and Laing (1993)	
	Thymol in water	Olfactory threshold test in 9 to 12 subjects	The olfactory threshold of thymol in water was found to be 500 µg/L Dietz and Traud (1978)	
Direct observation				
Publication : Review of published literature	Cold remedy that contained thymol, in addition to menthol and other volatile oils.	Inhalation A 3 week-old infant inhaling the cold remedy.	The infant suffered a respiratory collapse. The authors considered it very unlikely that the collapse was attributable to the remedy. No further details Davis and Livingstone (1986).	BG Chemie, (2000) (CA) B.6.9.3.1
	Mouthwash containing thymol	Three male patients following the use of the mouthwash for periods of 6 months – 3 years	Thyroid intoxication: weight loss, tremors, restlessness, sleeplessness and diarrhoea. Upon cessation of the use of the mouthwash the patients showed recovery and weight gain. Edens (1937)	
Published report: poisoning case	Mouthwash containing thymol (0.064%)	Fatal large-volume mouthwash ingestion in an adult. Ingestion of almost 3 litres of mouthwash by one patient (almost 1.92 g of thymol in the almost 7.74 g of non-alcoholic ingredients)	Cardiovascular collapse, multiorgan system failure and death. The patient presented a profound anion gap metabolic acidosis and a significant osmolar gap. It was concluded that exposure to phenolic ingredients of the mouthwash (i.e. eucalyptol, menthol and thymol) as well as alcohol accounted for the adverse effects.	Soo Hoo. *et al.* (2003) (CA) B.6.9.3.2

2.6.10 Toxicological end points for risk assessment (reference values)

Table 67: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat (Osborne-Mendel) male and female	19-week feed study	Thymol (commercially available. Purity not indicated)	No effect at 900 mg/kg bw/day (10000 ppm)	900 mg/kg bw/day (10000 ppm)	-	Hagan E.C., *et al.*, (1967) (CA) (B.6.3.2.1)
Rat SD (Crj:CD, SPF) male and female	Combined repeated dose and reproductive study, 43 days (7 weeks), oral gavage	Thymol (purity 99.6%)	Adult			[redacted], (1996) (CA) (B.6.3.1.1)
			Local: Histopathological changes in forestomach at 40 mg/kg bw/day	8 mg/kg bw/day	40 mg/kg bw/day	
			Systemic: Reduced motor activity and ataxic gait (CNS or muscular contraction depressive action) and increased thymus weight (absolute and relative) at 200 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	
			Reproductive			[redacted]., (1996) (CA) (B.6.6.1.1)
			No effect at 200 mg/kg bw/day	200 mg/kg bw/day	-	
			Offspring			
			↓pup weight ↓pup weight gain at 200 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The acceptable daily intake (ADI) for humans is normally derived from the NO(A)EL in the most susceptible species in long-term toxicity studies, and an appropriate safety factor.

No data on long-term toxicity of thymol is available. Short-term/subchronic data on thymol is limited and rat is the only species tested. A 19-weeks toxicity study in rats (Hagan E.C., *et al.*, 1967 – B.6.3.2.1) reported no effects after administration of 10000 ppm of thymol in diet (equivalent to 900 mg/kg bw/day). It should be noted that the tested substance was not pure thymol, but the commercially available material for its use as food additive.

A 43-day combined repeated dose and reproductive toxicity study in rats was also provided ([redacted], 1996), which lies between a sub-acute (28-d) and a subchronic (90-d) study. An ADI could be derived from the resulting offspring NOAEL of 40 mg/kg bw/day, based on the reduction of pup weights and weight gains at 200 mg/kg bw/day.

Considering the duration of the exposure of the available study, and following EFSA guidance on selected default values (EFSA Journal 2012;10(3):2579) a safety factor of 600 is deemed appropriate in this case. This value is derived from the usual 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability and a 6-fold factor is added to extrapolate from subacute to chronic (as used by ECHA (2010) for REACH chemicals). Thus, the ADI is calculated as follows:

ADI = (40 mg/kg bw/day)/ 600 = 0.07 mg/kg bw/day

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Thymol is classified as acute (oral) toxicity, category 4 (Acute Tox. 4; H302), corrosive to skin (Skin Corr. 1B; H314) and causes serious eye damage (Eye Dam.1).

Four acute oral toxicity studies performed with thymol (B.6.2.1.1 to B.6.2.1.3: McOmie *et al.*, 1949; Jenner *et al.*, 1964; Hasegawa *et al.,* 1989; and RIFM, 2001e) reported depression, decrease in spontaneous activity hypoactivity, ataxia and tremors (rats, guinea pigs and mice), among the clinical symptoms; also irritation/congestion of the gastro-intestinal tract were found at necropsy (guinea pig and mice). However no data on the tested dose levels are reported and only LD_{50} values are available for these studies (980 mg/kg bw for rat, 880 mg/kg bw for guinea pig and 640, 1050 or 1800 mg/kg bw for mice).

In addition, a combined repeated dose and reproductive toxicity test in rats ([redacted], 1996 - B.6.6.1.1) reported reduced motor activity and ataxic gait, from day 1 of administration, in females dosed with 200 mg/kg bw/day. Based on this effect a systemic NOAEL was established as 40 mg/kg bw for adult rats.

Regarding the gastro-intestinal effects, the study of [redacted]., 1996 (B.6.6.1.1) reported histopathological changes in the forestomach of rats at 40 mg/kg bw, leading to a local NOAEL of 8 mg/kg bw for this study. However, all the GI effects were observed after the administration of thymol by gastric gavage and, therefore, these effects are most likely due to a local irritant effect. According to OECD guidance 124 for the derivation of an ARfD: «since ARfD applies to ingestion of a compound in food or drinking water, local GI effects exerted by bolus administration at very high doses may not being considered relevant for setting an ARfD».

Altogether, the NOAEL of 40 mg/kg bw (based on reduced motor activity and ataxia) is considered the worst-case for acute effects and, therefore, the most relevant for setting the ARfD.

A safety factor of 100, derived from both 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability, is deemed appropriate. Thus, the ARfD for humans is calculated as follows:

ARfD = (40 mg/kg bw)/100= 0.4 mg/kg bw

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The AOEL is defined on the basis of short-term toxicity studies in the most sensitive species and with the application of an appropriate safety factor.

Available short-term/subchronic data on thymol is very limited and rat is the only species tested. A 19 weeks toxicity study in rats (Hagan E.C., *et al*., 1967 – B.6.3.2.1) reported no effects after administration of 10000 ppm of thymol in diet (equivalent to 900 mg/kg bw/day). It should be noted that the tested substance was not pure thymol, but the commercially available material for its use as food additive.

In addition, a 43-day combined repeated dose and reproductive toxicity study in rats was also provided ([redacted], 1996 – B.6.3.2.1). In this study, a systemic NOAEL of 40 mg/kg bw/day was established for adult rats (based on the reduction of motor activity, ataxia and increased thymus weight observed at 200 mg/kg bw/day), which coincides with the NOAEL of 40 mg/kg bw/day established for offspring (based on the reduction of pup weights and weight gains at 200 mg/kg bw/day).

This NOAEL of 40 mg/kg bw/day is considered the most representative for establishing the AOEL.

A safety factor of 100, derived from both 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability, is deemed appropriate. Thus, the AOEL is calculated as follows:

AOEL= (40 mg/kg bw/day)/ 100 = 0.4 mg/kg bw/day

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

For the first EU approval of thymol no value of acute acceptable operator exposure (AAOEL) was proposed.

Since an ARfD has been set, an AAOEL is also proposed in this RAR, based on the signs of reduced motor activity and ataxia after acute administration of thymol in several toxicity studies and species.

The data on the acute oral toxicity studies (B.6.2.1.1 to B.6.2.1.3: McOmie *et al.*, 1949; Jenner *et al.*, 1964; Hasegawa *et al.,* 1989; and RIFM, 2001e) is very limited and no information on the tested doses is available, but the LD_{50} values.

However, in the combined repeated dose and reproductive toxicity test in rats ([redacted], 1996 - B.6.6.1.1) reduced motor activity and ataxic gait was reported from day 1 of administration, in females dosed with 200 mg/kg bw.

Based on this effect a systemic NOAEL was established as 40 mg/kg bw for adult rats in this study.

A safety factor of 100, derived from both 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability, is deemed appropriate. Thus, the AAOEL for humans is calculated as follows:

AAOEL = (40 mg/kg bw)/100= 0.4 mg/kg bw

2.6.11 Summary of product exposure and risk assessment

The product Mevalone is a capsule suspension (CS) formulation containing 33,3 g/L eugenol, 67,3 g/L geraniol and 66,7 g/L thymol (technical) which is applied to grapes and pome fruit as a fungicide.

No dermal absorption studies have been performed with Mevalone. Following the EFSA Guidance on dermal absorption (2017) and the SANTE/2018/10591 rev. 1 (Guidance on dermal absorption) document, default dermal absorption values have been defined for thymol, geraniol and eugenol.

Considering that the preparation Mevalone is formulated as a capsule suspension (CS), and taking into account the concentration of the active substances in the formulation (66 g/L for thymol and geraniol, and 33 g/L for eugenol), the following values are established for these active substances:

- Concentrate: 25% for thymol and geraniol, and 70% for eugenol.
- Dilution: 70% for thymol, geraniol and eugenol.

To perform the assessment, EFSA model has been applied and the following conclusions have been raised:

- **OPERATOR:**

According EFSA model, operator exposure to MEVALONE (4 L/ha) from tractor mounted air assisted sprayer application outdoor to high crops is below the AOEL with the use of workwear (arms, body and legs covered) and chemical protective gloves during mixing/loading and application.

However, a safe use for the operator to thymol from manual application is not obtained.

In the opinion of the RMS, as according EFSA Guidance, 2014, the penetration factor of the workwear is 10 %, a type 6 protective coverall (or the equivalent according EN-ISO 27065 :2017/A1 :2019) should be used.

Besides, and due to the toxicological classification of the product as skin sensitiser, face shield (according EN 166:2002) is advisable during mixing/loading of the product.

In case of tractor spraying, the specific chemical protective gloves will be used only to handle the application equipment or contaminated surfaces.

Moreover, during cleaning and handling of the equipment, the same PPE as mixing/loading should be used.

- **WORKER:**

For worker, the results of the exposure risk assessment indicate that the risk to residues of eugenol, geraniol and thymol is acceptable with the following conditions:

- For pome fruit (4 L fp/ha), the exposure of workers is acceptable when they wear work clothing (arms, body and legs covered) and chemical protective gloves and the numbers of applications are reduced to two.
- For vineyard, (4 L fp/ha) workers need work clothing and chemical protective gloves and the numbers of applications are reduced to a single one.

Treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried.

- **RESIDENTS/BYSTANDERS**

For residents/bystanders, according EFSA model, and considering the SVC approach for vapour pathway, exposure to MEVALONE (4 L/ha) is below the AOEL of Eugenol, Geraniol, and Thymol, and AAOEL of Thymol.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

According to OECD Guideline No. 506, Stability of Pesticide Residues in Stored Commodities, apples (pome fruits) are considered representative of the storage stability group of predominantly water containing commodities whilst grapes are considered high acid content commodities. Therefore, high acid and high water content commodities are represented in this evaluation.

Grapes

A total of 3 storage stability studies (AF/11145/ED, AF/12351/ED and S20-06526) are available and can be considered acceptable and satisfying the guideline requirements for residue storage stability (OECD test guideline 506, 2007). Analytical methods supporting these storage stability studies were considered acceptable (See Vol 3 section B.7.1 for further details).

Study AF/11145/ED was evaluated and accepted in the DAR (2012). Samples were fortified with thymol, applied onto the surface of grapes at a level of 0.1 mg/kg and put into storage at -18°C. The results showed that 1 month after fortification, surface residues of thymol on grapes were not stable, with 42% thymol recovered. Moreover, the information provided is not relevant from a consumer point of view since data should be referred to the entire fruits and not to the surface residues.

Study AF/12351/ED was evaluated and accepted in the DAR (2012) (one-month interim report). The 1-year report has not been previously evaluated at EU level. Whole grapes were fortified at a rate of 1.0 mg/kg for thymol and placed directly into frozen storage at -18°C. Residues of thymol on whole grapes were stable for at least 12 months, with 79% of fortified thymol recovered at 1 year.

Study S20-06526 has not been previously evaluated at EU level. Homogenised grapes were fortified with thymol at a rate of 0.1 mg/kg. Samples were placed directly into frozen storage at ≤-18°C. Residues of thymol were stable at 278 days, with 78% of the nominal fortified amount of thymol recovered.

Available plant residue trials on grapes are covered with the results of these storage stability studies of thymol in grapes.

Apples (pome fruits)

A new storage stability study (S20-06527) is available and can be considered acceptable and satisfying the guideline requirements for residue storage stability (OECD test guideline 506, 2007). Analytical methods supporting this storage stability study were considered acceptable (See Vol 3 section B.7.1 for further details).

Study S20-06527 has not been previously evaluated at EU level. Homogenised apple samples were fortified with thymol at a rate of 0.1 mg/kg and placed directly into frozen storage at ≤-18°C. Residues of thymol on homogenised apples were stable for at least 161 days, with 81% of nominal fortified amount of thymol recovered.

Available plant residue trials on apples are covered with the results of this storage stability study of thymol in apples.

Extract stability

Two residue studies were submitted in the first EU Review (Bailey, 2008 CA 6.3.1/01 and 2007 CA 6.3.1/02). Extracts were stored frozen prior to quantification. The maximum storage interval between extraction and analysis was 30 days. Stability of residues in sample extracts was demonstrated because procedural recovery samples analysed as part of each analytical batch were within the acceptable limits of recovery, as defined in analytical method validation. These procedural recovery data are available in the crop residue reports.

Two new residue studies are included in this submission (Chadwick, 2021a CA 6.3.1/03 and 2021b CA 6.3.2/01). Stability of extracts in acetonitrile was demonstrated in the corresponding method validation studies S20-06528 and S20-06529. In the grape residue trials (Study CA 6.3.1/03), the maximum duration between extraction and analysis was 7 days and stability of extracts was demonstrated for 7 days in study S20-06528 by Driss, 2021. In the apple residue trials (Study CA 6.3.2/01), the maximum duration between extraction and analysis was 7 days and stability of extracts was demonstrated for 15 days in study S20-06529 by Driss, 2021 CA 6.1/04.

Animal commodities

Not any storage stability study for animal commodities has been submitted in order to support the intended uses. However, since not any animal feeding studies were required according to the intended uses, storage stability studies are not considered necessary.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Plants

During Annex I inclusion of thymol and in the Peer review of the pesticide risk assessment of the active substance thymol (EFSA Journal 2012;10(11):2916) plant metabolism studies were not submitted. According to that EFSA conclusion thymol occurs naturally in plants and very limited information from the published literature was reported in the DAR. Data on the natural background levels of thymol in grapes from retail samples were also submitted and gave indication of residue levels far below 0.05 mg/kg (validated LOQ of the method). EFSA was of the opinion that no metabolism data are required to conduct a reliable consumer risk assessment if the submitted residue trials are considered as acceptable.

Sufficient and acceptable residue trials on grapes and apples, both belonging to the fruits and fruiting vegetables metabolism group, in compliance with the representative uses have been reported in the context of this evaluation. These residue trials are supported by appropriate storage stability studies.

No metabolism studies following SANCO or OECD guidelines have been reported. As supporting information, 2 published papers have been reported:
- Biotransformation of thymol, carvacrol, and eugenol by cultured cells of *Eucalyptus perriniana*
- Biotransformation of monoterpenes and sesquiterpenes by cell suspension cultures of *Achillea millefolium* L. ssp. *millefolium*

No conclusive data can be obtained from these two reported studies/literature data covering the metabolism of Thymol in primary crops. Degradation of Thymol into glycosylate forms were investigated on culture cells of yarrow (Achillea millefolium L. ssp. Millefolium) and Eucalyptus (Eucalyptus perriniana). The studied species are not representative of the metabolism for the intended GAP crops but they can provide an estimation on the natural biodegradability of Thymol in plants and its potential metabolites. Thymol would be considered to be the appropriate residue to study in the residue trials.

Poultry

The only relevant feed commodity in the EU for the intended uses of thymol is the apple wet pomace. According to OECD Series on Pesticides No. 73 (ENV/JM/MONO(2013)8, apple wet pomace is not a

feed item relevant to poultry. Therefore, metabolism studies on poultry are not required and no metabolism studies following SANCO or OECD guidelines have been reported.

However, as supporting information, 4 not previously evaluated at EU level published papers have been reported (See Vol 3 CA-B-7 for further details):
- Effect of Thyme Essential Oil Supplementation on Thymol Content in Blood Plasma, Liver, Kidney and Muscle in Broiler Chickens.
- Identification and quantification of thymol metabolites in plasma, liver and duodenal wall of broiler chickens using UHPLC-ESI-QTOF-MS.
- Thymol in the intestinal tract of broiler chickens after sustained administration of thyme essential oil in feed.
- Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: a review. (This paper is a review of the available literature on the use of thymol (amongst other supplements) in poultry feed).

From these four reported studies/literature data it is estimated that main metabolites Thymol sulfate and Thymol glucuronide are formed during biotransformation of Thymol. Further literature data on feeding experiments indicate that the distribution of Thymol in poultry tissues and fluids is not strictly proportional to the administered dose concentration and may vary depending on the metabolic activity of each tissue. Thymol was found at low levels in poultry muscle suggesting a low potential risk to humans associated to poultry intake: this is supported by the assessment of thymol used as feed flavouring.

EFSA Journal 2012;10(3):2620 and EFSA Journal 2014;12(11):3896 considered the use of Crina® Poultry Plus, which contains thymol (minimum 1%) amongst other ingredients, for use as a feed additive in chicken feed. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that it is safe for chickens at the maximum recommended dose (300 mg/kg). In its assessment of thymol used as feed flavouring, the FEEDAP Panel concluded that up to the maximum concentration of 6 mg/kg complete feed for chickens, no safety concern would arise for the consumer (EFSA, 2012). The maximum dose of CPP, would deliver 8.6 mg thymol/kg complete feed, which is in the same order of magnitude as the figure derived for flavouring use.

Recently a preparation, Biomin® DC-P, containing thymol amongst other ingredients, was considered by EFSA and later approved for the use as a feed additive (65-105 mg/kg complete feed) in chickens (EFSA Journal 2019;17(6):5724). The FEEDAP Panel concludes that the use of Biomin® DC-P (containing thymol 1-3 mg/g additive) in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

Rumiants

The only relevant feed commodity in the EU for the intended uses of thymol is the apple wet pomace. According to OECD Series on Pesticides No. 73 (ENV/JM/MONO(2013)8, apple wet pomace is a feed item relevant to rumiants.

A ruminant metabolism study is not required since the maximum dietary intake is calculated to be 0.001 mg/kg bw/day (see section 2.7.5 from this report). According to Regulation (EU) 283/2013 a study is only required if the dietary intake is >0.004 mg/kg bw/day. Furthermore, it should be noted that no residues were found at or above the LOQ in apple commodities, which may be used as animal feed in the EU. Therefore, the dietary intake calculation is a worst case, since it assumed residues at the worst case LOQ of 0.01 mg/kg.

Therefore, metabolism studies on rumiants are not required and no metabolism studies following SANCO or OECD guidelines have been reported. However, as supporting information, 2 not previously evaluated at EU level published papers have been reported (See Vol 3 CA-B-7 for further details):
- Development of a method to determine essential oil residues in cow milk.

- Milk and plasma disposition of thymol following intramammary administration of a phytoceutical mastitis treatment.

According to these literature data Thymol residues are not found in milk samples following incorporation of thymol into cows' diets nor in milk or plasma samples after a goat´s mastitis treatment. It is however noted that the metabolism route is not specified and the distribution of Thymol into other ruminant matrices was not further studied.

Pigs

The only relevant feed commodity for the intended uses of thymol is the apple wet pomace. According to OECD Series on Pesticides No. 73 (ENV/JM/MONO(2013)8, apple wet pomace is not a feed item relevant to pigs. Therefore, metabolism studies on pigs are not required and no metabolism studies following SANCO or OECD guidelines have been reported. However, Notifier has submitted a not previously evaluated at EU level published paper which can be used only as supporting information for metabolism in pigs. In this study (*In vitro* degradation and *in vivo* passage kinetics of carvacrol, thymol, eugenol and trans-cinnamaldehyde along the gastrointestinal tract of piglets) (See Vol 3 CA-B-7 for further details), data *in vivo* suggest metabolism and elimination of thymol is relatively fast, when it is used as antimicrobial growth promoter in pig feeds.

Fish

According to SANCO 11187/2013 rev.3, grapes, pome fruits and their processing products are not considered as commodities commonly used for the formulation of aquaculture diets (see Annex 2. Feeding stuffs table). Therefore, the use of thymol according to the intended uses is not foreseen to affect fishes feeding. Therefore, metabolism studies for fishes are nor necessary.

2.7.3 Definition of the residue

No metabolism studies are required and no metabolism studies following SANCO or OECD guidelines have been reported. Available literature papers indicate that thymol is converted to corresponding β-glucosides and β-gentiobiosides, which are accumulated in the cells. Glycosylation by plant cells serves for the detoxification of phenolic compounds, which could arise from normal plant metabolism or the environment. It may also be metabolised into other related terpenes, including 1,8-cinole, limonene, carvacrol, α-pioene and terpinolene. Thymol would be considered to be the appropriate residue to study in the residue trials.
Thymol naturally occurring in a wide variety of fruits, vegetables, herbs and spices (refer to Introduction to M-CA Section 6). In this submission, use is supported on grapes and pome fruit. Residue trials have demonstrated residues to be <LOQ (<0.01 mg/kg) in both grapes and apples at harvest.

Thymol is naturally occurring in grapes. In a study to measure natural background concentrations in grapes (Jones, 2012 – KCA 6.10.2/01), in 20 different grape samples thymol was found to be present in the range ND to 0.016 mg/kg.

It is proposed that a residue definition for plant matrices is not required, since it is proposed to include thymol into Annex IV to Regulation (EC) No. 396/2005. Thymol meets criterion 4 (The consumer exposure to the compound linked to use as PPP is considered as negligible compared to other uses in the food chain and/or natural background) of SANCO/11188/2013 Rev. 2, 14 September 2015 (Guidance document on criteria for the inclusion of active substances into Annex IV of Regulation (EC) N° 396/2005).

Separate residue definitions for rotational crops and processed commodities are not required. Specific studies to cover these areas were not triggered.

A residue definition in animal matrices is not required. Only apple pomace is fed to livestock in the EU. Residue levels in apple commodities are all <LOQ (<0.01 mg/kg). Consequently, the livestock dietary intake of thymol is low and below the trigger of 0.004 mg/kg bw/d. Taking into account the likely dietary exposure of livestock by consumption of treated apple products, it is expected that there will be negligible exposure. Based on this information, it is considered not necessary to change the existing residue situation, therefore enforcement and risk assessment residue definitions are not proposed for livestock products.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Grapes

The proposed GAP for grapevines is up to 4 applications at 26.4 g thymol/hL (water 400-1000 l/ha) with a 7 day interval and 7 day PHI for the Southern residue zone. It is noted that the critical GAP supported for renewal is the same as the one supported for the first inclusion in the EU.

Residues of thymol in grapes were determined in 3 studies (S20-06337, AF/11125/ED and AF/10728/ED). Studies AF/11125/ED and AF/10728/ED were already evaluated in the DAR (2012). Study S20-06337 has not been previously evaluated at EU level. A total of 11 trials in grapes were conducted in the Northern EU (Austria, Germany and Northern France) and in the Southern EU countries (Spain, Portugal and Italy) in 2006 and 2020.

Regarding new study S20-06337, six residue trials were conducted during the 2020 growing season according to the critical GAP for the renewal and are therefore relevant to support the use of thymol in the EU. Three residue trials were conducted in the Northern EU and the other three in the Southern EU. Thymol was found to be naturally occurring in some control samples between ND and 0.01 mg/kg. Thymol was found to be present in treated grapes between ND and 0.06 mg/kg. In all cases, residues were at background concentrations by 7 DALA: N-EU (2 x ND, <0.01 mg/kg), S-EU (2 x ND, 0.01 mg/kg).

Regarding study AF/11125/ED containing three residue trials, conducted in the same location, only surface extractable residues were considered. Since the storage stability study on surface extractable residues did not demonstrate stability of surface extractable residues when stored at -18°C, this residue trial is considered not to be valid and only provides supporting information. Moreover from a consumer point of view, relevant information should be referred to the entire fruits and not to the surface residues.

Regarding study AF/10728/ED, Notifier stated that four residue decline trials for thymol on grapes were conducted in Spain (2 trials) and Italy (2 trials), during the 2006 growing season. However, it should be emphasized that trials conducted in Spain were carried out less than 20 km far from one another, and therefore different geographical sites could not be demonstrated. Dates of treatment are different but not more than 30 days apart. The same situation applies to the two Italian trials. Therefore, only 2 residue trials (one in Spain and one in Italy) can be considered, and the other two residue trials must be considered as replicates. At the critical GAP both trials showed residue levels < LOQ (0.05 mg/kg). These trials did not differentiate between ND and <LOQ.

A summary of the acceptable results, compliant with the current guidelines is given in the table below.

Table 2.7.4-01: Summary of valid residue trials

Commodity	Results relevant to the critical GAP (mg/kg)	STMR	HR
Grapes NEU	2 x ND, <0.01	<0.01	<0.01
Grapes SEU	2 x ND, 0.01[†], 2 x <0.05[*]		

[†] The residue is the same as the natural background concentration. There is no increase over natural background concentrations.

[*] The trials showing residues of <0.05 mg/kg (<LOQ) (AF/10728/ED) are supportive of the results of the new, guideline compliant trials (S20-06337), where residues are either <LOQ or equal to natural background levels. It is considered that there is no increase over natural background concentrations.

Pome fruits

The proposed GAP for pome fruit is up to 4 applications at 26.4 g thymol/hL (water 600-1000 l/ha) with a 7 day interval and 1 day PHI for the Northern residue zone. A 3 day PHI is being supported for the Southern residue zone in the MRL application that has been made, but it is not a representative use for the EU Review.

Residues of thymol in apples were determined in one study (S20-06361) that has not been previously evaluated at EU level. To evaluate total residues, 3AEY (Mevalone) was applied at the cGAP rate. A total of 6 trials in apples were conducted in the Northern EU (Austria, Germany and Northern France) and in the Southern EU countries (Spain, Italy and Sothern France) in 2020. Residues of thymol were all <LOQ in the Northern residue zone, except in one trial that a residue of 0.02 mg/kg was observed at 0 DALA, but this was <LOQ by 1 DAL. In two trials the Southern residue zone residues were 0.03 mg/kg at 0 DALA and all residues were <LOQ by 3 DALA.

Apple is the representative crop for pome fruit and the results can be extrapolated to the entire pome fruits, group. However it should be emphasized that black chokeberry (*Aronia melanocarpa*) and mountain ash (*Sorbus sp*.) have been applied for the Notifier, but they are not included in the pome fruits group (see Commission Regulation 2018/62). Therefore they cannot be supported.

A summary of the acceptable results, compliant with the current guidelines is given in the table below.

Table 2.7.4-02: Summary of valid residue trials

Commodity	Results relevant to the critical GAP (mg/kg)	STMR	HR
Apples NEU	ND, 2 x <0.01	< 0.01	< 0.01
Apples SEU	2 x ND, <0.01		

Sufficient trials are available to confirm that residues in apples will not exceed 0.01 mg/kg. Furthermore, it is proposed that thymol is included into Annex IV to Regulation (EC) No. 396/2005 meaning that MRLs are not required. Residues of thymol were in the range ND to 0.03 mg/kg in the treated samples. All residues had declined to <LOQ by 1 day after the last application in the northern residues zone and 3 days after last application in the southern residues zone. Taking into account its natural occurrence and residues below the LOQ, it is considered that 6 trials over the two zones are sufficient.

Grapes and pome fruits

No MRLs are required for thymol as it is listed in Annex IV to Commission Regulation (EC) No 839/2008. According to Commission Regulation (EC) No 839/2008 it was temporarily included in Annex IV.

It is proposed that thymol is included into Annex IV to Regulation (EC) No. 396/2005, meaning that MRLs are not required. Thymol is naturally occurring in grapes and apples, and in the worst case scenario no residues above background concentrations are found following application of Mevalone according to the cGAP for grapes and apples. Taking this into account, it is considered that the available trials over the two zones are sufficient for grapevines and apple trees.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

The only relevant feed commodity in the EU for the intended uses of thymol is the apple wet pomace (grape pomace is not fed to livestock in the EU). According to OECD Series on Pesticides No. 73 (ENV/JM/MONO(2013)8, apple wet pomace is not a feed item relevant to poultry nor pigs. Therefore, feeding studies on poultry and pigs are not required.

To assess the livestock dietary burden of thymol for EU livestock, the residues trials values (STMR) supporting the cGAP for renewal for the raw agricultural commodities (RAC) for apples were used as input into the 'Animal model 2017'

The currently calculated STMR is 0.01 mg/kg. A processing factor (PF) from apple to wet pomace should be used too. Default PF (5) is used in the calculation.

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0,001	0,001	0,03	0,03	Beef cattle	Apple	pomace, wet	No
Cattle (dairy only)	0,000	0,000	0,01	0,01	Dairy cattle	Apple	pomace, wet	No
Sheep (all diets)	0,001	0,001	0,01	0,01	Lamb	Apple	pomace, wet	No
Sheep (ewe only)	0,000	0,000	0,01	0,01	Ram/Ewe	Apple	pomace, wet	No
Swine (all diets)								No
Poultry (all diets)								No
Poultry (layer only)								No
(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed a								
(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".								

None of the diets for any of the EU livestock exceed the trigger value of 0.004 mg/kg bw/day, based on the predicted intakes of thymol via the consumption by livestock of treated apples.

On the basis of this information, it is considered that livestock metabolism studies are not required. No feeding studies are required.

According to SANCO 11187/2013 rev.3, grapes, pome fruits and their processing products are not considered as commodities commonly used for the formulation of aquaculture diets (see Annex 2. Feedingstuffs table). Therefore, the use of thymol according to the intended uses is not foreseen to affect fishes feeding. Therefore, feeding studies for fishes are not necessary.

2.7.6 Summary of effects of processing

A study on the nature of the residue is not required. One trial on grapes showed a residue value of 0.01 mg/kg, at the same level of background concentrations. Trials (2) from study AF/10728/ED showed residues of <0.05 mg/kg (<LOQ), these 2 old trials did not differentiate between ND and <LOQ and are considered as supportive information. So it can be considered that no residues above the LOQ (0.01 mg/kg) were found in grapes or apples (representative crop for pome fruit). According to Commission Regulation (EU) No 283/2013 a study on the nature of the residue is only required if residues above 0.01 mg/kg are found in the raw commodity to be processed.

Regarding fruit peeling, no data are required as the representative crops (grapes and pome fruits) do not have an inedible peel.

According to Commission Regulation (EU) No 283/2013, studies are only required if residues in the part of the plant to be processed are >0.1 mg/kg. If this trigger is not exceeded, then a study is necessary if the TMDI is greater than 10% of the ADI or the dietary intake is greater than 10% of the ARfD. Processing

or household studies are not required, since residues in the parts of the plant to be processed are lower than the trigger value of 0.1 mg/kg. No residues at or above the LOQ (0.01 mg/kg) were found in grapes or apples (representative crop for pome fruit). Furthermore, the TMDI is less than 10% of the ADI and the dietary intake is less than 10% of the ARfD for grapes and pome fruit. Therefore, a magnitude of the residue processing study is not triggered.

2.7.7 Summary of residues in rotational crops

A metabolism study in rotational crops is not triggered. Grapes and pome fruit are perennial crops and therefore not grown in rotation.

Furthermore, according to Regulation (EU) 283/2013 a study is only required if the parent or its metabolites are persistent or significant concentrations of metabolites will occur in soil. In a soil metabolism study (Jones, A. 2015), the rate of degradation in 4 laboratory soils was studied. The DT90 for thymol was clearly <3 days in all soils. A DT90 of 100 days is generally accepted as the trigger for persistence in soil. The DT90 for thymol in soil is clearly below the trigger and therefore a study is not required. No metabolites were formed in the soil metabolism study and therefore no metabolite uptake needs to be considered with respect to rotational crop studies.

Metabolism studies in rotational crops are not triggered and therefore magnitude of the residue studies are not required.

2.7.8 Summary of other studies

2.7.8.1. Effect on the residue level in pollen and bee products

According to SANTE/11956/2016, grapes and pome fruit may be foraged by bees. For grapes the timing of application (BBCH 60-89) is such that bees may potentially forage the crop following application. However, the timing of application for pome fruit (BBCH 75-87) is after the flowering stage (BBCH 60-69) and therefore the bees will not forage blossoms following application of Mevalone. Furthermore, thymol is not persistent and therefore potential exposure in subsequent seasons will not occur.

From reported trials only residues on the fruits have been analysed. According to SANTE/11956/2016 rev. 9 data from aerial parts sampled during the attractive period of the crop or its weeds should be used. Fruits are not considered aerial parts like leaves, flowers and/or nectar, but residues on fruits give clear indications about the behaviour of the residue. N-EU trials on grapes showed thymol residue levels on the day of the treatment: 2 x ND and 0.06 mg/kg (decreasing to 0.03 mg/kg the day after treatment) while residues from S-EU trials were (mg/kg) 0.02, 0.06 (background levels were 0.01 mg/kg) and < LOQ on the day of the treatment decreasing to < LOQ, 0.02 mg/kg and ND respectively the day after treatment. According to SANTE/11956/2016 rev. 9 for spray applications sampling can be done within 1 day after drying of the residue, thus so it is possible that thymol residues will not be above the trigger value 0.05 mg/kg in the aerial parts of the crop. However, there are no data from aerial parts of the crop and the relationship weight-volume makes residue on the fruit as a not worst case scenario. Although this could lead to some uncertainties, there are two additional factors to consider: thymol occurs naturally in plants foraged by bees and is present in authorised veterinary treatments for bees as the Notifier stands in the paragraphs below.

For both grapes and pome fruit, the timing of application is such that application may occur during the period April to September i.e. when in-field weeds or adjacent crops may be flowering. However, thymol is found in a wide variety of plants. Since it is naturally occurring in plants such as thyme, it may be expected that bees will naturally be exposed to thymol when foraging[3] and thymol has been demonstrated to be naturally present in pollen and nectar, on which bees will feed[4]. Indeed, thymol has been found to

[3] Palmer-Young *et al.* (2017) Synergistic effects of floral phytochemicals against a bumble bee parasite Ecology and Evolution. 2017;7:1836–1849. KCA 6.10.1/01

[4] Wiese, N. *et al.* (2018) The terpenes of leaves, pollen, and nectar of thyme (*Thymus vulgaris*) inhibit growth of bee disease-associated microbes. Scientific Reports (2018) 8:14634. – KCA 6.10.1/02

be naturally occurring in Spanish honeydew honey derived from holm-oak, oak and the forest at mean concentrations of 1.0 – 6.5 µg/kg[5,6]. Thymol was found to be present at 0 – 6 ppm in chestnut honey and 18 – 161 ppm in lime honey and also notable concentrations were observed in rape, rosemary and sunflower honeys[7]. In another paper, thymol was found to be present at up to 49 µg/kg in floral honey and 97 µg/kg in heather honey[8].

Products containing thymol may be used for the control of varroa mite on bees[9]. The use of thymol in the hive results in significant concentrations of thymol in wax and feed in the brood comb and low residues in honey[10,11]. The use of thymol as a varroa treatment was found to increase the concentration of thymol in honey from a natural concentration of 0.036 mg/kg to 0.384 mg/kg following treatment[12] and in another study an approximate 10-fold increase to 2.8 g/kg was found following treatment[13]. In a third study, no thymol residues were found in the honey control samples, but residues were found in up to 80% of the treated samples at concentrations up to 1.1 mg/kg[14]. Concentrations were below the flavour threshold. Thymol is also found in propolis, following anti-varroa treatment, with concentrations between 31 and 76% or 11 and 44% reported for analysis by HS-SPME or SDE respectively[15]. Low concentrations may also be found in bee pollen following treatment (up to 57 µg/kg)[16]. The US EPA has established an exemption from the requirement of a tolerance for residues of thymol on honey and honeycomb[12].

Given thymol's natural occurrence at high concentrations in plants such as thyme, resulting in natural concentrations of thymol in bee products and also that thymol may be applied directly to the hive, resulting in a far greater exposure of bees to thymol than from plant protection product use, it is considered that studies on residues in bee products are not necessary.

Thymol is rapidly degraded in soil with a DT_{90} <3 days (please refer to MCA Section 7 for further details). Therefore, residues in succeeding crops and weeds are not applicable. Furthermore, grapes and pome fruit are not grown in rotation.

Thymol is not intended to be used in forestry and therefore potential exposure to honeydew from plant sucking insects is not relevant.

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[5] Castro-Vazquez, L. *et al*. (2006) Volatile Composition and Contribution to the Aroma of Spanish Honeydew Honeys. Identification of a New Chemical Marker. J Agric Food Chem 54: 4809-4813 (2006) – KCA 6.10.1/03

[6] Pažitná A, Džúrová J, Spánik I. (2014) Enantiomer distribution of major chiral volatile organic compounds in selected types of herbal honeys. Chirality. 2014 Oct;26(10):670-4. – KCA 6.10.1/04

[7] Guyot, C. *et al*. (1998) Floral Origin Markers of Chestnut and Lime Tree Honeys. J. Agric. Food Chem. 1998, 46, 625−633 – KCA 6.10.1/05

[8] Bernal, J. *et al*. (2020) Determination of Carvacrol and Thymol in Honey by Using a Simple and Efficient Headspace-Gas Chromatography-Mass Spectrometry Method. Food Analytical Methods (2020) 13:2138–2146 – KCA 6.10.1/06

[9] Tonello *et al*. (2016) Square wave voltammetry with multivariate calibration tools for determination of eugenol, carvacrol and thymol in honey Talanta158(2016)306–314 – KCA 6.10.1/07

[10] Bogdanov, S. *et al*. (1998) Residues in wax and honey after Apilife VAR® treatment. Apidologie 29 (1998) 513-524 – KCA 6.10.1/08

[11] Charpentier *et al*. (2013) Lethal and sub-lethal effects of thymol on honeybee (*Apis mellifera*) larvae reared *in vitro*. Pest Manag Sci 2014; 70: 140–147. – KCA 6.10.1/09

[12] Donders, J. *et al*. (2006) Varroa control preceding honey flow; thymol and formic acid residue. Proc. Neth. Entomol. Soc. Meet. Volume 17 2006. – KCA 6.10.1/10

[13] Adamczyk, S. *et al*. (2005) Evaluation of Residues of Essential Oil Components in Honey after Different Anti-*Varroa* Treatments. J. Agric. Food. Chem. 2005, 53, 10085-10090. KCA 6.10.1/11

[14] Tananaki, C. *et al*. (2014) Evaluation of the impact of Exomite Pro™ on Varroa mite (*Varroa destructor*) populations and honeybee (*Apis mellifera*) colonies: efficacy, side effects and residues. Parasitol Res (2014) 113:1251–1259 – KCA 6.10.1/12

[15] Jerković, I. *et al*. (2016) Comprehensive Study of Mediterranean (Croatian) Propolis Peculiarity: Headspace, Volatiles, Anti-*Varroa*-Treatment Residue, Phenolics, and Antioxidant Properties. Chem. Biodiversity 2016, 13, 210 – 218. – KCA 6.10.1/13

[16] Ares, A.M. *et al*. (2020) Simultaneous determination of carvacrol and thymol in bee pollen by using a simple and efficient solvent extraction method and gas chromatography-mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 181 (2020) 113124. – KCA 6.10.1/14

It is concluded that studies on the residue level in pollen and bee products are not required. Residues of thymol according to the intended GAP are probably to be below the trigger of 0.05 mg/kg. Also the natural occurrence of thymol in many flowers to which bees are attracted. It is considered that MRLs are also not necessary for honey. Thymol is naturally occurring in a wide variety of plants, on which bees forage and furthermore is used for an in-hive treatment to control varroa mite on bees. Therefore, any residues found in bee products, including honey could not necessarily be concluded to be due to plant protection product use.

Therefore studies on residues in bee products are not considered necessary.

2.7.8.2. Natural background levels

According to reported study "*Determination of natural background level residues of thymol, eugenol and geraniol in grapes*" (See Volume 3 CA B-7 for further details) thymol is naturally occurring in grapes. When the study was conducted there were no approvals for thymol to be used as a plant protection product. Therefore, all amounts found represented natural background concentrations. In this study to measure natural background concentrations in grapes, in 20 different grape samples thymol was found to be present in the range ND to 0.016 mg/kg.

Reported study S20-06337 contains 6 residue trials of thymol on grapes. Control plots showed residue values that could be assimilated to background levels of this order: 3 x ND, 2 x <LOQ, 0.01 mg/kg.
From reported study AF/10728/ED no residues of thymol were found in grapes samples collected before treatment, that could be assimilated to background levels, but the LOQ was set at 0.05 mg/kg and no difference was made between ND and < LOQ.

Regarding apples, representative crop of pome fruits, from study S20-06361 containing 6 residue trials of thymol on apples it can be deduced that background levels of thymol on apples were negligible (5 x ND, 1 x < LOQ (0.01 mg/kg)) according to data from control plots.

Therefore it can be deduce that thymol is naturally occurring in grapes, regarding apples only one of six trials showed background traces of thymol on apples.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic dietary consumer risk due to exposure to residues of thymol were performed using the EFSA model for chronic and acute risk assessment (PRIMo rev. 3.1).

It is proposed that MRLs are not required for thymol, due to its natural occurrence in a wide variety of crops. Residue trials have demonstrated that the plant protection product use of thymol according to the intended GAP does not increase the concentration of thymol above natural background levels.

Nevertheless, as a worst-case a risk assessment has been performed for thymol. For each commodity, default residue values of 0.01 mg/kg for thymol have been used for the risk assessment, as indicated within Table 2.7.9-01. The calculation is performed with all inputs set at 0.01 mg/kg. Although for grapes, in one trial, residues were found to be at this level, they were the same as the natural background concentrations and so the plant protection product use does not result in any residues above natural concentrations. Therefore 0.01 mg/kg is considered to represent a worst-case. For apples, all residues were <LOQ and so again an input at LOQ is considered to be worst-case. For all other commodities this is a default value based on the default levels at which MRLs would be set, if they were required.

Table 2.7.9-01: Thymol input values for the consumer risk assessment

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	**Comment**	**Input** (mg/kg)	**Comment**
Thymol				
Products of plant origin				
Grapes	0.01	Default value*	0.01	Default value†
Apples	0.01	Default value†	0.01	Default value†
All other commodities listed within Regulation (EU) 2018/62	0.01	Default value	0.01	Default value
Products of animal origin				
All products of livestock origin (as listed within Regulation (EU) 2018/62)	0.01	Default value	0.01	Default value

* Residues in trials were all the same as the control samples and so a default value is used to represent residues from plant protection product use.
† Residues in all residue trials were <LOQ (0.01 mg/kg)

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation:

The ADI for thymol is proposed at 0.07 mg/kg bw/day.

The calculation of the TMDI was performed using the EFSA PRIMo model rev. 3.1, taking into account all the crops to which thymol may be applied. As an initial worst-case, the calculation was performed using a default value of 0.01 mg/kg for all commodities (See Table 2.7.9-02).

With the current EFSA model the chronic risk assessment constitutes 2 % of the ADI. The diet with the highest TMDI was the NL toddler diet. For this diet the highest contributor is milk, followed by apples.
No refinement with use of the STMR is required.
When only the intended uses were considered, TMDI was also performed using the EFSA PRIMo model rev. 3.1 (see Table 2.7.9-03).

Acute Reference Dose (ARfD) and Dietary Exposure Calculation:

The ARfD for thymol is proposed at 0.4 mg/kg bw/day

The calculation of the IESTI was performed using the EFSA PRIMo model rev. 3.1, taking into account the intended uses. (See Table 2.7.9-04).

With the current EFSA model no exceedance of the ARfD was found for adults or children consuming processed or unprocessed commodities. Highest IESTI was 0.3 % of ARfD for pears and apples for children.
No refinement is required.

Comparison to background exposure

Thymol is naturally occurring in a wide variety of fruits, vegetables, herbs and spices (refer to Introduction to Vol.3CA-B.7). In this submission, use is supported on grapes and pome fruit. For grapes,

thymol was found to be naturally occurring in one of the control samples and no residues above natural background concentrations were found at harvest. No residues at or above the LOQ (0.01 mg/kg) were found in apples (representative crop for pome fruit).

Thymol is naturally occurring in grapes. In a study to measure natural background concentrations in grapes ("*Determination of natural background level residues of thymol, eugenol and geraniol in grapes*" (See Volume 3 CA B-7 for further details)), in 20 different grape samples thymol was found to be present in the range ND to 0.0165 mg/kg. Thus, the level of thymol found within the residue trials according to the intended GAP is within this natural concentration range for grapes.

It is clear from the information presented in the Introduction that thymol is present in many plant species. Human dietary exposure can therefore occur by either consuming the 'raw' source of thymol (e.g. citrus fruits, herbs, walnuts and honey) or consumption of thymol in beverages, such as tea, herbal teas and infused gins, is also a likely exposure route for humans.

It is clear that the use of thymol on grapes and pome fruit will not increase exposure of humans to thymol above background levels. Residue trials demonstrated no residues were found at harvest and there is significant human exposure from other routes.

Nevertheless, since an ADI and ARfD are set for thymol, a conventional risk assessment is possible (see above) and the comparison to background is not necessary for the demonstration of safe use.

As part of the confirmatory data submission for the first EU Review, an assessment of the exposure to thymol was made based on naturally occurring concentrations reported in the literature. Although the assessment was questioned by EFSA, the calculation demonstrates the potential for significant, natural exposure to thymol. It is important to note that:

1. This assessment is not necessary in terms of risk, because a conventional risk assessment using an ADI, ARfD and the results of residue trials indicates an acceptable risk.

2. Proprietary data should that thymol is naturally occurring in grapes. The residue trials generally showed no residues at or above the LOQ. In one trial residues were found at 0.01 mg/kg in both the test and control samples and so there is no increase in concentrations of thymol following application.

3. Thymol is approved for use as a food additive at higher concentrations than any residues reported in the residue trials.

4. Thymol is approved for use in animal feed, resulting in a potential for exposure via non-plant protection product use.

5. The daily intake per capita is calculated to be 0.059 mg/day, equivalent to 0.001 mg/kg bw/day assuming a 60 kg body weight. This is lower than in the USA where the total annual volume of production of thymol is estimated to be 1240 kg and the daily intake per capita is calculated to be 0.16 mg/day, equivalent to 0.003 mg/kg bw/day assuming a 60 kg body weight (see Introduction).

   The highest calculated dietary exposure was 0.00124 mg/kg bw/day for the NL toddler, followed by 0.00065 mg/kg bw/day for the NL child, both based on milk. Residues are not expected in milk based on the representative uses and so this is clearly worst-case. If a refined calculation is made only considering the target crops (grapes and pome fruit), then the highest calculated dietary exposure is 0.00017 mg/kg bw/day for the NL toddler, based on apples. This calculation is also worst-case because it is based upon residues at the LOQ (0.01 mg/kg) and residues in trials were all either less than the LOQ or no higher than natural background concentrations.

   The estimated daily intake per capita is well in excess of the highest, worst-case calculations for dietary intake resulting from plant protection product use.

Table 2.7.9-02: Theoretical Maximum Daily Intake (TMDI) for thymol calculated using EFSA PRIMo rev.3.1 (normal mode)

European Food Safety Authority

EFSA PRIMo revision 3.1 2019/03/19

Thymol

LOQs (mg/kg) range from to

Toxicological reference values

ADI (mg/kg bw/day)	0,07	ARfD (mg/kg bw)	0,4
Source of ADI		Source of ARfD	
Year of evaluation		Year of evaluation	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments

Normal mode

Chronic risk assessment JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI: ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
2%	NL toddler	1,24	0,9%	Milk Cattle	0,2%	Apples	0,1%	Maize/corn		
0,9%	NL child	0,65	0,3%	Milk Cattle	0,1%	Sugar beet roots	0,1%	Apples		
0,9%	DE child	0,61	0,3%	Milk Cattle	0,2%	Apples	0,1%	Wheat		
0,9%	UK infant	0,61	0,6%	Milk Cattle	0,0%	Potatoes	0,0%	Wheat		
0,8%	FR toddler 2 3 yr	0,54	0,4%	Milk Cattle	0,0%	Apples	0,0%	Wheat		
0,8%	FR child 3 15 yr	0,53	0,3%	Milk Cattle	0,1%	Wheat	0,1%	Sugar beet roots		
0,6%	UK toddler	0,45	0,3%	Milk Cattle	0,1%	Wheat	0,0%	Potatoes		
0,6%	DK child	0,41	0,2%	Milk Cattle	0,1%	Rye	0,1%	Wheat		
0,6%	GEMS/Food G11	0,39	0,1%	Milk Cattle	0,1%	Potatoes	0,1%	Soyabeans		
0,5%	RO general	0,38	0,2%	Milk Cattle	0,1%	Wheat	0,1%	Potatoes		
0,5%	GEMS/Food G06	0,38	0,1%	Wheat	0,1%	Tomatoes	0,0%	Milk Cattle		
0,5%	SE general	0,37	0,2%	Milk Cattle	0,1%	Bovine Muscle/meat	0,1%	Potatoes		
0,5%	GEMS/Food G07	0,37	0,1%	Milk Cattle	0,1%	Wheat	0,1%	Potatoes		
0,5%	GEMS/Food G15	0,37	0,1%	Milk Cattle	0,1%	Wheat	0,1%	Potatoes		
0,5%	GEMS/Food G08	0,36	0,1%	Milk Cattle	0,1%	Wheat	0,1%	Potatoes		
0,5%	GEMS/Food G10	0,36	0,1%	Milk Cattle	0,1%	Wheat	0,0%	Soyabeans		
0,5%	ES child	0,35	0,2%	Milk Cattle	0,1%	Wheat	0,0%	Oranges		
0,5%	DE women 14-50 yr	0,34	0,2%	Milk Cattle	0,1%	Sugar beet roots	0,0%	Apples		
0,5%	DE general	0,34	0,2%	Milk Cattle	0,1%	Sugar beet roots	0,0%	Apples		
0,5%	IE adult	0,32	0,1%	Milk Cattle	0,1%	Sweet potatoes	0,0%	Wheat		
0,4%	FR infant	0,29	0,2%	Milk Cattle	0,0%	Potatoes	0,0%	Apples		
0,4%	NL general	0,28	0,1%	Milk Cattle	0,0%	Sugar beet roots	0,0%	Potatoes		
0,3%	PT general	0,21	0,1%	Potatoes	0,1%	Wheat	0,0%	Wine grapes		
0,3%	ES adult	0,20	0,1%	Milk Cattle	0,0%	Wheat	0,0%	Oranges		
0,3%	FR adult	0,19	0,1%	Milk Cattle	0,0%	Wine grapes	0,0%	Wheat		
0,2%	FI 3 yr	0,17	0,1%	Potatoes	0,0%	Bananas	0,0%	Wheat		
0,2%	IT toddler	0,16	0,1%	Wheat	0,0%	Other cereals	0,0%	Tomatoes		
0,2%	DK adult	0,16	0,1%	Milk Cattle	0,0%	Potatoes	0,0%	Wheat		
0,2%	LT adult	0,16	0,1%	Milk Cattle	0,0%	Potatoes	0,0%	Apples		
0,2%	UK vegetarian	0,14	0,0%	Milk Cattle	0,0%	Wheat	0,0%	Potatoes		
0,2%	UK adult	0,13	0,0%	Milk Cattle	0,0%	Wheat	0,0%	Potatoes		
0,2%	FI 6 yr	0,13	0,1%	Potatoes	0,0%	Wheat	0,0%	Bananas		
0,2%	FI adult	0,13	0,1%	Coffee beans	0,0%	Potatoes	0,0%	Rye		
0,2%	IT adult	0,12	0,1%	Wheat	0,0%	Tomatoes	0,0%	Apples		
0,1%	PL general	0,10	0,0%	Potatoes	0,0%	Apples	0,0%	Tomatoes		
0,1%	IE child	0,08	0,1%	Milk Cattle	0,0%	Wheat	0,0%	Potatoes		

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of Thymol is unlikely to present a public health concern.

Table 2.7.9-03: Theoretical Maximum Daily Intake (TMDI) for thymol calculated using EFSA PRIMo rev.3.1 (intended uses)

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

LOQs (mg/kg) range from	0,01	to	0,01

Toxicological reference values

ADI (mg/kg bw/day)	0,07	ARfD (mg/kg bw)	0,4
Source of ADI		Source of ARfD	
Year of evaluation		Year of evaluation	

Input values

Details - chronic risk assessment

Supplementary results - chronic risk assessment

Details - acute risk assessment/children

Details - acute risk assessment/adults

Comments

Normal mode

Chronic risk assessment JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI: ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
0,2%	NL toddler	0,17	0,2%	Apples	0,1%	Pears	0,0%	Table grapes	0,2%	
0,2%	DE child	0,15	0,2%	Apples	0,0%	Table grapes	0,0%	Pears	0,2%	
0,1%	NL child	0,08	0,1%	Apples	0,0%	Pears	0,0%	Table grapes	0,1%	
0,1%	PT general	0,04	0,0%	Wine grapes	0,0%	Apples	0,0%	Pears	0,1%	
0,1%	DE women 14-50 yr	0,04	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,1%	
0,1%	FR toddler 2 3 yr	0,04	0,0%	Apples	0,0%	Pears	0,0%	Wine grapes	0,1%	
0,1%	DE general	0,04	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,1%	
0,0%	RO general	0,03	0,0%	Wine grapes	0,0%	Apples	0,0%	Table grapes	0,0%	
0,0%	FR adult	0,03	0,0%	Wine grapes	0,0%	Apples	0,0%	Pears	0,0%	
0,0%	DK child	0,03	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	GEMS/Food G11	0,03	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	GEMS/Food G07	0,03	0,0%	Wine grapes	0,0%	Apples	0,0%	Table grapes	0,0%	
0,0%	GEMS/Food G08	0,03	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	GEMS/Food G15	0,03	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	FR child 3 15 yr	0,03	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	IE adult	0,03	0,0%	Wine grapes	0,0%	Apples	0,0%	Pears	0,0%	
0,0%	PL general	0,03	0,0%	Apples	0,0%	Table grapes	0,0%	Pears	0,0%	
0,0%	NL general	0,02	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	DK adult	0,02	0,0%	Apples	0,0%	Wine grapes	0,0%	Pears	0,0%	
0,0%	GEMS/Food G06	0,02	0,0%	Table grapes	0,0%	Apples	0,0%	Pears	0,0%	
0,0%	UK toddler	0,02	0,0%	Apples	0,0%	Table grapes	0,0%	Pears	0,0%	
0,0%	LT adult	0,02	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	FR infant	0,02	0,0%	Apples	0,0%	Pears	0,0%	Wine grapes	0,0%	
0,0%	UK infant	0,02	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	GEMS/Food G10	0,02	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	ES child	0,02	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	UK adult	0,02	0,0%	Wine grapes	0,0%	Apples	0,0%	Pears	0,0%	
0,0%	ES adult	0,02	0,0%	Apples	0,0%	Wine grapes	0,0%	Pears	0,0%	
0,0%	UK vegetarian	0,02	0,0%	Wine grapes	0,0%	Apples	0,0%	Pears	0,0%	
0,0%	SE general	0,01	0,0%	Apples	0,0%	Pears	0,0%	Quinces	0,0%	
0,0%	FI 3 yr	0,01	0,0%	Apples	0,0%	Table grapes	0,0%	Pears	0,0%	
0,0%	IT toddler	0,01	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	IT adult	0,01	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	FI adult	0,01	0,0%	Apples	0,0%	Wine grapes	0,0%	Table grapes	0,0%	
0,0%	FI 6 yr	0,01	0,0%	Apples	0,0%	Pears	0,0%	Table grapes	0,0%	
0,0%	IE child	0,00	0,0%	Apples	0,0%	Table grapes	0,0%	Pears	0,0%	

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of is unlikely to present a public health concern.

Table 2.7.9-04: International Estimate of Short-Term Intake (IESTI) for thymol calculated using EFSA PRIMo rev.3.1 (intended uses)

Acute risk assessment /children	Acute risk assessment / adults / general population	Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults	Hide IESTI new calculations	Show IESTI new calculations

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

IESTI new calculations:
The calculation is performed with the MRL and the peeling/processing factor (PF), taking into account the residue in the edible portion and/or the conversion factor for the residue definition (CF). For case 2a, 2b and 3 calculations a variability factor of 3 is used. Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.
Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.

Show results for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,3%	Pears	0,01 / 0,01	1,4
0,3%	Apples	0,01 / 0,01	1,1
0,2%	Table grapes	0,01 / 0,01	0,73
0,06%	Quinces	0,01 / 0,01	0,25
0,03%	Medlar	0,01 / 0,01	0,14
0,02%	Wine grapes	0,01 / 0,01	0,09

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,08%	Table grapes	0,01 / 0,01	0,34
0,08%	Pears	0,01 / 0,01	0,31
0,07%	Apples	0,01 / 0,01	0,28
0,06%	Wine grapes	0,01 / 0,01	0,24
0,04%	Quinces	0,01 / 0,01	0,15
0,02%	Medlar	0,01 / 0,01	0,07

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new) ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,2%	Apples	0,01 / 0,01	0,62
0,1%	Pears	0,01 / 0,01	0,59
0,1%	Table grapes	0,01 / 0,01	0,44
0,04%	Quinces	0,01 / 0,01	0,15
0,03%	Medlar	0,01 / 0,01	0,10
0,02%	Wine grapes	0,01 / 0,01	0,09

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new) ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,09%	Pears	0,01 / 0,01	0,36
0,08%	Apples	0,01 / 0,01	0,30
0,06%	Wine grapes	0,01 / 0,01	0,24
0,05%	Table grapes	0,01 / 0,01	0,20
0,02%	Quinces	0,01 / 0,01	0,09
0,01%	Medlar	0,01 / 0,01	0,05

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,1%	Apples / juice	0,01 / 0,01	0,54
0,1%	Wine grapes / juice	0,01 / 0,01	0,44
0,1%	Pears / juice	0,01 / 0,01	0,33
0,0%	Quinces / jam	0,01 / 0,01	0,03
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Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,1%	Apples / juice	0,01 / 0,01	0,33
0,05%	Wine grapes / juice	0,01 / 0,01	0,21
0,02%	Wine grapes / wine	0,01 / 0,01	0,09
0,01%	Table grapes / raisins	0,01 / 0,05	0,06
0,00%	Quinces / jam	0,01 / 0,01	0,01
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#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
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#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
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Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new) ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,1%	Apples / juice	0,01 / 0,01	0,54
0,1%	Wine grapes / juice	0,01 / 0,01	0,44
0,08%	Pears / juice	0,01 / 0,01	0,33
0,01%	Quinces / jam	0,01 / 0,01	0,03
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#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
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#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new) ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,08%	Apples / juice	0,01 / 0,01	0,33
0,05%	Wine grapes / juice	0,01 / 0,01	0,21
0,02%	Wine grapes / wine	0,01 / 0,01	0,09
0,01%	Table grapes / raisins	0,01 / 0,05	0,06
0,00%	Quinces / jam	0,01 / 0,01	0,01
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
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#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Expand/collapse list

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of is unlikely to present a public health risk.
For processed commodities no exceedance of the ARfD/ADI was identified.

2.7.10 Proposed MRLs and compliance with existing MRLs

Thymol is temporarily included into Annex IV to Regulation (EC) No. 396/2005

In residue trials, for grapes, no residues above natural background concentrations were found at harvest. No residues at or above the LOQ (0.01 mg/kg) were found in apples (representative crop for pome fruit) at harvest. Thymol is naturally occurring in grapes, it is clear that the exposure due to plant protection product use is not higher than natural exposure to thymol. Furthermore, thymol is naturally found in a wide variety of plants in addition to grapes and apples (refer to Introduction to Vol 3 CA B-7), and therefore natural background exposure is further increased via consumption of other foodstuffs containing thymol.

It is proposed that thymol should be fully included into Annex IV to Regulation (EC) No. 396/2005. MRLs are not required for thymol. Thymol meets criterion 4 (The consumer exposure to the compound linked to use as PPP is considered as negligible compared to other uses in the food chain and/or natural background) of SANCO/11188/2013 Rev. 2, 14 September 2015 (Guidance document on criteria for the inclusion of active substances into Annex IV of Regulation (EC) N° 396/2005).

Proposed MRLs for commodities relevant to this submission are detailed in the table below.

Table 2.7.10-01: Proposed MRLs for thymol for commodities in this submission

Code	Commodity	Proposed EU MRL	Current MRL*
0151010	Table grapes	No MRL required	No MRL required
0151020	Wine grapes	No MRL required	No MRL required
0130000	Pome fruit	No MRL required	No MRL required
1000000	Products of animal origin	No MRL required	No MRL required

* Temporary inclusion into Annex IV

2.7.11 Proposed import tolerances and compliance with existing import tolerances

No imported products are considered in this submission hence proposed MRLs for import tolerances are not required.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Route of degradation in soil

2.8.1.1.1 Aerobic degradation in soil

The route and rate of degradation of thymol were studied in four soils in the laboratory under aerobic conditions (Jones, A., 2015a). Soil samples were treated with [^{14}C]-thymol at a nominal concentration of 1.04 mg/kg, approximately equivalent to a use rate of 1040 g ai/ha. The samples were incubated under aerobic conditions in the dark at about 20°C at a moisture content equivalent to pF 2 for periods of up to 120 days after application. Combined soil extracts were analysed by HPLC with fraction collection.

The overall recoveries of applied radioactivity (AR) were in the range 90 – 110% AR. The variable recoveries of radioactivity may be due to the inhomogeneous nature of the non-extractable residue, and the difficulties in accurately quantifying this fraction.

The proportion of radioactivity extracted from soil decreased with time with a corresponding increase in the levels of non-extractable radioactivity and $^{14}CO_2$ evolution. Extractable radioactivity declined with time from mean values of 80.8 – 91.8% AR in each soil at zero-time to mean values of 11.4 – 25.5% AR after 3 days incubation and then remained at a similar level throughout the incubation period. Non-extractable radioactivity accounted for mean values of 72.4 – 78.2% AR after 3 days and accounted for 62.0 – 78.2% AR after 120 days. Volatile radioactivity, characterised as $^{14}CO_2$, represented 11.9 – 28.2% AR after 120 days. Thymol declined rapidly in each soil type. The DT_{50} and DT_{90} values were 0.6-0.8 days and 1.8-2.6 days respectively.

Thymol degraded to five minor unidentified degradates (none of which individually accounted for >10% AR), bound residues and carbon dioxide.

2.8.1.1.2 Anaerobic degradation in soil

No studies on anaerobic degradation have been performed. Based on the fast aerobic degradation and the application timing of Mevalone, it is unlikely that thymol residues would be found in anaerobic conditions.

2.8.1.1.3 Photodegradation in soil

No data has been submitted. The following justification has been submitted by applicant: based on the fast degradation rate of thymol it is unlikely that thymol will be present long enough for photolysis to occur.

However, according to section 7.1.1.3 of Regulation (EU) No. 283/2013 a soil photolysis study shall be submitted unless the applicant shows that deposition of the active substance on the soil surface is unlikely to occur or that photolysis is not expected to contribute significantly to the degradation of the active substance in soil for example due to low light absorbance of the active substance. **Therefore, RMS has identified a data gap for a soil photolysis study.**

2.8.1.1.4 Overall route of degradation

In studies designed to investigate the route and rate of aerobic soil degradation of thymol, no major soil metabolites or degradation products were observed at levels exceeding 10% of the applied amount, nor at above 5% of the applied amount for two sequential measurements, nor above 5% of the applied amount at the final measurement.

Thymol degraded rapidly to multiple minor unidentified degradates, bound residues and carbon dioxide.
The proposed degradation pathway is shown below in Figure 1.

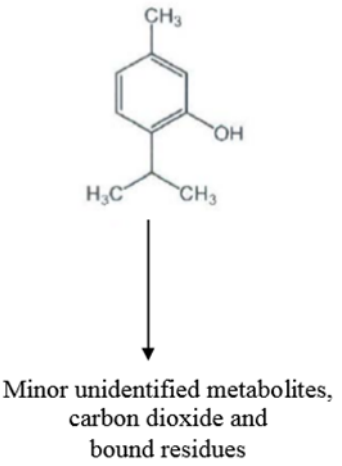


Figure 1: Transformation pathway

2.8.1.2 Rate of degradation in soil

2.8.1.2.1 Laboratory conditions

Thymol declined rapidly in each soil type. The DT50 and DT90 values were <1 day and <3 days respectively. The decline of thymol in aerobic soil with time was well described by the single first order (SFO) kinetic model. The persistence and modelling endpoints are summarized in the table below.

Table 68: Persistence and Modelling endpoints for thymol in soil

Soil	OC (%)	pH*	T. °C / % moisture	DT_{50} /DT_{90} (d)	DT_{50} (d) 20 °C pF2/10kPa[b)]	Kinetic model	Visual goodness-of-fit
Brierlow (Jones 2015)	3.5	5.3	20°C / pF2	0.6 / 2.1	<1	SFO	Good
Calke (Jones, 2015)	3.4	5.1	20°C / pF2	0.6 / 2.1	<1	SFO	Good
Ingleby (Jones, 2015)	1.8	4.7	20°C / pF2	0.8 / 2.6	<1	SFO	Good
Empingham (Jones, 2015)	4.4	7.1	20°C / pF2	0.6 / 1.8	<1	SFO	Good

[a] the visual fit was based on 2 sampling intervals only
NA insufficient data points to calculate Chi^2 value
*0.1M $CaCl_2$
For modelling purposes, a conservative DT 50 of 1 day has been proposed

2.8.1.2.2 Field dissipation studies

Based on the laboratory DT90 of < 3 days, field studies are not triggered for thymol and therefore no data is submitted.

2.8.1.2.3 Soil accumulation studies

Not required.

2.8.1.2.4 Assessment of Persistence in soil (P) in soil

Based on the rapid degradation rates of thymol in soil, it is not expected to be persistent in a viable soil environment.

2.8.1.3 Mobility in soil

2.8.1.3.1 Adsorption/Desorption

Two adsorption/desorption studies have been submitted. The first study by Jones, 2015b was previously peer reviewed in the context of the confirmatory data. Several deviations were found with respect to the OECD 106 guideline and it was considered that the study by Jones, 2015b was not robust enough to be used to derive the sorption endpoint for thymol.
The Annex I inclusion RMS (UK) suggested that due to the rapid degradation of thymol, it's stability during a batch equilibrium adsorption/desorption study could not be relied upon. Additionally, the indirect method was employed, while the OECD106 specifically recommends as not appropriate for this type of substance. Therefore, it was concluded that the adsorption calculated in this study using the indirect method, was not representative of thymol alone. Instead, the values calculated were mean characteristics for the range of compounds shown on the radiochromatograms.
The second study has been submitted for the renewal process since the previous one was not considered valid. Cashmore, 2021 studied the adsorption/desorption characteristics of thymol in four soils using a batch equilibrium method, in the dark at 20 ± 2°C. The test was performed with non-radiolabelled thymol and direct method was employed.
Freundlich coefficients (KF) were in the range 1.021 to 5.361 L/kg for adsorption and 1.624 to 5.816 L/kg for desorption. When corrected for organic carbon, the corresponding KFOC values were in the range of 92.8 to 171.0 L/kg for adsorption and 126.4 to 250.7 L/kg for desorption. The corresponding 1/n values ranged from 0.830 to 0.958.
Using the McCall Classification scale to assess a chemical's potential mobility in soil (based on its mean KOC), Thymol can be classified as having a "high" potential

Table 69: Freundlich coefficients and exponents for thymol in sterilised soils

Parent							
Soil Type	OC %	Soil pH[a)]	K_d (mL/g)	K_{doc} (mL/g)	K_F (mL/g)	K_{Foc} (mL/g)	1/n
RefeSol 01-A, Sandy loam	1.0	5.4	-	-	1.710	171.0	0.908
RefeSol 03-G, Silty clay loam	4.6	6.0	-	-	5.361	116.6	0.929
Kenslow, Clay loam	3.8	5.0	-	-	4.137	108.9	0.958
La Reina, Clay	1.1	7.7	-	-	1.021	92.8	0.830
Geometric mean (if not pH dependent)*						119.14	
Arithmetic mean (if not pH dependent)							0.906

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Available environmental fate studies have been considered and summarised in the Thymol Monograph (Volume 3, Annex B8, May 2011) and in the renewal of approval dossier (dRAR, Volume 3, Annex B8).

The key information pertinent to determine the environmental hazard classification for Thymol is presented below.

Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the representative test guideline, if applicable. Full robust summaries of these studies are presented in Annex 1 to this dossier.

2.8.2.1 2.8.2.1 Rapid degradability of organic substances

Table 70: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
Ready biodegradability. OECD 301F,	87 % degradation after 28 days.	Key. The study is considered acceptable.	Test substance: Thymol Purity: 99.7%	CA 7.2.2.1/01 Seyfield, B (2007) Study number B34435

Method	Results	Key or Supportive study	Remarks	Reference
C.4-D Manometric Respirometry Test, 1992			Ready biodebradable	
Ready biodegradability. OECD 301F, C.4-E Manometric Respirometry Test, 1992	83 % degradation after 28 days.	Key. The study is considered acceptable.	Test substance: Thymol Purity: 99.5% Ready biodebradable	Anonymous (2010)
Ready biodegradability. OECD 301C	82 % degradation after 28 days but a concentration of 30 g/L.	Non GLP Supportive study	Test substance: Thymol Purity: > 95% Ready biodebradable	Nabeoka, R. et al. (2016)
Ready biodegradability. No guideline	Removal efficiency was around 95%.	Non GLP Supportive study	Test substance: eighteen compounds from five municipal sewage treatment plants Purity: - Ready biodebradable	Nakada, N., et al (2006)
Aerobic mineralisation in surface water. OECD 309	<u>SFO</u> 7.9-16.3 days <u>HS</u> 42.8-60.3 days (slow phase low/high conc) 3.42-1.24 days (fast phase low/high conc) tB (Lag phase) 9.675-17.78 days (low/high conc)	The study is considered acceptable to establish the DT50.	Test substance: Thymol Purity: 99.58% The route of degradation has not been addressed.	Anonymous (2021)
Hydrolysis OECD 111	Stable at 50 ºC and pH 4 and pH 7. $DegT_{50}$ 1048.7 days at 25ºC, pH 9.	Key The study is considered acceptable.	Test substance: Thymol Purity: 99.58%	Anonymous (2021)
Photolysis in water. OECD 316	DT50: 33.9/60.6 days (normalised / unnormalised) Summer sunlight 30-50°N	The study is considered acceptable to establish the DT50.	Test substance: Thymol Purity: 99.58%	Anonymous (2022)

Method	Results	Key or Supportive study	Remarks	Reference
			The route of degradation has not been addressed.	

2.8.2.1.1 Ready biodegradability

The ready biodegradability of thymol has been previously evaluated, and the study remains valid (Seyfield, 2007). There is a study from REACH Registration dossier (Anonymous, 2010) and there are also two supplentary studies from literature review, which all of them are summarised below. Thymol is readily biodegradable.

Seyfield, B., (2007)

The ready biodegradability of thymol was determined in a manometric respirometry test over 28 days in accordance with OECD test guideline 301 F and to GLP. The percent biodegradation of the test item was calculated based on the theoretical oxygen demand (ThOD) of 2.77 mg O2/mg test item.

Aqueous test solutions of thymol (purity 99.7%) at a concentration of 103 mg/L were inoculated with aerobic activated sludge from a wastewater treatment plant treating predominantly domestic wastewater. Samples were incubated in airtight flasks under aerobic conditions in the dark at 22°C. Prior to the test, the pH was measured in each test flask before the addition of the activated sludge inoculum. The pH was between 7.2 and 7.3 in all test flasks and thus was not adjusted. At the end of incubation, the pH was measured again in each test flask.

In the toxicity control, containing both thymol and the reference item sodium benzoate, biodegradation was >25% within 14 days of exposure. Thus, thymol had no inhibitory effect on the activity of activated sludge microorganisms at the concentration of 98 mg/L.

In the procedure controls, the reference item sodium benzoate was degraded by an average of 94% by exposure day 14, and reached an average biodegradation of 99% by the end of the test (day 28), thus confirming suitability of the activated sludge.

The biochemical oxygen demand (BOD) of thymol in the test media significantly increased from about Exposure Day 3 until test termination after 28 days. At the end of the 28-day exposure period, the mean biodegradation of thymol amounted to 87%. Consequently, thymol was found to be biodegradable under the test conditions within 28 days. Moreover, the pass level for ready biodegradability, i.e., biodegradation of at least 60% of the ThOD in a 10-day window within the 28-day period of the test, was reached.

The study is considered acceptable and Thymol can be classified as readily biodegradable under the test conditions.

Anonymous (2010)

The objective of this study was to assess the ready biodegradability of the test material thymol. The study was conducted according to EU Method C.4-E (Determination of the "Ready" Biodegradability - Closed Bottle Test) under GLP requirements. The test item thymol (purity: 99.5%) in a mineral medium was inoculated with secondary effluent to result in completely filled, closed flasks and incubated under aerobic conditions at 22°C for 28 days in the dark.

During this period, the biodegradation was followed by analysis of dissolved oxygen. The amount of oxygen taken up by the test chemical, corrected for uptake by the blank inoculum run in parallel, was expressed as a percentage of theoretical oxygen demand (ThOD). A degradation of 83 % within 28 days was observed for thymol; and hence thymol is considered readily biodegradable (Anonymous, 2010). The reference compound sodium benzoate showed 78 % degradation after 14 days.

The study is considered acceptable.

Nabeoka, R., et al. (2016).

The authors performed test guideline 301C tests at test concentrations of 30 mg/L for 13 substances that were readily biodegradable in ready biodegradability tests but not in test guideline 301C tests. Of the 5 substances with

potential to cause microbial toxicity at 100 mg/L, the percentage of biodegradation of sodium dimethyldithiocarbamate, 4-chloro-3-cresol (CC), thymol (THY), and p-tert-butyl-a-methylbenzenepropionaldehyde measured by biochemical oxygen demand (BOD) increased in the test guideline 301C test at 30 mg/L, suggesting a reduction in toxicity effects.

Activated sludge was prepared and cultivated according to the protocol specified in guideline 301C. None of the prepared activated sludge was acclimated to the test substances. Mineral medium at pH 7 was prepared according to test guideline 301C.

The test solutions were prepared by using 30 mg/L and 100 mg/L of thymol (purity > 95%), incubated at 25±1°C under dark conditions for 28 d while being continuously stirred with a stir bar. Biochemical oxygen demand was continuously measured using a manometric respirometer. Theoretical oxygen demand was calculated on the assumption that no nitrification had occurred.

The validity criteria concerning the oxygen uptake of the blank control and the reference substance reaching the pass levels by day 14, as stipulated by the OECD guideline, were met for all test substances. The results of the validity test were acceptable with sodium benzoate and aniline degrading at >80%.

The ready biodegradability of thymol was determined in accordance with the OECD test guideline 301C, but at a lower concentration (30 g/L). Thymol was readily biodegradable under the conditions of the test; the mean degradation was 82% in 28 days.

The study is non GLP and it is considerd as supporting information.

Nakada, N., et al (2006)

The authors measured eighteen compounds in 24-h composite samples of influents and secondary effluents collected seasonally from five municipal sewage treatment plants in Tokyo.

Thymol was extracted using SPE under neutral conditions. The recovery of thymol in spiked STP effluent was 56% and concentrations were not corrected for recovery.

The reduction in the concentration of thymol observed between the data measurements in the influent and effluent of the STPs, supports that thymol is readily degraded. Removal efficiency was around 95%.

The study is considerd as supporting information.

2.8.2.1.2 BOD5/COD

No data available.

2.8.2.2 2.8.2.2 Other convincing scientific evidence

No other data available.

2.8.2.2.1 Aquatic simulation tests

Anonymous, (2021).

The degradation rate of thymol in a surface water test system (Oak Beck) was studied at 20 ± 2°C in the dark (.Anonymous, 2021). The test was performed using two application rates (95 and 10 µg/L) Due to problems with synthesis of the radiolabelled thymol, this study was performed with non-labelled thymol (purity 99.58%). This meant that released CO_2 (mineralisation) could not be assessed. Sterilised samples were also tested at the higher application rate.

Sub-samples of non-sterile water were removed for analysis at 0, 1, 2, 4, 7, 15, 21 and 30 DAT (days after treatment) at the low application rate and 0, 1, 2, 4, 7, 15, 21, 30 DAT and 35 DAT at the high application rate. The sterile vessels were sampled at 35 DAT.

The validation of the analytical method was assessed against the linearity, matrix assessment, specific, extract stability, precisions and accuracy. The LOQ was the lowest validation level where the validation criteria were met, which was 0.001 µg/mL.

Mean recoveries from fortified samples were within the acceptable range of 70-120% and precision (% RSD) was

within the acceptable limit of ≤ 20% at each fortification level.

The mineralisation of sodium [14C]-benzoate to $^{14}CO_2$ from the surface water exceeded 50% AR by 7 DAT, the surface water used was microbially active. However, the extent of mineralization of thymol could not be calculated since non-radiolabelled thymol was used.

The degradation of thymol was significantly slower under sterile conditions. Levels of thymol were <LOD at the end of the respective incubation periods (30 DAT and 35 DAT at the low and high application rates, respectively) in the non-sterile vessels and present at 80% (nominal application rate) at 35 DAT in the sterile vessels.

Concentrations of thymol (% nominal application rate) for individual vessels (0 and 30 DAT for the low application rate and 0 and 35 DAT for the high application rate) were fitted to SFO and HS kinetic models in accordance to FOCUS guidelines. The existence of a lag phase required modelling of individual vessels using HS kinetics.

Aerobic degradation of thymol in a surface water test system was characterised by a variable length lag phase, with slow degradation, followed by a sudden and much more rapid degradation. The data did not fit SFO models well but were fitted to HS models to obtain the following DT50 values for the slow and rapid phases for the two application rates tested. Lag phase duration was 5 and 14 days for the low application rate vessels and was 15 and 21 days for the high application rate vessels.

SFO kinetics gave DT_{50} values of 7.9-16.3 days.

Degradation was significantly slower under sterile conditions showing that the degradation was primarily microbial.

According to Regulation 283/2013, the rate of degradation and the pathway or pathways shall be reported either for a ‘pelagic’ test system or for a ‘suspended sediment’ system.

Results obtained shall be presented in the form of schematic drawings showing the pathways involved, and in the form of balance sheets which show the distribution of radio-label in water and, where relevant, sediment as a function of time, as between: (a) active substance; (b) CO2; (c) volatile compounds other than CO2; and (d) individual identified transformation products. Therefore, it is considered that this data requirement is not fulfilled.

A new aerobic mineralization study using radiolabelled thymol is required.

The study could be valid to satablish the rate but not the degradation pathway.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No data available.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

No studies provided.

2.8.2.2.4 Soil and sediment degradation data

No studies available.
Based on Ready Biodegradability, DT_{50}’s from ReACH guidance of 15 days in water and 300 days in sediment have been used for the risk assessments.

2.8.2.2.5 Hydrolysis

This study has not been previously evaluated and it was provided in support of the assessment for the renewal of Thymol according to Regulation (EC) No 1107/2009.

. Anonymous (2021)

The hydrolysis of thymol was studied in the dark in sterile aqueous buffered solutions at pH 4 (acetate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer) by Anonymous, 2021 according to OECD guideline 111 and GLP. The study was performed with non-radiolabelled thymol (purity 99.58%).
According to the study report, in the Tier I test, duplicate vessels were analysed at the start (0 days) and after incubation at 50ºC for 0.1, 1 and 5 days. Thymol was shown to be stable at 50 ± 0.5°C in pH 4 and 7 buffers (no significant change in the levels of thymol was observed (< 10% hydrolysis of thymol)), and unstable in pH 9 buffer (see Table below).

pH	Mean Recovery of thymol	
	0 Day	5 Day
4	101.5	92.1
7	94.5	88.2
9	100.1	89.3

Tier II was only performed at pH 9 since > 10% decrease in the levels of thymol was observed. At Tier II test, concentration of 1.0 mg thymol/L was treated in pH 9 buffer at 40, 50 and 60ºC for up to 30 days. Thymol decreased from mean values of 86.8 to 87.8% at 0 DAT to between 46.3% at 60°C and 81.0% at 40°C at the end of incubation (see table below).

Nominal Sampling Interval (days)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Replicate	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
Temperature (°C)							
40	86.8	87.1	83.4	81.8	80.0	80.0	81.0
50	86.9	84.7	78.2	76.9	69.4	72.3	67.7
60	87.8	75.7	69.9	67.0	59.5	53.6	46.3

The degradation rate ($DegT_{50}$) was determined using non-linear regression and single first-order kinetics model and it was estimated to be 1048.7 days at 25ºC, using an Arrhenius plot.

The study did not include the identification of any degradation products, however, since thymol could be considered stable under natural conditions, no metabolites are expected to appear at concentrations of significance.

The study is considered acceptable. Thymol is considered hydrolytically stable at pH4 and 7 and the $DegT_{50}$ was estimated as 1048.7 days, at pH 9.

2.8.2.2.6 Photochemical degradation

This study has not been previously evaluated and it was provided in support of the assessment for the renewal of thymol according to Regulation (EC) No 1107/2009.

Anonymous (2022).

The photodegradation of non-radiolabelled thymol was investigated in sterile aqueous buffered solutions according to OECD 316. Thymol (purity 99.58%) was applied, at a rate of 2 µg/mL, to sterile pH 7 buffer solutions in individual photolysis vessels or serum vials for incubation in the dark. The treated irradiated buffer solutions were continuously irradiated using light from a xenon arc lamp. Treated vessels were maintained at *ca* 25 ± 2°C in either dark or light conditions. Duplicate samples were taken from the dark vessels for analysis immediately after treatment and from both dark and light vessels at the other sampling times. Vessels were treated on six occasions and sampled on two occasions to allow incubation times of 0, 1, 3, 8, 14, 21 and 28 days.

Recoveries of thymol ranged from 91.3% at 0 day to 69.1% at 28 days (irradiated unamended) and from 91.3% at 0 days to 112.2% at 28 days (dark control unamended)

The rate of transformation of Thymol in pH 7 buffer under dark and irradiated conditions, was modelled using single first-order (SFO) kinetics and the results were accepted based on visual fit and chi^2 values.

The mean fortification recoveries were 111.4% (sampling intervals 14, 21 and 28 DAT) and 88.3% (sampling intervals 0, 1, 3 and 8 DAT), which is in the range of recoveries for non-labelled chemicals (70-120%). The LOQ was the lowest validation level where the validation criteria were met, which was 0.1 µg/mL.

Recoveries of thymol ranged from 103.4% at 0 day to 62.0% at 28 days (irradiated normalised) and from 103.4% at 0 days to 100.7% at 28 days (dark control normalised).

Normalization of the data was done in view of the dark controls plot, since the sampling points, below and above the degradation curve, could have varied with the fortification's recoveries for these measurements.

As a result, the data (percent of nominal concentration) were normalised using the mean fortification recovery for the relevant analysis occasion as follows:

Normalised value = Unamended value × 100 / Mean fortification recovery

Thymol degraded very slowly in the dark but more rapidly when irradiated with light simulating sunlight.

Single first-order kinetics provided a good fit for the data. The corrected $DegT_{50}$ values for photolysis of Thymol applicable to Europe and North America (latitudes 30°, 40° and 50°) were 60.6 and 33.9 days for unamended and normalised values respectively. In Japanese spring sunlight the corrected $DegT_{50}$ values for Thymol in irradiated samples were 196 and 110 days for unamended and normalised values respectively.

Some deviations were found with respect to the OECD 316 guideline. First of all, the guide states that the major degradation products should be isolate and identify. Additionally, reference substances should be used for the characterisation and/or identification of phototransformation products by spectroscopic and chromatographic methods. No other reference standard than thymol was employed and therefore, the study did not investigate the possible photodegradation products. Labelled material is required for studying the pathway of phototransformation and for establishing a mass balance.

According to Regulation 283/2013, the identity of breakdown products formed which exceed 10 % of the applied test substance at any time during the study, a mass balance to account for at least 90 % of the applied radioactivity, as well as photochemical half-life (DT50) shall be reported.

This study is acceptable to stablish the rate of degradation under irradiated conditions but not the route.

A new direct photochemical study with radio-labelled thymol is requested in order to address the route of degradation in water.

2.8.2.2.7 Other / Weight of evidence

No other data available.

2.8.3 Summary of fate and behaviour in air

The vapour pressure of thymol is 3.4 Pa at 20°C (CA 2.2) and the water solubility at 20°C is 596 mg/l (EFSA Conclusion 2012). A Henry's Law constant of 0.86 $Pa.mol^{-1}.m^3$ was derived (CA 2.2).
An estimation of the photochemical oxidative degradation rate (using the Atkinson equation) has estimated that the expected half-life in air to be 1.197 hours. This indicates that any volatilised thymol will be extremely short-lived in the atmosphere, therefore there will be no local or global effects.

2.8.3.1 Hazardous to the ozone layer

Table 71: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
No data	-	-	-

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Based on the vapor pressure (3.4 Pa at 20°C) thymol is highly volatile and losses due to volatilization would not be excluded. An estimation of the photochemical oxidative degradation rate (using the Atkinson equation) has estimated that the expected half-life in air to be 1.197 hours. This indicates that any volatilised thymol will be extremely short-lived in the atmosphere. Therefore, global warming potential, ozone depleting potential, photochemical ozone creation potential and accumulation in the troposphere are all unlikely to occur following use of Thymol according to good agricultural practice.

There are no data provided regarding the hazard of Thymol to the ozone layer, the Ozone Depleting Potential (ODP) of Thymol has not been measured.

2.8.3.1.2 Comparison with the CLP criteria

A substance is considered hazardous to the ozone layer if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.
Any substances having an ODP of greater than or equal to the lowest ODP (i.e., 0.005) of the substances currently listed in Annex I to Regulation EC No 1005/2009 should be classified as hazardous to the ozone layer (category 1).
Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physicochemical properties, thymol is not expected to be hazardous to stratospheric ozone.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No monitoring data is available and based on the short half-life of thymol is not required.

2.8.5 Definition of the residues in the environment requiring further assessment

The residue definition for the risk assessment:

Soil:	Thymol
Groundwater:	Thymol
Surface water:	Thymol
Sediment:	Thymol
Air:	Thymol

Definition of the residue for monitoring:

Soil:	Thymol
Groundwater:	Thymol
Surface water:	Thymol
Sediment:	Thymol
Air:	Thymol

2.8.6 Summary of exposure calculations and product assessment

2.8.6.1 Predicted environmental concentration in soil

The predicted environmental concentrations in soil (PEC_{soil}) have been calculated according to the guidance in SANCO/9188/VI/97. The worst-case laboratory DT50 of 1 day was set to 1 day for thymol. All PECsoil calculations were performed assuming a soil bulk density of 1.5 g/cm³ and an equal distribution in the top 5 cm. The crop interception value was set to 60% for grapes and 65% for pome fruits. The following results were obtained:

Table 72: PECsoil values for thymol on grapes

PEC_{soil} (mg/kg)	**Grapes**			
	Single application		**Multiple Application**	
	Actual	**TWA**	**Actual**	**TWA**
Initial	0.141	-	0.142	-

Table 73: PECsoil values for thymol on apples

PEC_{soil} (mg/kg)	**Pome Fruit**			
	Single application		Multiple Application	
	Actual	TWA	Actual	TWA
Initial	0.123	-	0.124	-

2.8.6.2 Predicted environmental concentration in groundwater

The predicted environmental concentrations in groundwater (PECgw) of thymol were calculated using the environmental fate model FOCUS-MACRO (v5.5.4), FOCUS-PEARL (v5.5.5) and FOCUS-PELMO (v6.6.4) and in line with the current FOCUS guidelines.
The following PECgw values were obtained:

Table 74: Summary of PEARL and PELMO PECgw values after application to vines

Crop	**Scenario**	**80th Percentile PEC_{gw} at 1 m Soil Depth (µg/L)**	
		PEARL (v5.5.5)	**PELMO (v6.6.4)**
		Thymol	**Thymol**
Vines	**Châteaudun**	0.000000	<0.0005
	Hamburg	0.000000	<0.0005
	Kremsmünster	0.000000	<0.0005
	Piacenza	0.000000	<0.0005
	Porto	0.000000	<0.0005
	Sevilla	0.000000	<0.0005
	Thiva	0.000000	<0.0005

Table 75: Summary of PEARL and PELMO PECgw values after application to apples

Crop	**Scenario**	**80th Percentile PEC_{gw} at 1 m Soil Depth (µg/L)**	
		PEARL (v5.5.5)	**PELMO (v6.6.4)**
		Thymol	**Thymol**
Apples	**Châteaudun**	0.000000	<0.0005
	Hamburg	0.000000	<0.0005
	Jokioinen	0.000000	<0.0005
	Kremsmünster	0.000000	<0.0005
	Okehampton	0.000000	<0.0005
	Piacenza	0.000000	<0.0005
	Porto	0.000000	<0.0005
	Sevilla	0.000000	<0.0005

	Thiva	0.000000	<0.0005

Table 76: Summary of MACRO PECgw values after application to vines and apples

Crop	**Scenario**	**80th Percentile PEC_{gw} at 1 m Soil Depth (µg/L)**
		MACRO (v5.5.4)
		Thymol
Vines	**Châteaudun**	0.0
Apples	**Châteaudun**	0.0

The 80th percentile PEC_{gw} of thymol is lower than the 0.1 µg/L regulatory threshold in groundwater at 1 m depth after simulations with the models PEARL, PELMO and MACRO, for both crops (vines and apples).
The risk to groundwater was determined to be acceptable for all uses of Mevalone containing thymol.

2.8.6.3 Predicted environmental concentration in surface water

The predicted environmental concentrations in surface water (PECsw) and sediment (PECsed) of thymol, was assessed through simulations using STEPS 1-2 calculator (version 3.2), FOCUS-SWASH (version 5.3) and followed the recommendations of the FOCUS Working Group on Surface Water Scenarios (SANCO/4802/2001).

The worst-case PEC values are summarised below. The maximum PEC_{sw} values are provided, plus the 21-day time weighted average and the maximum PEC_{sed}.

Table 77: Summary of Worst-Case PEC Values for thymol at STEPS 1-3

FOCUS STEP / Crop	**Scenario**	**Max PEC_{sw} (µg/L)**	**Dominant entry route**	**21 d- $PEC_{sw,twa}$ (µg/L)**	**Max PEC_{sed} (µg/kg)**
STEP 1					
Vines	332.06	-	312.64	361.52	332.06
Apples	359.15	-	334.92	361.52	359.15
STEP 2					
Vines	Southern Europe Mar-May	14.85	-	6.20	14.64
Apples	Southern Europe Mar-May	22.67	-	9.22	21.76
STEP 3					
Vines	R3 stream	4.636	Drift	1.415*	2.608*
Apples	R3 stream	10.41	Drift	1.030*	2.871*

*D6 ditch

2.8.6.4 Predicted environmental concentration from airborne transport

For information on local in-field or edge-of-field exposure for operators or bystanders, please refer to data submitted under datapoint Vol 3 CP B.6.2.

The vapors pressures 3.4 Pa at 20ºC of thymol, are above the triggers for volatilization of 1 x 10 4 Pa for soil and 1 x 10 5 Pa for plants. Therefore, the short-range transport needs to be addressed.

Based on the DT50 on air of thymol, it can be concluded that the substance is not persistent in the atmosphere and would not be subject to significant concerns related to long-range atmospheric transport and atmospheric accumulation.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

The previous EU-agreed acute oral avian LD_{50} value of >10000 mg Mevalone/kg bw (corresponding to >640 mg thymol/kg bw) for Northern bobwhite quail *Colinus virginianus* (EFSA Journal 2012; 10(11):2916) is considered acceptable to support renewal of thymol. The data on the representative formulation, Mevalone, are sufficient to address the active substance data requirement. No data are available for the long-term avian toxicity of thymol.
The applicant has requested a waiver for long-term reproductive toxicity data to mammals based on a weight of evidence. The weight of evidence included data of residues of thymol in a range of plants other than grapes or pomes from "Dr Duke's phytochemical and ethnobotanical databases" (https://phytochem.nal.usda.gov/phytochem/search), residue data of thymol on fruits (grapes and pomes) from residue trials of the residue section (Vol. 3 CA B.7) and in the short persistence of the compound, its natural occurrence and volatility, and known low acute oral avian toxicity. Furthermore,the appicant proposed to conduct long-term avian risk assessment using the acute toxicity value LD_{50}/10 as a surrogate.

However, the residue data database showed deficiencies that question their reliability. Furthermore, the range of estimated residues indicates that the dietary exposure, assuming 100% consumption of the food items, would be between 1 – 111000 mg/kg for thymol, thus, in most cases this would result in background exposure greater the predicted exposure following the proposed use. Moreover, the residue trials data from the residue section are relevant specifically to consideration of frugiovorus birds.The applicant states that due to the high volatilisation (Vp = 3.4 Pa at 20º C) and the rapid degradation (DT_{50} soil 1 < day) initial environmental exposure of thymol will decline rapidly. However, these data are not sufficient to demostrate a negligible exposure.

Additionally, a literature paper (Labaque *et al*., 2013; Study B.9.1.1.2/01) has been considered considered as supporting information by the RMS. In this study, female Japanese quail birds (*Coturnix coturnix japonica*) were fed a diet supplemented with 2 g of thymol per kg of feed, equivalent to a dose of 80 mg of thymol/animal/day. The Co-RMS has indicated that the results of this study suggest that there is an effect on the behavior of birds fed with 80 mg of thymol/animal/day even after only 2 days of administration. More specifically, the birds in the treated group exhibited significantly ($p < 0.05$) reduced stress and fear responses compared to the control during short periods of mechanical restriction after 2 and 15 days of consecutive feeding.

The co-RMS agrees with the opinion of RMS that the high volatility of thymol and its rapid degradation in soil and air are not sufficient to demonstrate a negligible exposure. Thus, it cannot be excluded that birds may experience reduced fear and anxiety from exposure to thymol in nature. In that case, reduced fear and anxiety responses could interfere with the normal behavioral activities of birds, such as the fear of predators, leading to less chances of survival. Consequently, the effects on birds' behavior shown in the dietary study by Labaque *et al.* (2013) support the need for further information on the presumable reproductive toxicity of thymol to birds.

Moreover, the applicant proposed the use of LD_{50}/10 as surrogate of the long-term endpoint for risk assessment since no avian reproduction study is avaiable and a low acute toxicity was observed (LD_{50} > 640 mg thymo/kg bw). Birds and Mammals EFSA Guidance (2009) considers the use of the lowest acute LD_{50}/10 and the NOAEL from avian reproduction studies for long-term risk assessment. However, no long-term endpoint on birds for thymol is available for comparison. Therefore, the weight of evidence submitted by the applicant is not sufficient to address the avian long term risk. Consequently, **further information should be submitted to address reproductive risk to birds.**

The previous EU-agreed mammalian (rat) acute oral LD_{50} value of 980 mg thymol/kg bw (EFSA Journal 2012; 10(11):2916) is considered acceptable to support renewal of thymol. No data are available for the long-term mammalian toxicity of thymol. However, for the renewal of thymol, a waiver is requested by the applicant for long-term reproductive toxicity data to mammals based on the same data of concentration of thymol in terrestrial plants and residue data (from the Residue Section, vol. 3 CA B.7) on vines and apples from birds (see above).
The applicant states that due to the high volatilisation (Vp = 3.4 Pa at 20º C) and the rapid degradation (DT_{50} soil 1 < day) initial environmental exposure of thymol will decline rapidly. However, reliable data with regards to the background level of thymol in the environment has not been provided. Moreover, the applicant proposed the use of LD_{50}/10 as surrogate of the long-term endpoint for risk assessment since no mammals reproduction study is avaiable and a low acute toxicity was observed (LD_{50} > 980 mg thymol /kg bw). However, the use of LD50/10 for mammals is not especifically envisaged by EFSA Guidance (2009). Therefore, the long-term risk to mammals could not be considered addressed. Consequently, **further information should be submitted to address the long term risk to mammals.**

Additioinally, considering that developmental toxicity studies on mammals (i.e., rat and rabbit) are available in the DAR of eugenol, May 2011, the Co-RMS proposed a read-across approach from the developmental toxicity data of eugenol to thymol since these two substances share common characteristics. The RMS has request to the applicant the read-across approach.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

Available ecotoxicology studies have been considered and summarised in the Thymol Monograph (Volume 3, Annex B9, May 2011) and in the renewal of approval dossier (dRAR, Volume 3, Annex B9). There have been also some studies that are included in the REACH Registration dossier of Thymol and they are considered acceptable.

The key information pertinent to determine the environmental hazard classification for thymol is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the representative test guideline, if applicable. Studies from REACH Registration dossier considered as not reliable for classification in the table 79) are not summarised into the descriptive text.

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 78: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study[1]	Remarks	Reference
Partition co-efficient n-octanol/water EEC A8, OECD 107	-	log P_{ow} value for thymol = 3.43 (at pH 4) log P_{ow} value for thymol = 3.44 (at pH 7) log P_{ow} value for thymol = 3.41 (at pH 9)	Key The study is considered acceptable	Thymol purity 99.69% w/w	Anonymus (2020)
Bioconcentration test OECD TG 305	*Cyprinus carpio*	At 1 µg/L BCF < 48 At 10 µg/L BCF = 7.9 - 19	The study is considered acceptable	Thymol purity 99.9% w/w No potential for bioacumulation	MITI (1996)

2.9.2.1.1 Estimated bioaccumulation

Not relevant. Please refer to section 2.9.2.1.2 below.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

The log P_{ow} values for thymol are 3.43, 3.44 and 3.41 at pH values 4,7 and 9, respectively (Vol. 3 CA B.2). The pH value has no impact on the octanol/water partition coefficient of thymol. In line with Annex I, Section 4.1.2.8.1 of the CLP Regulation[17], these log P_{ow} values are less than the CLP cut-off criteria of 4, indicating thymol does not show potential to bioaccumulate. Studies on active substance bioconcentration in organisms are therefore not required, as previously agreed during the EU review for the EU inclusion of thymol (EFSA Journal 2012;10(11):2916). Regarding the PBT criteria, thymol is therefore not considered B since the log P_{ow} values at varying pH values are all lower than 4.

Bioconcentration test.

[17] Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

A bioaccumulation study with thymol was included in **REACH Registration dossier** and it is summarised below.

MITI (1996).

A bioaccumulation study was carried out to evaluate the bioaccumulation potential of the test material thymol. The study was conducted in accordance with the Japanese guideline "Bioconcentration test of chemical substances in fish and shellfish" specified in 'The test method relating to new chemical substances' and OECD Guideline 305C following GLP.

The BCF in *Cyprinus carpio* was determined over a 6-week exposure period at concentrations of 1 µg/L and 10 µg/L thymol, respectively. The exposure method was a continuous flow-through system. The test material showed no relevant bioaccumulation at concentrations at or above the water solubility. The steady state BCF values were 7.8 - 19 at a concentration of 10 µg/L and ≤ 48 at a concentration of 1 µg/L, respectively. The measured BCF is far below 500; and hence thymol has no significant bioaccumulation potential.

The study is considered acceptable.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 79: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Acute toxicity to fish OECD 203 (1992)	*Oncorhynchus mykiss* (Rainbow trout)	Thymol technical (99.7 % w/w)	96-hour LC_{50} = 3.0 mg thymnol/L (nom) (semi-static)	Key. The study is considered acceptable.	-	Anonymous (2008a)
Acute toxicity to fish OECD 203 (1992)	*Danio rerio* (Zebra fish)	Thymol technical (99.7 % w/w)	96-hour LC_{50} = 7.1 mg thymol/L (nom) (semi-static)	Key. The study is considered acceptable.	-	Anonymous (2008b)
Acute toxicity to fish OECD 203 (1992)	*Oryzias latipes*	Thymol technical (100 % w/w)	96-hour LC_{50} = 4.67 mg thymol/L (nom) (semi-static)	Key The study is considered acceptable.		Anonymous, 2005.
Acute toxicity to fish US-EPA, 1975	*Pimephales promelas*	Not specified	96-hour LC_{50} = 3.2 mg thymol/L	Supportive information		Anonymous (1986)
Acute toxicity to fish Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"	*Oryzias latipes*	Thymol technical (99.9 % w/w)	48-hour LC_{50} = 9.35 mg thymol/L (nom)	Non standar guideline has been follwed and GLP are not specified. There is no information about the test item concentrations, and there is no reference substance. Supportive information.		Anonymous (1996)

Acute toxicity to fish OECD 203 (1992)	*Brachydanio rerio*	Thymol technical (99.9 % w/w)	96-hour LC_{50} = 5.7 mg thymol/L (nom)	Non GLP Supportive information		Anonymous (2009)
Acute toxicity to fish No guideline	*Leuciscus idus*	Not specified	48-hour LC_{50} = 10 mg thymol/L	Non guideline and Non GLP Supportive information		Anonymous (1978)
Acute toxicity to fish No guideline	*Ptychocheilus oregonensis Oncorhynchus tshawytscha, Oncorhynchus kisutch* and *Salmo gairdneri.*	Not specified	Effect for loss-of-equilibrium, and death time have been calculated. There is no a LC_{50} value.	Non GLP. Disregarded due to major methodological deficiencies. Non reliable for classification		Anonymous, (1969).
Acute toxicity to fish OECD 203 (1992)	*Oryzias latipes*	Thymol technical (98 % w/w)	96-hour LC_{50} = 5.1 mg thymol/L	Non GLP. Disregarded due to major methodological deficiencies. Non reliable for classification		Anonymous, (1995)
Acute toxicity to *Daphnia* OECD 202 (2004)	*Daphnia magna*	Thymol technical (99.7 % w/w)	48-hour EC_{50} = 4.9 mg thymol/L (nom) (static)	Key. The study is considered acceptable.	-	Grade, R., Wydra, V. 2008.
Acute toxicity to *Daphnia* OECD 202 (2004)	*Daphnia magna*	Thymol technical (100 % w/w)	48-hour EC_{50} = 4.46 mg thymol/L (nom) (static)	The study is considered acceptable.	-	MITI (National Institute of Technology and Evaluation, Japan), (2005)
Acute toxicity to Daphnia EPA-600/3-75-009	*Daphnia magna*	Not specified	96-hour LC_{50} = 3.2 mg thymol/L	Supportive information		Ewell et al. (1986)
Growth inhibition to green algae OECD 201 (2006)	*Pseudokirchneriella subcapitata*	Thymol technical (99.7 % w/w)	<u>Growth rate</u> 72-hour E_rC_{50} = 11.1 mg thymol/L (mm) <u>Biomass</u> 72-hour EbC50 = 5.14 mg thymol/L (mm)	Key. The study is considered acceptable.		Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c).

			Yield 72-hour EyC50 = 4.89 mg thymol/L (mm)			
Growth inhibition to green algae OECD 201 (2006)	*Pseudokirchneriella subcapitata*	Thymol technical (100 % w/w)	Growth rate 72-hour E_rC_{50} = 13.5 mg thymol/L (nom) Biomass 72-hour EbC50 = 7.73 mg thymol/L (nom)	Key. The study is considered acceptable.		MITI (National Institute of Technology and Evaluation, Japan), (2005).

Two acute fish toxicity studies with thymol were previously evaluated as part of the EU review for the EU inclusion of thymol (DAR, Volume 3, Annex B.9, 2011, B.9.2.1.1) (in Table 79,. Anonymous (2008a) and. Anonymous (2008b)). These studies are considered appropriate for the current assessment to support renewal of thymol; full summaries are provided in Vol. 3 CA B.9 (CA 8.2.1/01 and CA 8.2.1/02). Moreover, another five acute fish toxicity studies (Anonymous (2005), Anonymous (1986), Anonymous (1996), Anonymous (2009) and Anonymous (1978))) with thymol was included in **REACH** Registration dossier and are summarised below.

Anonymous (2008a)

The 96-hour acute toxicity of thymol to *Oncorhynchus mykiss* was studied under semi-static conditions in accordance with OECD 203. Groups of seven rainbow trout (*Oncorhynchus mykiss*) were exposed to thymol at nominal concentrations of 0.625, 1.25, 2.5, 5 and 10 mg thymol/L for 96 hours under 16 hours light and 8 hours dark per day. There was also a control of test medium only. The test media were renewed after 48 hours. The temperature, which was measured daily in all test units, ranged between 13 and 14°C and the dissolved oxygen, which was measured in each vessel every 24 hours, was >91 %. Test concentrations were analysed at the start and the end of each renewal period. Mortality and other observations were made after 1, 24, 48, 72 and 96 hours.

The range of light intensity applied to the study (453-552 lux) surpassed in 87 lux the lower limit of the range recommended in the OECD test guideline 203 update of 2019 (540-1000 lux). However this little deviation probably does not affect the results.

The measured concentrations of the samples of nominal concentrations of 0.625 mg thymol/L were below the NOEC and below the Limit of Quantification. At the start of the test and after renewal of the test media 78% of the nominal test concentrations were found (average for the test concentrations 1.25, 2.5, 5 and 10 mg thymol/L). In the aged test media 77% of the nominal values were found (average for the test concentrations 1.25, 2.5, 5 and 10 mg thymol/l.). Thus, during the test period the fish were exposed to an overall mean measured concentration of 77% of nominal (average for the test concentrations 1.25, 2.5, 5 and 10 mg thymol/L). Thus, results were expressed based on mean measured concentrations.

No mortality or sublethal effects were observed in the controls or at nominal test concentrations up to 2.5 mg thymol/L. Sublethal symptoms were observed at nominal test concentrations of 2.5 mg thymol/L and above. The sublethal effects observed at nominally 2.5 mg thymol/L were tumbling during swimming (observed in 29% of fish at 96 hours only), and additional effects observed after 2 hours or more of exposure at nominally 5 mg thymol/L and higher included convulsions, dark coloration, apathy and lateral position. At nominal test concentrations of 5 and 10 mg thymol/L, 86% and 100% mortality, respectively, were recorded after 96 hours. The 96-hour LC_{50} value for thymol to *Oncorhynchus mykiss* was calculated to be 3.0 mg thymol/L and the NOEC was 1.06 mg thymol/L, based on mean measured concentrations.

All validity criteria were met in accordance with OECD test guideline 203 (update 2019): in the control, the mortality did not exceed one fish at the end of the test ; the dissolved oxygen concentration in the test media was above 60% of air saturation value during the test ; analytical measures were conducted.

The study is considered acceptable. The EU-agreed LC_{50} value of 3.0 mg thymol/L for rainbow trout (EFSA Journal 2012; 10(11):2916) (from CA 8.2.1/01) is considered the appropriate critical endpoint for acute toxicity to fish.

Anonymous (2008b)

The 96-hour acute toxicity of thymol to *Danio rerio* was studied under semi-static conditions in accordance with OECD 203 (1992). Groups of seven zebra fish were exposed to thymol at nominal concentrations of 2.5, 5.0, 10, 20 and 40 mg thymol/L for 96 hours in a semi-static system under 16 hours light and 8 hours dark per day. There was also a control of test medium only. The test media were renewed after 48 hours. The temperature, pH and dissolved oxygen were measured daily in all test units. The temperature values ranged between 21 and 25 ºC. The dissolved oxygen concentration in the test media did not fall below 60% of air saturation.

Test concentrations were analysed at the start and the end of each renewal period, except where all test fish were dead at a particular test concentration where aged samples were taken at the time of observation. Mortality and other observations were made after 2, 24, 48, 72 and 96 hours. Statistical analysis was performed using ToxRat Professional v2.09. EC_{50} values and 95% confidence limits were calculated, where possible, by Probit analysis. The NOEC and LOEC values were determined directly from the raw data.

Mortality and sub-lethal effects were observed daily and at test termination. Analytical verification of test concentrations confirmed measured concentrations were maintained within 86 – 99% of nominal throughout the exposure period and biological endpoints were based on nominal concentrations.

All validity criteria were met in accordance with OECD test guideline 203 (update 2019): in the control, the mortality did not exceed one fish at the end of the test (no fish died during the test in control or solvent control), and the dissolved oxygen concentration in the test media was above 60% of air saturation value during the test (actual values ≥7.6 mg/L, equivalent to 90%, at 22 °C, sea level, 1 atm).

The 96-hour LC_{50} value was calculated to be 7.1 mg thymol/L based on nominal concentrations. The corresponding NOEC value was 2.5 mg thymol/L, based on nominal concentrations. The sub-lethal effects observed included tumbling during swimming, convulsions, apathy and fish lying on side or back.

This study is considered as acceptable.

Anonymous (2005).

The 96-hour acute toxicity of thymol to Oryzias latipes was studied under semi-static conditions in accordance with OECD 203. The study was conducted under GLP. Fish were exposed to the substance at a range of nominal concentrations of 0, 0.911, 3.64, 5.10, 7.14 and 10 mg/L in freshwater. The test was performed with 4 fish per vessel and concentration. Symtoms observed during the exposure perios were superficial concentration, loss of equilibrium, lethargy and decreased activity. The following OECD 203 validity criteria were met: mortality in the control did not exceed 10% at the end of the test; the dissolved oxygen concentration did not drop below 60% throughout the test; constant conditions (temperature between 23.5°C and 24.8°C and pH between 7.1 and 7.7) were within specified deviations; and the concentrations of the substance were maintained within ± 20 % of the nominal concentration throughout the test. Under the conditions of the test, the 96-hour LC50 was 4.67 mg/L.

The study is considered acceptable.

Anonymous, (1986).

The acute toxicity of thymol to juvenile fish (*Pimephales promelas*) was determined together with seven species according to the Methods of US-EPA, 1975. Simultaneously seven species from five phyla were exposed to the test item concentrations of nominal 0.1, 1.0, 10 and 100 mg/L in the multi-species test.

A duplicate single species test was also performed with thymol. The 96 h LC50 of thymol was 3.2 mg/L. Due to deviations from standard guidelines for acute toxicity to fish such as less than 5 test concentrations, missing analytics and protocol, the 96-h LC50 value has not been chosen as key value for risk assessment but it does strongly support the key value.

The study is considered as supporting information.

Anonymous (1996).

The acute toxicity of thymol to juvenile fish (*Oryzias latipes*) was determined in a semi-satatic test for 48 hours of exposure. Non standar guideline has been follwed and GLP are not specified. There is no information about the test item concentrations and there is no reference substance.

The test substance concentrations were confirmed to be stable within +/- 20% of initial nominal concentration via analytical measurement.

The 48 h-LC50 of thymol was 9.35 mg/L. Due to lack of information the study is considered as supporting information.

Anonymous (2009).

The acute toxicity of thymol to juvenile fish (*Danio rerio*) was determined in a satatic test for 96 hours of exposure according to OECD 203. Non GLP. Fish were exposed to the substance at a range of nominal concentrations of 2.3, 3.2, 4.2, 5.6, 7.5 and 10 mg/L in freshwater. There is no analytical monitoring.

The 96-h LC50 of thymol was 5.7 mg/L. Due to lack of information and Non GLP, the study is considered as supporting information.

Anonymous (1978).

The acute toxicity of thymol to juvenile fish (*Leuciscus idus*) was determined in a satatic test for 48 hours of exposure. Non guideline has been follwed and Non GLP. There is no analytical monitoring.

The 48-h LC50 of thymol was 510 mg/L. Due to lack of information and Non GLP, the study is considered as supporting information.

2.9.2.2.1 Acute (short-term) toxicity to aquatic invertebrates

One acute *Daphnia* toxicity study with thymol was previously evaluated as part of the EU review for the EU inclusion of thymol (DAR, Volume 3, Annex B.9, 2011, B.9.2.1.2) (in Table 79, Grade, R., Wydra, V., 2008). This study is considered appropriate for the current assessment to support renewal of thymol; a full summary is provided in Vol. 3 CA B.9 (CA 8.2.4.1/01). Moreover, another toxicity study with *Daphnia magna* (MITI-National Institute of Technology and Evaluation, Japan, (2005)) with thymol was included in REACH Registration dossier and they are also summarised below.

Grade, R., Wydra, V. (2008).

The 48-hour acute toxicity of thymol to *Daphnia magna* was studied under static conditions in accordance with OECD Guideline 202 (2004). Daphnids were exposed to nominal concentrations of 0.625, 1.25, 2.5, 5 and 10 mg thymol/L and an untreated control and solvent control for 48 hours. Five individual daphnids were added to each test vessel, with four replicate vessels per treatment group. Duplicate analytical samples were taken from all test concentrations at test start (0 hours) and after 48 hours. From the control samples only one of the duplicate samples was analysed at both sampling times.

Analytical verification of test concentrations confirmed measured concentrations were maintained within 86 – 111% of nominal throughout the exposure period and biological endpoints were based on nominal concentrations.

No immobilisation was observed in the control and in the three lowest test concentrations (0.625, 1.25 and 2.5 mg thymol/L). A single specimen was immobile in the solvent control and at a test concentration of 2.5 mg thymol/L after 48 hours; however, these observations were not considered to be treatment-related. Significant immobilisation was observed in the two highest test concentrations after 48 hours (45% and 100% at test concentrations of 5.0 and 10 mg thymol/L, respectively). As a result, the following endpoints were obtained, the 48-hour EC_{50} (immobilisation) = 4.9 mg thymol/L and the NOEC (48-hour) = 2.5 mg thymol/L, based on nominal concentrations.

All validity criteria were met in accordance with OECD test guideline 202 (2004): immobilisation in the control group was ≤ 10% (actual value: 0% in control; 5% in solvent control); the dissolved oxygen concentration at the end of the test was ≥ 3 mg/L in all test vessels (actual values: ≥ 8.2 mg O_2/L); and analytical measurement of test concentrations was included.

This study is considered acceptable.

The EU-agreed EC_{50} value of 4.9 mg thymol/L for *Daphnia magna* (EFSA Journal 2012; 10(11):2916) (from CA 8.2.4.1/01) is considered the appropriate critical endpoint for acute toxicity to aquatic invertebrates.

MITI, (2005)

The acute toxicity of thymol to *Daphnia magna* was determined over a 48-hour exposure period under static conditions in accordance with OECD Guideline 202 (2004). *Daphnids* were exposed to five nominal concentrations of 0, 2.08, 2.92, 4.08, 5.71 and 8.0 mg thymol /L in quadruplicate (20 daphnids per concentration). The test was performed with five daphnia per vessel (20 daphnids per concentration); the photoperiod was 16 hours light and 8 hours dark. During the exposure period, symptoms observed were lethargy, swimming inhibition, and decreased activity. The nominal concentration of the test substance in the test solution was kept within ± 20% of the set concentration and the environmental conditions were within the appropriate range. Under the conditions of the test, the 48-hour LC50 was 4.46 mg/L.

The study is considered acceptable.

Ewell et al. (1986).

The acute toxicity of thymol to invertebrates (*Dapnia magna*) was determined together with seven species according to the Methods of US-EPA from 1975. No study took place before GLP became official standard. Simultaneously seven species from five phyla were exposed to the test item concentrations of nominal 0.1, 1.0, 10 and 100 mg/L in the multi-species test. A duplicate single species test was also performed with thymol.

In this multispecies test, a 96 h LC50 of 3.2 mg/L of thymol to *Daphnia magna* was obtained.

Due to deviations from standard guidelines such as less than 5 test concentrations, missing analytics and protocol, the 48-h LC50 value has not been chosen as key value.

The study is considered as supporting information.

2.9.2.2.2 Acute (short-term) toxicity to algae or aquatic plants

One green algal study with thymol was previously evaluated as part of the EU review for the EU inclusion of thymol (DAR, Volume 3, Annex B.9, 2011, B.9.2.1.3) (in Table 79, Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c)). Another alga toxicity study (MITI-National Institute of Technology and Evaluation, Japan, (2005)) with thymol was included in REACH Registration dossier and it is summarised below.

Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c).

The toxicity of thymol to *Pseudokirchneriella subcapitata* was tested in an algal growth inhibition test in accordance with OECD 201. Test species were exposed to control, solvent control, and test chemical at nominal concentrations of 1, 3.2, 10, 32 and 100 mg thymol/L, a control containing culture medium only, and a solvent control. Three replicates of each test concentration were used and exponentially growing cultures of *P. subcapitata* were inoculated at 5×10^3 cells/mL, and cultured for 96 hours at temperatures of 23°C to 24°C.

Measured concentrations of thymol were determined at 0 and 96 hours and analytical verification of test concentrations confirmed measured concentrations were maintained within 69 – 100% of nominals throughout the 96-hour test period. Biological endpoints are reported based on mean measured concentrations. The test concentration of 1.0 mg thymol/L could not be quantified with the available analytical method. Therefore, the overall geometric mean value was assumed as concentration for this treatment level. Since the geometric mean values in the treatment levels of 32 – 100 mg thymol/L were all close to 80% of nominals, this approach was considered to be justified. Algal cell numbers were determined spectrophotometrically after approximately 24, 48, 72 and 96 hours.

All validity criteria were met in accordance with OECD test guideline 201 (2006): the biomass in the control cultures increased exponentially by a factor of at least 16 within 72 hours (87.6); the mean coefficient of variation for section-by-section specific growth rates in the control cultures did not exceed 35% (10.5%, 0 – 72h); the coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7% (3.3%; (0 – 72h).

The 72-hours results were:

72-hour E_rC_{50} (growth rate) = 11.1 mg thymol/L (mean measured).

72-hour E_bC_{50} (biomass) = 5.14 mg thymol/L (mean measured).

72-hour E_yC_{50} (yield) =4.89 mg thymol/L (mean measured).

72-h ErC_{10} (growth rate) = 3.81 mg thymol/L (mean measured).

72-h EbC_{10} (biomass) = 1.54 mg thymol/L (mean measured).

72-h EyC_{10} (yield) = 2.23 mg thymol/L (mean measured).

72-hour NOE_rC (growth rate) = 8.10 mg thymol/L (mean measured).

72-hour NOE_bC (biomass) = 0.82 mg thymol/L (mean measured).

72-hour NOE_yC (yield) = 0.82 mg thymol/L (mean measured).

The study is considered acceptable.

MITI, (2005)

The toxicity of thymol to *Pseudokirchneriella subcapitata* was tested in an algal growth inhibition test in accordance with OECD 201. The study was conducted in a static system and algae were exposed to the substance at a range of concentrations of 0, 0.854, 1.88, 4.13, 9.09 and 20 mg/L in freshwater for 72 hours. The test was performed with 3 replicates per test concentration and 6 replicates for the control. The following OECD 201 validity criteria were met: cell concentration in the control increased by a factor of 38 within 72h; the mean coefficient of variation of the daily growth rates were 33% and the coefficient of variation of average growth rate during the whole test period was 4%.

Under the conditions of the test, the nominal concentration of the test substance in the test solution was kept within ± 20% of the set concentration and the 72-hours results were:

72-hour E_rC_{50} (growth rate) = 13.5 mg thymol/L (nominal).

72-hour E_bC_{50} (biomass) = 7.73 mg eugenol/L (nominal).

72-h NOEC = 1.88 mg eugenol/L (nominal)

The study is considered acceptable.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 80: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Chronic toxicity to *Daphnia* OECD 211 (2004)	*Daphnia magna*	Thymol technical (99.58 % w/w)	21-day EC_{10} (reproduction) = 0.292 mg thymol/L (mm) 21-day EC_{20} (reproduction) = 0.554 mg thymol/L (mm) 21-day NOEC (reproduction) = 0.137 mg thymol/L (mm)	Key. The study is considered acceptable.	-	Anonymous, (2021)
Chronic toxicity to *Daphnia* OECD 211 (2004)	*Daphnia magna*	Thymol technical (100 % w/w)	21-day NOEC (reproduction) = 2.0 mg thymol/L (mm)	Key. The study is considered acceptable.		MITI (National Institute of Technology and Evaluation, Japan), (2005).
Growth inhibition to green algae	*Pseudokirchneriella subcapitata*	Thymol technical (99.7 %	Growth rate 72-hour E_rC_{10} = 3.81 mg	Key. The study	-	Hoffman, K., Wydra, V.

OECD 201 (2006)		w/w)	thymol/L (mm) 72-hour NOE_rC = 8.10 mg thymol/L (mm) <u>Biomass</u> 72-hour EbC10 = 1.54 mg thymol/L (mm) 72-hour NOEbC = 0.82 mg thymol/L (mm) <u>Yield</u> 72-hour EyC10 = 62.23 mg thymol/L (mm) 72-hour NOEyC = 0.82 mg thymol/L (mm)	is considered acceptable.		(2011; revised report) (original report Grade, R., Wydra, V., 2008c).
Growth inhibition to green algae OECD 201 (2006)	*Pseudokirchneriella subcapitata*	Thymol technical (100 % w/w)	72-hour NOE_rC = 1.88 mg thymol/L (nom)	The study is considered acceptable.	-	MITI (National Institute of Technology and Evaluation, Japan), (2005).

2.9.2.3.1 Chronic toxicity to fish

No data are available on the chronic toxicity to fish. According to the acute toxicity endpoints obtained from EFSA Journal 2012; 10(11):2916 (see also Section 2.9.2.2 above), it is observed that fish are no more sensitive to thymol than *Daphnia magna* (acute LC_{50} and EC_{50} values for fish and *Daphnia* are in same order of magnitude). However, according to the Regulation 283/2013 the long-term and chronic toxicity study on fish is a data requirement. The study should be provided unless it is proved that the substance is unstable in water, that is to say there is less than 90% loss of the original substance over 24 hours via hydrolisis. Since, thymol was considered hydrolytically stable at pH 4 and 7 (Vol. 3 CA Study B.8.2.1.1/01 ; Anonymous, 2021) the data gap for further information to address the chronic risk to aquatic organisms has not been addressed. Thus, **a data gap** has been identified **to submit a chronic toxicity study on fish**.

Considering the suggestion of the Co-RMS, an ELS test (OECD TG 210) could be perform to study the chronic risk to fish and EAS-adversity of the active substace thymol.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

A new chronic toxicity study with *Daphnia magna* has been provided to support the renewal of thymol; a full summary is provided Vol. 3 CA B.9 (Study B.9.2.5.1/01). Another toxicity study with *Daphnia magna* (National Institute of Technology and Evaluation, Japan, (2005)) with thymol was included in REACH Registration dossier and it is also summarised below.

Anonymous, (2021)

The chronic toxicity of thymol to *Daphnia magna* was studied under semi-static conditions in accordance with OECD Test Guideline 211 (2012). *Daphnids* were exposed to nominal concentrations of 0.0563, 0.141, 0.352,

0.880 and 2.20 mg thymol/L (corresponding to mean measured concentrations of 0.0344, 0.137, 0.300, 0.767 and 2.0 mg thymol/L) and an untreated control (Elendt medium M4) for 21 days.

Adult daphnids were observed daily for immobility, presence of eggs and mortality. They were transferred to fresh media 3 times per week. 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. Any juveniles produced were counted and removed daily.

Analytical verification of the test item concentrations indicated they were stable in the test solutions, at > 80% of the nominal concentrations, except for the lowest test concentration, where the time-weighted mean (TWM) of the measured concentrations was < 80% of nominal concentrations (61.1%). Therefore the biological endpoints were based on TWM of the measured concentrations : 0.0344, 0.137, 0.300, 0.767 and 2.00 mg test item/L.

The validity criteria according to OECD guideline 2011 were met: mortality of the parent animals (female Daphnia) in the controls at the end of the test should be ≤ 20% (0.0%) and mean number of live offspring produced per surviving parent animal in the controls at the end of the test should be ≥ 60 (135.4).

The 21-day $EC_{10,}$ EC_{20} and EC_{50} 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).

This study is considered acceptable and satisfies the requirements for a chronic toxicity study with freshwater invertebrates (OECD test guideline 211, 2012).

MITI, (2005).

This study was carried out to determine the effect of the test substance thymol on reproduction in the test organism Daphnia magna according to OECD Guideline 211 (Daphnia magna Reproduction Test) and following GLP. *Daphnids* were exposed to nominal concentrations of 0 (control), 0.25, 1.0, 2.0, and 4.0 mg thymol/L The concentration of the test substance in the test solution was maintained within ±20% of the established concentration during the exposure period, and the environmental conditions were within the appropriate range, indicating that the test was appropriate in accordance with the test method.

The validity criteria were met: at the end of the exposure, the mortality rate of *Daphnia magna* in the control group does not exceed 20% (0% in the estudy) and at the end of the exposure, the average cumulative number of surviving litters per surviving *Daphnia magna* in the control group was 134, meeting the requirement of 60 or more litters.

Each daphnid was held in a separate vessel (10 vessels per concentration) and exposed under semistatic conditions for 21 days to concentrations of the test substance of nominal 4.00, 2.00, 1.00, 0.25 mg/L (nominal) and control group (0 mg/L). The 21d-NOEC of thymol towards Daphnia magna was 2.0 mg/L. The EC50 (21d) was calculated to be 3.49 mg/L (95% confidence intervall: 3.27 - 3.78 mg/L).

This study is considered acceptable.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

One green algal study with thymol was previously evaluated as part of the EU review for the EU inclusion of thymol (DAR, Volume 3, Annex B.9, 2011, B.9.2.1.3). This study is considered appropriate for the current assessment to support renewal of thymol ; a full summary is provided in Vol. 3 CA B.9.2.6.1/01.

Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c).

Please refer to Section 2.9.2.2.3 ‘Acute (short-term toxicity to algae or aquatic plants’ where the data for both acute (short-term) and chronic toxicity to algae are discussed. For the chronic aquatic hazard assessment, the EU-agreed 72-hour ErC10 and NOErC values of 3.81 and 8.10 mg thymol/L, respectively, for *Pseudokirchneriella subcapitata* are considered the appropriate critical endpoints for chronic effects on growth of green algae

For the chronic aquatic hazard assessment, the 72-hour E_rC_{10} value of 3.81 mg thymol/L for *Pseudokirchneriella subcapitata* is considered the appropriate critical endpoint for chronic effects on growth of green algae.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

There are no further studies on other aquatic organisms that are considered relevant to the classification of thymol.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 81: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to fish OECD 203 (1992)	*Oncorhynchus mykiss* (rainbow trout)	Thymol technical (99.7% w/w)	96-hour LC_{50} = 3.0 mg thymol/L mm (semi-static)	Key (acceptable)	Anonymous, (2008a)
Acute toxicity to *Daphnia* OECD 202 (2004)	*Daphnia magna*	Thymol technical (99.7% w/w)	48-hour EC_{50} = 4.46 mg thymol/L nom (static)	Key (acceptable)	(National Institute of Technology and Evaluation, Japan), (2005)
Growth inhibition to green algae OECD 201 (2011)	*Pseudokirchneriella subcapitata*	Thymol technical (99.7% w/w)	72-hour E_rC_{50} = 11.1 mg thymol/L mm	Accepted	CA 8.2.6.1/01 Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c)

nom: based on nominal concentrations; mm: based on mean measured concentrations

Full acute set was available for thymol as there were acute studies on fish, aquatic invertebrates and algae and aquatic plants, covering the three tropich levels (see Table 81). The acute toxicity (LC_{50} / EC_{50}) values for all three trophic levels are >1 mg eugenol/L, and fish is the most sensitive trophic level with the 96h-EC_{50} of 3.0 mg thymol/L.

For classification of a substance in relation to acute aquatic hazard, table 4.1.0 (a) of Annex I of Regulation (EC) No. 1272/2008 should be used. The acute endpoint selected has to be compared with the cut-off value (acute toxicity values ≤ 1 mg/l). The 96h- EC50 of 3.0 mg/L is > 1 mg/L. Therefore, *Thymol* is **Not classified** for acute aquatic hazard.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The actual harmonized classification of Thymol for environmental hazards is Aquatic Chronic 2. This classification arises from an automatic translation between classification in accordance with Directive 67/548/EEC (as R51/53) and Regulation 1272/2008 when entering into force Regulation 1272/2008.

Commission Directive 98/73/EC harmonizaed classification as R51/53. The data supporting this classification are not available, however, new data warranted a new assessment and classification following the CLP criteria.

Table 82: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
Chronic toxicity to *Daphnia* OECD 211 (2004)	*Daphnia magna*	Thymol technical (99.58% w/w)	21-day NOEC = 0.137 mg thymol/L mm **21-day EC_{10} = 0.292 mg thymol/L mm**	Accepted	CA 8.2.5.1/01 Egeler, P. (2021) Report number 20GC2DB
Growth inhibition to green algae OECD 201 (2011)	*Pseudokirchneriella subcapitata*	Thymol technical (99.7% w/w)	Growth rate 72-hour E_rC_{50} = 11.1 mg thymol/L mm 72-hour E_rC_{10} = 3.81 mg thymol/L mm 72-hour NOE_rC = 8.10 mg thymol/L mm	Accepted	CA 8.2.6.1/01 Hoffman, K., Wydra, V. (2011; revised report) (original report Grade, R., Wydra, V., 2008c) Report number 34284210

nom: based on nominal concentrations; mm: based on mean measured concentrations

Degradability

Thymol can be considered to be **readily biodegradable** since there was a ready biodegradation study available which demonstrated a high level of degradation within the 10-d window. Therefore, thymol can also be considered as rapidly degradable substance.

Bioaccumulation

The experimental BCF in fish was determined to be less than 48. In line with Annex I, Section 4.1.2.8.1 of the CLP Regulation, the BCF values are below the threshold of ≥ 500, indicating thymol does **not show potential to bioaccumulate**. Supporting this conclusion, the log P_{ow} value for thymol is 3.43, 3.44 and 3.41 at pH values 4,7 and 9, respectively, less than the CLP cut-off criteria of 4.

Chronic aquatic hazard

A full set of chronic data for three trophic levels is not available however, adequate chronic toxicity data with thymol are available for two trophic levels (invertebrates and algae). Therefore, in line with Annex I, Section 4.1 (Figure 4.1.1) of the CLP Regulation, the long-term aquatic hazard classification is assessed according to the following two methods, with the classification determined according to the most stringent outcome :

(a) using the chronic toxicity data for invertebrates and algae according to the criteria given in Table 4.1.0(b)(ii) (for rapidly degradable substances)

As demonstrated in the table above (Table 82), the chronic toxicity (21-d EC_{10}) value for invertebrates (crustacea) of 0.292 mg thymol/L is ≤1 mg/L (but > 0.1 mg/L). Therefore, in line with Annex I, Section 4.1 (Table 4.1.0(b)(ii)) of the CLP Regulation, thymol is classified for long-term aquatic hazard as **Category Aquatic Chronic 3**.

(b) using the acute toxicity data for fish according to the criteria given in Table 4.1.0(b) (iii).

To classify according to these criteria, substances should be classified as not rapidly degradable and/or the experimentally deetermined BCF ≥ 500 (or, is absent, the logKow ≥ 4). Taking into account these values, thymol could not be classified according to these criteria since it is rapidly degradable and the BCF is < 500/logKow < 4.

Therefore, in line with Annex I, Section 4.1 (Figure 4.1.1) of the CLP Regulation, the proposed classification of thymol according to the most stringent approach for long-term aquatic hazard is **Category Aquatic Chronic 3**.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Taking into account all the information and the assessment summarized in the previous sections 2.9.2.4, the following classification class and category can be concluded for this active substance thymol, in accordance with Regulation (EC) 1272/2008:

CLP Annex ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1]	Reason for no clasification[2]
4.1	Hazardous to the aquatic environment	Aquatic Chronic 3 H412	-	-	-
5.1	Hazardous to the ozone layer	-	-	-	Chemical structure and physicochemical properties warrants no classification

Labelling : Signal word: -

Hazard statements : Harmful to aquatic life with long lasting effects (H412)

Precautionary statements :

P273: Avoid release to the environment

P391 : Collect spillage

P501 : Dispose of contents/container in accordance with national hazardous waste regulations

Pictogram : -

The following additional statements are required when used as a plant protection product (CLP Annex II, part 4).

- EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

2.9.3 Summary of effects on arthropods

The data on the representative formulation, Mevalone, are considered sufficient to address the active substance data requirements. The previous EU-agreed (EFSA Journal 2012; 10(11):2916) acute oral LD_{50} value of >224.6 µg Mevalone/bee and acute contact LD_{50} value of >200 µg Mevalone/bee are considered the appropriate critical endpoints for acute and contact toxicity to honey bees. New chronic oral adult and repeated exposure larval honey bee (*Apis mellifera*) toxicity tests are submitted to support renewal of thymol with a 10-day (adult) LDD_{50} value of 123.53 µg Mevalone/bee/day and a 22-day (larva) NOED value of 1300 µg Mevalone/larva/developmental period.

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. It is noted that several literature papers are available assessing the short-term and long-term effects of thymol (or veterinary/bee-health formulations containing thymol) on honey bee colonies when it is applied as an acaracide treatment. The most relevant of these literature papers are summarised Vol. 3 CA B.9 (Studies B.9.3.1/01 to B.9.3.1/04) as supporting information. The remaining papers are summarised in Vol. 3 CA B.9 Appendix I, but these papers are considered of limited relevance or reliability to the risk assessment of bees. Please note, the critical endpoints from the standard OECD studies presented in the paragraph above are considered more relevant and reliable for the quantitative risk assessment. No further studies are considered necessary.
The previous EU-agreed (EFSA Journal 2012; 10(11):2916) LR_{50} values of >12420 g Mevalone/ha for both *Aphidius rhopalosiphi* and *Typhlodromus pyri* are considered the appropriate critical mortality endpoints based on glass plate studies for non-target arthropods other than bees. No further studies are considered necessary.

In addition, it is noted that two literature papers were identified during the literature search (Vol. 3 CA B.9.11.1), assessing the effects of topical/contact exposure of thymol on *Chrysoperla* (lacewing) and *Podisus* (predatory bug) species. Results of these papers are summarised in Vol. 3 CA B.9 Appendix I for completeness (CA 9.6.3.4/01 and CA 9.6.3.4/17), but these are considered of limited relevance and reliability for use in the risk assessment, principally as a non-standard study design was used and test item purity was not reported. The critical endpoints summarised in Vol. 3 CP B.9.5.2, from the standard glass plate (IOBC) studies with *Typhlodromus* and *Aphidius* species, testing the representative product, Mevalone, are considered more relevant and reliable for the risk assessment. No further studies are considered necessary.

2.9.4 Summary of effects on non-target soil meso- and macrofauna

The data on the representative formulation, Mevalone, are considered sufficient to address the active substance data requirements. The requirement for acute toxicity data for earthworms is now obsolete under Regulation (EU) No 283/2013, but the previous EU-agreed acute LC_{50} value for *Eisenia fetida* of >1000 mg Mevalone/kg soil (EFSA Journal 2012; 10(11):2916) is presented in Vol. 3 CP (Lührs, 2007 : Study B.9.7.1.1/01) as supporting information to support renewal of thymol. A new chronic reproduction toxicity study for earthworm with formulation Mevalone, including eugenol, geraniol and thymol, has been provided to meet new data requirements under Regulation (EU) No 284/2013. The new chronic reproduction toxicity endpoint to earthworm (*Eisenia andrei)* 56-day NOEC (reproduction) value of 52.9 mg Mevalone/kg dry soil ($NOEC_{corr}$ = 26.5 mg Mevalone/kg dry soil) ($NOEC_{corr}$ corresponding to 1.65 mg thymol/kg dry soil) is considered to be the appropriate critical reproduction endpoint.

In addition, one new chronic reproduction toxicity test with the soil macro-organisms *Folsomia candida* and the representative product, Mevalone, has been submitted to support renewal of thymol. It is noted that *Hypoaspis acuelifer* 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. The new *Folsomia candida* 28-day EC_{10} value of 37.3 mg Mevalone/kg dry soil ($EC_{10\ corr}$ = 18.7 mg Mevalone/kg dry soil) ($EC_{10\ corr}$ corresponding to 1.15 mg thymol/kg dry soil) is considered the appropriate critical reproduction endpoint to be used in the risk assessment.

No further studies are considered necessary.

2.9.5 Summary of effects on soil nitrogen transformation

The data on the representative formulation, Mevalone, are considered sufficient to address the active substance data requirements. The previous EU-agreed conclusion (EFSA Journal 2012;10(11):2916) is considered appropriate for effects on nitrogen transformation: No significant effects (<25% relative to the control) on nitrogen transformation were observed after 56 days at 54.4 mg Mevalone/kg dry soil (corresponding to 3.5 mg thymol/kg dry soil). No further studies are considered necessary.

2.9.6 Summary of effects on terrestrial non-target higher plants

Thymol does not exhibit herbicidal activity or a plant growth regulator mode of action and the available screening data on the representative formulation, Mevalone are expected to be sufficient to address the active substance data requirement. The previous information on plant screening was provided in the DAR (eugenol, geraniol and thymol DAR, Volume 3, Annex B.9, 2011, B.9.9.1) and is considered sufficient to demonstrate a lack of effects on non-target plants. Limit tests at rates including 4 x 4 L Mevalone/ha (equivalent to 4 x 4120 g Mevalone/ha based on a density of 1029 g/L) and higher were conducted with Mevalone. Effects were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).
In addition, it is noted that several literature papers were identified during the literature search (Vol. 3 CA B.9.11.1), assessing the effects of thymol on various plant terrestrial species. Results of these papers are summarised in Document Vol. 3 CA B.9 Appendix I for completeness, but these are considered of limited relevance and reliability for use in the risk assessment, principally as non-standard study designs were used. The available screening data summarised in Vol. 3 CP B.9.11, testing the representative product, Mevalone, are considered more relevant and reliable for the risk assessment of terrestrial non-target higher plants. No further studies are considered necessary.

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No other groups of terrestrial organisms are considered to be at risk and no concerns were raised from a review of the open literature. No further data are considered necessary.

2.9.8 Summary of effects on biological methods for sewage treatment

The data on the representative formulation, Mevalone, are considered sufficient to address the active substance data requirements. One new study based on an activated sludge respiration inhibition test with Mevalone, is submitted to support the renewal of thymol. The critical (lowest) 3-hour EC_{50} value, based on nitrification respiration, was calculated to be 204.9 mg product/L (CI: 115.5 – 361.7 mg product/L), corresponding to 12.8 g thymol/L. No further studies are considered necessary.

2.9.9 Summary of product exposure and risk assessment

The representative product, Mevalone, contains three active substances at nominally 6.4% w/w thymol, 6.4% w/w geraniol and 3.2% w/w eugenol. Mevalone is the common representative product of all three active substances (thymol, geraniol and eugenol), which are intended to be renewed at the same time. The three active substances share the same Vol. 3 CP B.9 and the same studies for Mevalone.

The risk assessments have been carried out considering the representative GAP of four applications (7-day interval) of 4.12 kg Mevalone/ha (based on a density of 1.029 g/L), which corresponds to 4 x 0.264 kg thymol/ha, 4 x 0.264 kg geraniol/ha and 4 x 0.132 kg eugenol/ha, in vineyards (BBCH 60-89) and pome fruit (BBCH 75-87).

Birds (Vol. 3 CP B.9.2.1)

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

All acute TER values for birds were above the relevant trigger values at the screening step.Screening step for the acute oral risk to birds due to the use of Mevalone in vineyards: DDD_{90} = 707; TER_a >14. . In the case of the active substance thymol, a DDD_{90} of 45.3 mg/kg bw/d and a TER_a >14 were found.

However, no avian reproductive toxicity data for the active substance thymol or formulation Mevalone are available, consequenlty, **birds reproductive risk assessment cannot be finalised**. Therefore, further information should submitted.

The acute risk to birds is considered to be acceptable without the need for any mitigation. The acute risk to birds *via* consumption of contaminated water and the risk *via* secondary poisoning is considered to be acceptable.

Terrestrial vertebrates other than birds (Vol. 3 CP B.9.2.1)

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

All acute TER values for mammals were above the relevant trigger value at the screening step when considering the available acute toxicity data for each active substance. Screening step for the acute oral risk to mammals due to the use of thymol in orchard/vineyards: DDD_{90} = 64.8; TER_a = 15.

When considering the acute mammalian toxicity value of >2000 mg Mevalone/kg bw for the representative product, the Tier 1 TER values were below the trigger of 10 for the two generic focal species, ‘vole’ and ‘dormouse’:

First tier assessment for the acute oral risk to mammals due to the use of Mevalone (LD_{50} >2000 mg Mevalone/kg bw):

Orchards/vineyards BBCH ≥40 ‘vole’ DDD_{90} = 303.3; TER_a >6.6;
Orchards BBCH 71-79 ‘dormouse’ DDD_{90} = 355.2; TER_a >5.6.

However, 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. Since the acute formulation toxicity study did not derive an actual value for use as an endpoint (i.e. LD_{50} >2000 mg Mevalone/kg bw), the combined toxicity of the three active substance components was calculated using the Finney (1942) equation. Using the predicted mixture toxicity endpoint of 10033.7 mg Mevalone/kg bw, the resulting Tier 1 TER values clearly indicate a low acute risk to mammals:

First tier assessment for the acute oral risk to mammals due to the use of Mevalone (LD_{50} = 10033.7 mg Mevalone/kg bw):

Orchards/vineyards BBCH ≥40 ‘vole’ DDD_{90} = 303.3; TER_a = 33;

Orchards BBCH 71-79 'dormouse' DDD_{90} = 355.2; TER_a = 28.

However, no long-term toxicity data on mammals for the active substance thymol or formulation Mevalone are available, consequenlty, **mammals reproductive risk assessment cannot be finalised**. Therefore, further information should submitted.

Therefore, the acute risk to mammals is considered to be acceptable without the need for any mitigation. The acute risk to mammals *via* consumption of contaminated water and the risk *via* secondary poisoning is considered to be acceptable.

Aquatic organisms (Vol. 3 CP B.9.4)

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the EFSA Journal 2013, 11(7):3290.

The relevant global maximum FOCUS PEC_{sw} values for risk assessments covering the proposed use pattern are calculated in Vol. 3 CP B.8 for each active substance.

An acceptable risk to Mevalone is concluded at the first-tier based on instantaneous formulation PEC_{sw} calculations (spray drift only) for aquatic organisms (PEC/RAC values greater than the trigger of 1).

Table 2.9.9-1: 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 apples 4 applications at 4120 g product/ha

Group		**Fish acute**	**Inverteb. acute**
Test species		***O. mykiss***	***D. magna***
Endpoint (µg/L)		LC_{50} 31100	EC_{50} 35400
AF		100	100
RAC (µg/L)		311	354
Crop	**PEC $_{gl-max}$ (µg/L)**	PEC/RAC	PEC/RAC
Vines (late)	110.034	0.354	0.311
Apples (late)	215.816	0.694	0.610

For the intended uses of Mevalone in vines and apples, 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.

Table 9.4-4: 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 apples 4 applications at 264 g thymol/ha

Group		**Fish acute**	**Inverteb. acute**	**Inverteb. chronic**	**Algae**
Test species		***O. mykiss***	***D. magna***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	NOEC	E_rC_{50}
(µg/L)		3000	4900	137	11100
AF		100	100	10	10
RAC (µg/L)		30	49	13.7	1110
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC
STEP 1					
Vines	332.1	**11.07**	**6.78**	**24.24**	0.299
Apples	359.2	**12.00**	**7.33**	**26.2**	0.324
STEP 2 1.08					
Vines (S-Europe)	14.85	0.459	0.303	**1.08**	-
Apples (S-Europe)	22.67	0.756	0.460	**1.65**	-
STEP 3					
Vines R3 stream*	4.636	-	-	0.338	-
Apples R3 stream*	10.41	-	-	0.760	-

* For simplicity, only the worst-case FOCUS STEP 3 scenario in vines and apples is presented here; this covers all other FOCUS STEP 3 scenarios in vines and apples

For the intended uses of Mevalone in vines and apples, 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.

Bees (Vol. 3 CP B.9.6.)

The acute risk to honey bees from the use of Mevalone was first assessed using the maximum single application rate and the respective LD_{50} values to calculate hazard quotients (HQ) 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) as follows. 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:

First tier assessment for the acute oral risk to adult honey bees due to the use of Mevalone in orchard/vineyards: maximum single application rate = 4120 g product/ha; HQ <18.3.

First tier assessment for the acute contact risk to adult honey bees due to the use of Mevalone in orchard/vineyards: maximum single application rate = 4120 g product/ha; HQ <20.6.

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.

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).

In accordance with EFSA/2013/3295, the hazard quotient (HQ) is well below the trigger of 85 (for sideward sprays), indicating an acceptable acute contact risk to bees following the proposed use of Mevalone in vineyards and orchards:

Screening assessment for the acute contact risk to adult honey bees due to the use of Mevalone in orchard/vineyards: maximum single application rate = 4120 g product/ha; HQ <20.6.

In accordance with EFSA/2013/3295, the ETR_{oral} values for the acute oral risk to adult honey bees and chronic risk to larval honey bees are below the relevant trigger of 0.2 at the screening step indicating acceptable risk following the proposed use of Mevalone in vineyards and orchards:

Screening assessment for the acute oral risk to adult honey bees due to the use of Mevalone in orchard/vineyards: maximum single application rate = 4120 g product/ha; SV = 10.6; ETR_{oral} = 0.194.

Screening assessment for the chronic risk to larval honey bees due to the use of Mevalone in orchard/vineyards: maximum single application rate = 4120 g product/ha; SV = 6.1; ETR_{oral} = 0.019.

Tier 1 assessments were conducted in Vol. 3 CP B.9.6.1, to assess the chronic oral risk to adult honey bees in accordance with EFSA/2013/3295. Acceptable risk (ETR_{oral} >0.03) was demonstrated for all relevant scenarios in pome fruit (BBCH 75-87) and vineyards (BBCH ≥70). For the proposed uses in vineyards at BBCH 60-69, the Tier 1 chronic oral adult ETR_{oral} is above the trigger value of 0.03 only for the treated crop scenario:

Worst-case scenario in the first-tier assessment for the chronic oral risk to adult honey bees due to the use of Mevalone. Vineyards BBCH 60-69 treated crop scenario: maximum single application rate = 4120 g product/ha; EF = 1; SV = 8.2; TWA = 0.72; ETR_{oral} = 0.197.

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. The same conclusions were found in two open literature papers (Attractiveness of Agriculture Crops to Pollinating Bees- USDA Report 2017[18]). Insect Pollination of Crops - FAO[19]) which indicates that vineyards are not attractive for nectar.. Therefore, vineyards can be considered attractive only for collection of pollen and the risk assessment for treated crop scenario can be conducted with the short-cut value indicated in Table J_x of EFSA Journal 2013;11(7):3295. EF = 1, SV = 0.06, TWA = 0.72, ETR_{oral} = 0.0014.

Overall, the risks to bees are considered acceptable following the proposed representative uses of Mevalone in vineyards and pome fruit without the need for mitigation.

Non-target arthropods other than bees (Vol. 3 CP B.9.6.2)

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. According to the drift values obtained from ESCORT 2, orchards represent the worst-case drift values. Therefore, the risk assessment of orchards also covers the risk assessment for vineyards applying the risk envelope approach.

All $HQ_{in\text{-}field}$ and $HQ_{off\text{-}field}$ values for both indicator species are below the relevant trigger value at the first tier, indicating acceptable in-field and off-field risk to non-target arthropods:

First tier assessment for the in-field risk to NTAs due to the use of Mevalone in orchards: $PER_{in\text{-}field}$ (foliar) = 11124 g product/ha; $HQ_{in\text{-}field}$ (foliar) <0.9; $PER_{in\text{-}field}$ (soil) = 14008 g product/ha; $HQ_{in\text{-}field}$ (soil) <1.1.

First tier assessment for the off-field risk to NTAs due to the use of Mevalone in orchards: $PER_{off\text{-}field}$ (foliar) = 2626.3 g product/ha; $HQ_{off\text{-}field}$ (foliar) <0.21; $PER_{off\text{-}field}$ (soil) = 3307.3 g product/ha; $HQ_{off\text{-}field}$ (soil) <0.27.

Overall, acceptable in-field and off-field risk for non-target arthropods is concluded following the representative uses of Mevalone in vineyards and orchards without the need for mitigation measures.

Earthworms (Vol. 3 CP B.9.8.1)

The evaluation of the risk for earthworms 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 initial PEC_{soil} values for risk assessments covering the proposed use pattern are calculated in Vol. 3 CP

[18] https://www.ars.usda.gov/ARSUserFiles/OPMP/Attractiveness%20of%20Agriculture%20Crops%20to%20Pollinating%20Bees%20Report-FINAL_Web%20Version_Jan%203_2018.pdf

[19] FAO, Insect Pollination of Crops. Academic Press, London, UK (1993)

B.8 for each active substance (eugenol, geraniol and thymol).

Acceptable chronic risk to earthworms is concluded at the first-tier based on a worst-case initial PEC_{soil} value (TER value greater than the trigger of 5):

First-tier assessment of the chronic risk for earthworms due to the use of Mevalone in vineyards: PEC_{soil} = 2.195 mg Mevalone/kg dw; TER_{lt} = 12.1.

Therefore, the chronic risk to earthworms is considered to be acceptable without the need for mitigation measures.

Non-target soil meso- and macro- fauna other than earthworms (Vol. 3 CP B.9.8.2)

The evaluation of the risk for other non-target soil meso- and macrofauna (other than earthworms) 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 initial PEC_{soil} values for risk assessments covering the proposed use pattern are calculated in Vol. 3 CP B.8.

Acceptable chronic risk to non-target soil meso- and macrofauna is concluded at the first-tier based on a worst-case initial PEC_{soil} value (TER value greater than the trigger of 5):

First-tier assessment of the chronic risk for *Folsomia candida* due to the use of Mevalone in vineyards: PEC_{soil} = 2.195 mg Mevalone/kg dw; TER_{lt} = 8.5

Soil nitrogen transformation (Vol. 3 CP B.9.10)

The evaluation of the risk for soil micro-organisms 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 initial PEC_{soil} values for risk assessments covering the proposed use pattern are calculated in Vol. 3 CP B.8.

The relevant PEC_{soil} value for Mevalone of 2.195 mg Mevalone/kg dry soil is well below the appropriate critical endpoint of 54.4 mg Mevalone/kg dry soil.Therefore, the risk for soil nitrogen transformation is considered to be acceptable without the need for mitigation.

Terrestrial non-target plants (Vol. 3 CP B.9.12)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). Limit tests at rates including 4 x 4 L product/ha (equivalent to 4 x 4120 g product/ha based on a density of 1029 g/L) and higher were conducted with Mevalone. Effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). The limit test rates equal/exceed the highest field application rate in vineyards and orchards of 4 x 4120 g Mevalone/ha and are thus considered an indicator for an acceptable risk without the need for mitigation. No further risk assessment is considered necessary.

2.10 ENDOCRINE DISRUPTING PROPERTIES

Introduction to this chapter

The scientific criteria for the determination of endocrine disrupting properties are set out in Commission Regulation (EU) 2018/605. The European Chemicals Agency (ECHA) and European Food Safety Authority (EFSA) provide guidance for the identification of endocrine disruption (ED) in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (2018). Based on the available information, data on thymol for potential ED in mammalian are summarized and assessed in this chapter according to the EFSA/ECHA guidance.

2.10.1. Gather all relevant information

The applicant has provided information on the endocrine disrupting properties of the active substance thymol: only one 19-weeks sub-chronic published study, and one combined repeated dose and reproductive/developmental

toxicity study, both conducted in rats, were fully provided. The applicant also provided an assessment of the endocrine disruption properties of thymol (Staphyt Ltd, Eden Research plc., 2021, «Thymol: Assessment of endocrine disrupting properties»). In addition, other sources of relevant data to endocrine disruption properties were presented (e.g. the US EPA paper and CompTox Chemicals Dashboard, and BG-Chemie toxicological published review).

The endocrine disruption assessment itself carried out by the RMS can be found on this section, while relevant data has been compiled using the Excel Spreadsheet in Appendix E, where each study was given an identification number (Study ID Matrix) that is important for its identification in the data-matrix of the Excel.

2.10.1.1. Non-test information

In accordance with the OECD Conceptual Framework and the ED Guidance, Level 1 information was gathered by the RMS for thymol:

- Qualitative structural activity relationship (QSAR) data was obtained for thymol from the Danish QSAR database and the OECD QSAR Toolbox v.4.5. The Danish (Q)SAR Database was released in November 2015 and has since then been expanded and updated a number of times. It currently holds many predictions for around 650000 substances and is a tool that allows industry, research, authorities and others to search for hazard information on chemical substances, especially those with little or no testing data. While the Danish (Q)SAR Database contains around 650000 substances, there may still be substances of interest to some users which are not contained in the database. Or even when contained, there may in some cases be interest to obtain more detailed predictions in the QSAR Prediction Reporting Format, which includes information on prediction probability, possible alerts, nearest analogs in the training set etc. The Danish (Q)SAR Models website offers users to make on-the-fly predictions for user-defined chemical structures by use of >30 models developed by DTU in the Leadscope software. The use of the commercial Leadscope® Enterprise Server software as a back-end to this website was made possible based on a collaboration agreement between Leadscope Inc. and the National Food Institute at the Technical University of Denmark.
- Further information predicting the potential for thymol to demonstrate estrogenic and androgenic activity was sourced from the United States Environmental Protection Agency (US EPA) ToxCast modelling data presented in the CompTox Dashboard.

  The ToxCast Model tab in the CompTox Dashboard includes predictions of the estrogen receptor activity of thymol, based on the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP; Mansouri *et al*., 2016[20]). The CERAPP is a large-scale modelling project which has investigated the efficacy of using predictive computational models trained on high-throughput screening data (e.g. from the EDSP21 initiative) to evaluate the ER -related activity of thousands of chemicals, and identify priorities for further testing.

  The ToxCast Model tab in the CompTox Dashboard includes predictions of the androgen receptor activity of thymol based on the COMPARA project. COMPARA is a large-scale collaboration between 35 international groups using QSAR models to predict androgen receptor activity using a common training set of 1746 compounds provided by the US EPA. The result is a consensus model of AR agonist activity that is run against the DSSTox chemical library, and aims to identify priorities for further testing.

The results of the QSAR analysis and predictive computational models are presented and discussed on sections 2.10.2.1 and 2.10.2.2. This information is not included in the ED Excel spreadsheet since *in vitro* an *in vivo* Level 2, and 4 data are available.

2.10.1.2. *In vitro* mechanistic data – US EPA CompTox Chemicals Dashboard

In accordance with the OECD Conceptual Framework and the ECHA/EFSA GD on ED, available Level 2 data was gathered for thymol. Level 2 data includes *in vitro* assays that provide data on selected endocrine mechanisms and pathways.

In vitro mechanistic data for thymol was sourced from the US EPA Endocrine Disruptors Screening Program (EDSP). The EPA's EDSP is designed to detect the intrinsic ability of chemicals to interact with the endocrine

[20] Mansouri K, Abdelaziz A, Rybacka A, Roncaglioni A, Tropsha A, Varnek A, Zakharov A, Worth A, Richard AM, Grulke CM, Trisciuzzi D, Fourches D, Horvath D, Benfenati E, Muratov E, Wedebye EB, Grisoni F, Mangiatordi GF, Incisivo GM, Hong H, Ng HW, Tetko IV, Balabin I, Kancherla J, Shen J, Burton J, Nicklaus M, Cassotti M, Nikolov NG, Nicolotti O, Andersson PL, Zang Q, Politi R, Beger RD, Todeschini R, Huang R, Farag S, Rosenberg SA, Slavov S, Hu X, Judson RS. CERAPP: Collaborative Estrogen Receptor Activity Prediction Project. Environ Health Perspect. 2016 Jul;124(7):1023-33.

system, specifically for chemicals that can interact with the estrogen, androgen or thyroid pathways. The US EPA initially developed high throughput data for approximately 1800 chemicals by using a series of 700 high throughput screens. These data were available for certain chemicals on the US EPA Aggregated Computational Toxicology Resource (ACToR) website. To further refine the activity specifically related to the EDSP Program, and to aid in screening the large number of chemicals subject to regulation in the US, the EPA developed the EDSP21 Dashboard to provide access to new chemical data on over 1800 chemicals of interest, many of which are the same chemicals evaluated through the ToxCast program. In August 2019, the EDPS21 and ToxCast Dashboards have been discontinued, and the updated the EDSP21, ToxCast/Tox21 bioassay data has been updated and integrated into US EPA CompTox Chemicals Dashboard Version 4. The bioassay data can be accessed at https://comptox.epa.gov/dashboard and https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data. Published ER (PMID 26272952) and AR (PMID 27933809) model results are available for citation. ED bioassay data can be accessed using the CompTox Chemicals Dashboard via a bioactivity tabs: EDSP21 and TOXCAST/TOX21.

The data from the EDSP21 and ToxCast/Tox21 tabs provide information regarding the biological activity of a chemical in an *in vitro* system. The results summarised below (data also included in the ED Excel spreadsheet in appendix E) indicate whether a chemical has the potential to interact with a particular signalling cascade, and do not indicate with certainty whether a substance would cause adverse effects via that signalling pathway in experimental animals and/or humans.

2.10.1.3. Summary of all studies considered for the assessment of endocrine disrupting properties of thymol

Study type (results, source)		Reference	Study ID Matrix
***In vitro* ToxCast mechanistic - OECD framework Level 2**			
Thyroid			
ATG_THRa1_TRANS_up	Toxcast T-Bioactivity Model		1
ATG_THRa1_TRANS_dn	Toxcast T-Bioactivity Model		2
LTEA_HepaRG_THRSP_dn	Toxcast T-Bioactivity Model		3
LTEA_HepaRG_THRSP_up	Toxcast T-Bioactivity Model		4
Tox21_TSHR_Agonist_ratio	Toxcast T-Bioactivity Model		5
Tox21_TSHR_Antagonist ratio	Toxcast T-Bioactivity Model		6
Tox21_TR_LUC_GH3_Agonist	Toxcast T-Bioactivity Model		7
Tox21_TR_LUC_GH3_Antagonist	Toxcast T-Bioactivity Model		8
Tox21_TR_LUC_GH3_Antagonist_viability	Toxcast T-Bioactivity Model		9
TOX21_TSHR_HTRF_Agonist_ratio	Toxcast T-Bioactivity Model		10
TOX21_TSHR_HTRF_Antagonist_ratio	Toxcast T-Bioactivity Model		11
TOX21_TSHR_HTRF_wt_ratio	Toxcast T-Bioactivity Model		12
Estrogen			
ACEA_ER_80hr	Toxcast ER Bioactivity Model		13
ACEA_ER_AUC_viability	Toxcast ER Bioactivity Model		14
ATG_ERa_TRANS_up	Toxcast ER Bioactivity Model		**15**
ATG_ERE_CIS_up	Toxcast ER Bioactivity Model		16
NVS_NR_hER	Toxcast ER Bioactivity Model		17
OT_ERa_EREGFP_0120	Toxcast ER Bioactivity Model		18
OT_ERa_EREGFP_0480	Toxcast ER Bioactivity Model		19
OT_ER_ERaERa_0480	Toxcast ER Bioactivity Model		20
OT_ER_ERaERa_1440	Toxcast ER Bioactivity Model		21
OT_ER_ERaERb_0480	Toxcast ER Bioactivity Model		22
OT_ER_ERaERb_1440	Toxcast ER Bioactivity Model		23
OT_ER_ERbERb_0480	Toxcast ER Bioactivity Model		**24**
OT_ER_ERbERb_1440	Toxcast ER Bioactivity Model		25
TOX21_ERa_BLA_Agonist_ratio	Toxcast ER Bioactivity Model		26
TOX21_ERa_BLA_Antagonist_ratio	Toxcast ER Bioactivity Model		27
TOX21_ERa_BLA_Antagonist_viability	Toxcast ER Bioactivity Model		28
TOX21_ERa_LUC_VM7_Agonist	Toxcast ER Bioactivity Model		29
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Toxcast ER Bioactivity Model		30
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability	Toxcast ER Bioactivity Model		31

Study type (results, source)		Reference	Study ID Matrix
Androgen			
ATG_AR_TRANS_up	Toxcast AR Bioactivity Model		32
OT_AR_ARELUC_AG_1440	Toxcast AR Bioactivity Model		33
OT_AR_ARSRC1_0480	Toxcast AR Bioactivity Model		34
OT_AR_ARSRC1_0960	Toxcast AR Bioactivity Model		35
TOX21_AR_BLA_Agonist_ratio	Toxcast AR Bioactivity Model		36
TOX21_AR_BLA_Antagonist_ratio	Toxcast AR Bioactivity Model		37
TOX21_AR_BLA_Antagonist_viability	Toxcast AR Bioactivity Model		38
TOX21_AR_LUC_MDAKB2_Agonist	Toxcast AR Bioactivity Model		39
TOX21_AR_LUC_MDAKB2_Antagonist_ 0.5nM_R1881	Toxcast AR Bioactivity Model		40
TOX21_AR_LUC_MDAKB2_Antagonist_ 0.5nM_R1881_viability	Toxcast AR Bioactivity Model		41
TOX21_AR_LUC_MDAKB2_Antagonist_ 10nM_R1881	Toxcast AR Bioactivity Model		42
TOX21_AR_LUC_MDAKB2_Antagonist_ 10nM_R1881_viability	Toxcast AR Bioactivity Model		43
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist	Toxcast AR Bioactivity Model		44
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_ Antagonist	Toxcast AR Bioactivity Model		45
Steroidogenesis			
TOX21_Aromatase_Inhibition	Toxcast S-Bioactivity Model		46
TOX21_Aromatase_Inhibition_viability	Toxcast S-Bioactivity Model		47
In vitro mechanistic study - OECD framework Level 2			
In-vitro androgen. Chen *et al.* (2007)		B.6.8.3.5	48
In-vitro estrogen. Michalikova *et al.* (2019)		B.6.8.3.6	49
Mammalian toxicologic studies			
Repeated dose toxicity studies - OECD framework Level 4			
19-week range-finding study on rats, Hagan *et al.* (1967). Checked for compliance with OECD TG 408.		B.6.3.2	50
Short term toxicity studies on guinea pig, BG-Chem; from Moller *et al.* (1939)		B.6.8.3.7	51
Developmental studies - OECD framework Level 4			
Combined repeated dose and reproduction/developmental toxicity screening test, checked for compliance with OECD TG 422, [redacted] (1996)		B.6.3.1 B.6.6.1.1	52
Reproductive/developmental toxicity study on rats, US EPA (2006)		B.6.6.1.2	53

2.10.2. ED assessment for humans

2.10.2.1. ED assessment for T-modality

2.10.2.1.1. Analysis of non-experimental data

In accordance with the OECD Conceptual Framework and the ECHA/EFSA GD on ED, Level 1, T-related non-test information was gathered for thymol. Qualitative structural activity relationship (QSAR) data was obtained for thymol from the Danish QSAR database.

The results of the Danish QSAR database for thymol in respect of T-mediated endocrine endpoints are summarised below.

Table 2.10.2.1.1: The results of Danish QSAR database for thymol regarding T-modality

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Thyroperoxidase (TPO) inhibition QSAR1 (Rat in vitro)	NEG	NA	NA	POS_IN	NA
Thyroperoxidase (TPO) inhibition QSAR2 (Rat in vitro)	NEG	NA	NA	POS_OUT	NA
Thyroid Receptor α Binding (Human in vitro)					
mg/L		12083.43	24028.56	83.39842	138.3017

µM		80438.23	159955.8	555.1752	920.6613
Positive for IC50 ≤ 10 µM					
Positive for IC50 ≤ 100 µM					
Domain		IN	IN	OUT	OUT
Thyroid Receptor β Binding (Human in vitro)					
mg/L		2895.454	4861.026	8.20234	929.8816
µM		19274.76	32359.38	54.60218	6190.132
Positive for IC50 ≤ 10 µM					
Positive for IC50 ≤ 100 µM					
Domain		IN	IN	OUT	OUT

Key POS = Positive; NEG = Negative; IN = within the applicability domain of the model; OUT = outside of the applicability domain of the model

The Danish QSAR Database reports that thymol is within the applicability domain of the model for thyroid receptor binding when the "battery" prediction is made. The battery approach can give more reliable predictions and reduces "noise" from the individual models estimates and thereby improve accuracy and/or broaden the applicability domain. However, when predictions were analysed individually through the three available models (CASE Ultra, Leadscope Predictive Data Miner and SciQSAR), only Case Ultra displayed that thymol was within the applicability domain (Table 2.10.2.1.1).

On the other hand, the Danish QSAR Database reports that thymol gave positive results in respect of thyroid peroxidase (TPO) inhibition in two respective Leadscope global, binary composite QSAR models: QSAR1 and QSAR2 (but only QSAR1 was within the applicability domain of these models). These models were developed to predict the potential of substances to inhibit thyroperoxidase using the US EPA ToxCast data sets (training set for QSAR1 = 877 chemicals; training set for QSAR2 = 1519 chemicals) and the commercial software Leadscope® Predictive Data Miner. The highest ranking structural features associated with activity were versions of phenols, anisole and aniline whereas the most frequent structural features associated with inactivity included ethers, esters, aryl halides and a tertiary amine [21]. The structure of thymol only includes a phenol group, so the substance has one feature associated with TPO inhibition activity.

2.10.2.1.2. US EPA CompTox Chemicals Dashboard

The EDSP1 tab in the CompTox Chemicals Dashboard includes 12 thyroid bioassays for thymol. These assays are summarized in the table below and have been included in the ED Excel spreadsheet (Study ID matrix nos.: 1-12). Negative results were obtained in all of them.

Table 2.10.2.1.2: Summary of 13 EDSP21 endocrine activity screening assays included in the ToxCast Thyroid Bioactivity Model

Assay endpoint	Assay type	Organism	Result	Study ID Matrix
ATG_THRa1_TRANS_up	mRNA induction	human	Inactive	1
ATG_THRa1_TRANS_dn	mRNA induction	human	Inactive	2
LTEA_HepaRG_THRSP_up	mRNA induction	human	inactive	3
LTEA_HepaRG_THRSP_dn	mRNA induction	human	inactive	4
TOX21_TRHR_HEK293_Agonist	intracellular calcium	human	inactive	5
TOX21_TRHR_HEK293_Antagonist	intracellular calcium	human	Inactive	6

[21] Rosenberg SA, Watt ED, Judson RS, Simmons SO, Paul Friedman K, Dybdahl M, Nikolov NG, Wedebye EB. QSAR models for thyroperoxidase inhibition and screening of U.S. and EU chemical inventories. *Computational Toxicology*. 2017, Vol 4,pp 11-21. https://doi.org/10.1016/j.comtox.2017.07.006.

TOX21_TR_LUC_GH3_Agonist	Luciferase induction	Rat	Inactive	7
TOX21_TR_LUC_GH3_Antagonist	Luciferase induction	Rat	Inactive	8
TOX21_TR_LUC_GH3_Antagonist_viability	cell viability	Rat	Inactive	9
TOX21_TSHR_HTRF_Agonist_ratio	cAMP measurement	human	Inactive	10
TOX21_TSHR_HTRF_Antagonist_ratio	cAMP measurement	human	Inactive	11
TOX21_TSHR_HTRF_wt_ratio	cAMP measurement	human	Inactive	12

2.10.2.1.3. Have T-mediated parameters been sufficiently investigated?

The available dataset of *in vivo* mammalian toxicology studies for thymol consists mainly in a combined repeated dose and reproduction/developmental toxicity study (ID: 52) conducted in rats (43 and 40 days for males and females, respectively), and a 19-week sub-chronic toxicity study conducted in rats (ID: 50, no guideline, checked for compliance with OECD TG 408). On the other hand, very short summaries of non-guideline published studies were also found within the documentation provided in the dossier [e.g. 8/9 and 16 days repeated subcutaneous injection study in guinea pigs (ID: 51), and a 14-days repeated toxicity study in rats (ID: 53)] No 28 and 90 days short term toxicity studies, long term-carcinogenicity or generational studies were provided. These studies pre-date OECD test guidelines to include EATS-mediated parameters.

Table 14 of the ECHA/EFSA GD on ED provides a list of T-mediated parameters that should be investigated in the OECD CF Level 4 and 5 *in vivo* OECD TG compliant mammalian toxicology studies. Using the currently available set of toxicological data for thymol, the Table 2.10.2.1.3/1 summarises the available information on T-mediated parameters.

Table 2.10.2.1.3/1: Summary of T-mediated parameters investigated in mammalian toxicology studies

T-mediated parameters	OECD Test guideline	**Sufficiently investigated?** **Overall conclusion: No (not sufficiently investigated)** Based on the absence of the following studies: OECD TG 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3, and the absence of data from most T-mediated parameters in the studies provided: thyroid-hormones measurements (T3/T4/TSH), thyroid weight and histopathology, HDL/LDL ratio, colloid area histopathology, and follicular cell height. Therefore, a comprehensive evaluation of T-modality cannot be performed.
T3/T4 levels	407 (optional), 408, 414, 421, 422, 443	Measurement of a single hormone on its own, without complementary parameters such as TSH, thyroid weight, histopathology of thyroid and pituitary, should not be used to draw conclusion regarding changes in the hypothalamus-pituitary-thyroid axis, but raises a concern for effects on the thyroid hormone system. T3 and/or T4 levels were not measured in sub-chronic toxicity (19-weeks) and combined repeated dose and reproduction/developmental toxicity studies provided. There were no OECD TG 414, 421, and 443 studies available.
Thyroid-stimulating hormone level (TSH)	407 (optional), 408, 414, 421, 422, 443	Measurement of a single hormone on its own, without complementary parameters such as T3/T4, thyroid weight, histopathology of thyroid and pituitary, should not be used to draw conclusion regarding changes in the hypothalamus-pituitary-thyroid axis, but raises a concern for effects on the thyroid hormone system. TSH levels were not measured in in sub-chronic toxicity (19-weeks) and combined repeated dose and reproduction/developmental toxicity studies provided. There were no OECD TG 414, 421, and 443 studies available.

Colloid area (thyroid histopathology)	407, 422 (optional)	Studies conducted according to the recent versions of these guidelines are not available for thymol. Thyroid histopathology was not conducted in the studies provided, so colloid area was not analysed.
Follicular cell height (thyroid histopathology)	407, 422, 416	Studies conducted according to the recent versions of these guidelines are not available for thymol. Thyroid histopathology was not conducted in the studies provided, so follicular cell height was not analysed.
HDL/LDL ratio	408	This parameter is considered to be T-mediated only when a change is observed in other T-mediated parameters. The ratio of high density lipoprotein to low density lipoprotein is not investigated in sub-chronic toxicity study presented (19-weeks in rat).
Liver weight	407, 408, 422, 451-3, 416, 443	This parameter is considered to be T-mediated only when a change is observed in other T-mediated parameters. No liver weights changes were found in the sub-chronic (19-weeks) and combined repeated dose and reproduction/developmental toxicity studies. There were no OECD TG 407, 451-3, 416 and 443 studies available.
Thyroid histopathology	407, 408, 414, 421 (optional), 422 (optional), 451-3, 416 (optional), 443.	Thyroid histopathology was not examined in any of the studies provided. There were no OECD TG 407, 414, 451-3, and 443 studies available.
Thyroid weight	407 (optional), 408, 414, 421 (optional), 422 (optional), 451-3, 416, 443.	Thyroid weights were not reported in any of the available studies. There were no OECD TG 414, 416, 451-3, and 443 studies available.

None of the most relevant T-*in vivo* mechanistic and T-mediated parameters were measured in the studies provided. Limited information was described and showed in these studies. Only livers were weighted in the 19-weeks (ID: 50) and in the combined repeated dose and reproduction/developmental toxicity studies (ID: 52), in which no changes were reported.

Table 2.10.2.1.3/2: T-mediated parameters not measured

OECD TG 422 - T-mediated parameters not investigated	
- T3 and/or T4 level - Thyroid stimulating hormone level (TSH) -Follicular cell height (thyroid histopathology)	
OECD TG 408 - T-mediated parameters not investigated	
- Thyroid weight - T3 and/or T4 level - Thyroid stimulating hormone level (TSH)	- Low-density lipoproteins (LDL) - High-density lipoproteins (HDL) -Thyroid histopathology

2.10.2.1.4. Lines of evidence for adverse effects and endocrine activity related to T-modality

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	1	Thyroid receptor	human	24 hour	Uptake from the medium (in vitro)		No effect	No effect	Negative results were obtained in Toxcast Thyroid bioactivity model.	Negative results were obtained in T-*in vitro* mechanistic assays. No *in vivo* mechanistic parameters were measured.	T
	2	Thyroid receptor	human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	3	Thyroid receptor	human	48 hour	Uptake from the medium (in vitro)		No effect	No effect			
	4	Thyroid receptor	human	48 hour	Uptake from the medium (in vitro)		No effect	No effect			
	7	Thyroid receptor	Rat	28 hour	Uptake from the medium (in vitro)		No effect	No effect			
	8	Thyroid receptor	Rat	28 hour	Uptake from the medium (in vitro)		No effect	No effect			
	9	Thyroid receptor	Rat	28 hour	Uptake from the medium (in vitro)		No effect	No effect			
	10	thyroid stimulating hormone receptor	human	0.5 hour	Uptake from the medium (in vitro)		No effect	No effect			
	11	thyroid stimulating hormone receptor	human	0.5 hour	Uptake from the medium (in vitro)		No effect	No effect			
	12	thyroid stimulating hormone receptor	human	0.5 hour	Uptake from the medium (in vitro)		No effect	No effect			
	5	thyrotropin releasing hormone receptor	human	20 hour	Uptake from the medium (in vitro)		No effect	No effect			
	6	thyrotropin releasing hormone receptor	human	20 hour	Uptake from the medium (in vitro)		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
T-mediated	51	Thyroid activation	Guinea pig	8_9 day	injection		Change	Clear thyroid activation was seen in 2 animals and weakly in a third animal.	Equivocal evidences of thyroid activity were observed in guinea pigs after subcutaneous injections of thymol (Möller et al., 1939).	Thyroid activation was observed in guinea pigs. However, the term "thyroid activation" was not specified in the documentation provided within the dossier. No more T-mediated parameters were measured. The role of thymol in T-adversity and activity should be further investigated.	
	51	Thyroid activation	Guinea pig	16 day	injection		Change	There was thyroid activation in the 60 mg (243 mg/kg/day) dose after two injections, 80 mg (313 mg/kg/day) dose after three injections, and 100 mg (375 mg/kg/day) dose after four injections			
	50	Liver weight	Rat	19 week	Oral		No effect	No effect			
	52	Liver weight	Rat	43 day	Oral		No effect	No effect	No treatment related effect was observed		
Sensitive to, but not diagnostic of, EATS	52	Adrenals histopathology	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Histopathological analysis showed an increase in lipid droplets in the fascicular zone of the adrenal gland in 10% (1/10) of females of high dose group.	Low incidence of lipid droplets was observed in adrenal at high dose tested.	No adverse effects were detected in sensitive EATS-related parameters.	N
	52	Adrenals weight	Rat	43 day	Oral		No effect	No effect			
	52	Brain histopathology examination	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		
	52	Dystocia	Rat	43 day	Oral	200 mg/kg bw/day	Increase	1 female failed to deliver in the high dose group. This finding was considered incidental.	No treatment related effect was observed.		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Fertility (mammals)	Rat	43 day	Oral		No effect	No effect	No treatment related effect was observed.		
	52	Number of implantations, corpora lutea	Rat	43 day	Oral		No effect	No effect	No treatment related effect was observed.		
	52	Number of live births	Rat	43 day	Oral		No effect	No effect			
	52	Pituitary histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		
	52	Pituitary weight	Rat	43 day	Oral		No effect	No effect			
	52	Presence of anomalies (external, visceral, skeletal	Rat	43 day	Oral	40 mg/kg bw/day	Increase	One pup with missing tail and one pup with protrusion of the naval region were described in the low dose group.	No treatment related effect was observed.		
	53	Presence of anomalies (external, visceral, skeletal	Rat	14 day	Oral		No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	Rat				No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	mice				No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	hamster				No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	rabbit				No effect	No effect			
	52	Sex ratio	Rat	43 day	Oral		No effect	No effect	No treatment related effect was observed.		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Time to mating	Rat	43 day	Oral	8 mg/kg bw/day	Increase	Time to mating was longer in the treated groups showing a non-statistically significant dose-increased trend (17, 30 and 56% for low, mid and high dose groups, compared with controls).	No treatment related effect was observed.		
Target organ toxicity	52	Adrenal gland necropsy	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Whitening of the adrenal gland was observed in 20% of females (2/10) in the high dose group; these findings were considered incidental due to their low incidence	No treatment related effect was observed.	Target organ toxicity was mainly observed in forestomach of both sexes at mid and high dose tested (hyperplasia of mucosa). Equivocal effects were noted in thymus (reduction in females at mid and high dose group, and increased weight on males at high dose).	
	50	Heart histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Heart histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Heart weight	Rat	19 week	Oral	-	No effect	No effect			
	50	Kidney histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Kidney histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Kidney weight	Rat	19 week	Oral	-	No effect	No effect			
	52	Kidney weight	Rat	43 day	Oral	-	No effect	No effect			
	50	Liver histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Liver histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	52	Lung histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Spleen histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Spleen histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Spleen weight	Rat	19 week	Oral	-	No effect	No effect			
	52	Stomach histopathology	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Histopathological examinations revealed hyperplasia of the mucosal epithelium and was observed in both sexes in 40 mg/kg bw/day (90% of males and 40% of females, respectively) and 200 mg/kg bw/day dose groups (100% of males and 67% of females, respectively).	A dose related increase of hyperplasia on the mucosa of forestomach was observed in both sexes.		
	52	Stomach necropsy	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Thickening of the forestomach wall (thickening of the proventricular wall) was observed in 100% of males (10/10) and in 11% of females (1/9) of the high dose group.			
	52	Thymus histopathology	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Shrinkage of the thymus was further confirmed after histopathological examination in 10% females of 40 and 200 mg/kg bw/day dose groups.	Increased in the thymus weight (absolute and relative) was observed in males at high dose, whereas reduction of the thymus was		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Thymus necropsy	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Reduction in the size of the thymus was observed in 10% of females (1/10) from each of the 40 and 200 mg/kg bw/day dose groups	observed in females at mid and high dose groups (10%).		
	52	Thymus weight	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Increased absolute and relative thymus weight was observed in males (9.8% and 15.3%, respectively) at high dose tested.			
Systemic toxicity	52	Body weight	Rat	43 day	Oral		No effect	No effect	No treatment related effect was observed.	Signs of neurotoxicity (decrease motor activity and ataxia) were observed in females were observed at high dose tested. Both effects were mainly observed during the first 13 days of the treatment	
	52	Body weight	Rat	43 day	Oral	200 mg/kg bw/day	Decrease	Decrease on pups weights at birth (10.3% and 9.4% for males and females, respectively), and on Day 4 (10.2% and 8.8% for males and females, respectively) were found at the high dose level compared with controls, whereas body weight gains decreased up to 14.6% for males and 10.5% for females, compared with controls. However, these differences were not statistically significant.			
	50	Mortality	Rat	19 week	Oral		No effect	No effect	No effect		
	52	Mortality	Rat	43 day	Oral	200 mg/kg bw/day	Increase	1/10 male died	1/10 male died at high dose group		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Mortality	Rat	43 day	Oral		No effect	No effect	No effect		
	52	Clinical signs	Rat	43 day	Oral	200 mg/kg bw/day	Decrease	At high dose group, 90% (9/10) of females showed decrease in spontaneous motor activity just after administration at least one day throughout the treatment. This effect was observed over 10 days in 2 females, over 3 days in 2 animals and only one day in 5 females, respectively			
	52	Clinical signs	Rat	43 day	Oral	200 mg/kg bw/day	Increase	At high dose group, 60% (6/10) females showed ataxic gait throughout the treatment, mostly accompanied by a decrease on spontaneous motor activity.			

2.10.2.1.5. Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

WoE for T-mediated adversity

Regarding T-mediated parameters:

-None of the most relevant T-*in vivo* mechanistic and T-mediated parameters were measured in the studies provided. Limited information was described and showed in these studies. Only data from livers weights were presented in the 19-weeks (ID: 50; no guideline, checked for compliance with OECD TG 408), and in the combined repeated dose and reproduction/developmental toxicity studies (ID: 52; no guideline stated, checked for compliance with OECD TG 422), in which no changes were reported. The other T-mediated parameters were not evaluated neither in the combined repeated dose and reproduction/developmental toxicity study (ID: 52) conducted in rats, nor in the 19-weeks study in rats, which were the only two studies fully provided.

Additional data regarding sensitive to, but not diagnostic of EATS and target organ toxicity parameters, were extracted from short summaries that were found within the documentation provided in the dossier [e.g. 8/9 and 16 days repeated subcutaneous injection study in guinea pigs (ID: 51), and 14-days (ID: 53) repeated toxicity study in rats].

-In the repeated dose toxicity study conducted in guinea pigs (ID: 51, from Möller *et al*., 1939), two experiments were carried out:

Experiment 1
Four young male guinea pigs received daily subcutaneous injections of thymol at 106 or 233 mg/kg bw/day (20 or 40 mg/day, respectively), for 8-9 days.

Clear thyroid activation was seen in 2 animals and weakly in a third animal. The dose in which the effects were noted was not indicated.

Experiment 2
Four young male guinea pigs received 9 subcutaneous injections over 16 days of thymol at 243, 313 and 375 mg/kg bw/day (60, 80 and 100 mg/day, respectively).

There was thyroid activation in the 60 mg/day (243 mg/kg/day) dose group after two injections, in the 80 mg/day (313 mg/kg/day) dose group after three injections, and in the 100 mg/day (375 mg/kg/day) dose group after four injections.

The term “thyroid activation” was not described nor specified in the summary found in the dossier, so the parameters that determine this activation were unknown.

Möller *et al* findings should be investigated in further studies in order to clarify whether thymol can exert a potential thyroid activity.

Regarding sensitive to, but not diagnostic of EATS parameters:

-Effects regarding sensitive to, but not diagnostic of EATS parameters were exclusively noted in the combined repeated dose and reproduction/developmental toxicity study (ID: 52):

. Time to mating was longer in the treated groups showing a non-statistically significant dose-increased trend (17, 30 and 56% for low, mid and high dose groups, compared with controls).

. In the high dose group (200 mg/kg bw/day), the delivery rate was slightly lower than control group (12%). This reduction was not statistically significant and was caused by one female at high dose group that failed to delivery, in which 3 dead foetuses were found in the uterus at necropsy.

. At high dose group, 90% (9/10) of females showed decrease in spontaneous motor activity, at least one day throughout the treatment, just after chemical administration. This effect was observed over 10 days in 2 females, over 3 days in 2 animals and only one day in 5 females, respectively. On the other hand, 60% (6/10) females showed ataxic gait throughout the treatment, mostly accompanied by a decrease on spontaneous motor activity. Both effects were mainly observed during the first 13 days of the treatment.

. Regarding developmental parameters (e.g. skeletal and visceral abnormalities, pup weight, foetal development and post-implantation loss), no evidence of a treatment-related effect on development was observed in the combined repeated dose and reproduction/developmental toxicity study. Some minor effects on skeletal development and mating were observed in one pup with missing tail and another pup with protrusion of the naval region (confirmed at necropsy as naval hernia) at 40 mg/kg bw/day dose group. Moreover, 1 female failing to deliver at 200 mg/kg bw/day dose groups, however, since all other pairings in this group delivered a normal-sized litter this is considered to be a chance finding.

.Whitening of the adrenal gland was observed in 20% of females (2/10) in the high dose group; these findings were considered incidental due to their low incidence. Histopathological analysis showed an increase in lipid droplets in the fascicular zone in one female, whereas no abnormalities were found in the other affected adrenal gland.

Target organ toxicity

-Effects on target organ toxicity were exclusively noted in the combined repeated dose and reproduction/developmental toxicity study (ID: 52):

. Thickening of the forestomach wall (thickening of the proventricular wall) was observed in 100% of males (10/10) and in 11% of females (1/9) of the high dose group. The mucosa of the thickened forestomach mucosa was whitened and rough. Histopathological examinations revealed hyperplasia of the mucosal epithelium and was observed in both sexes in 40 mg/kg bw/day (90% of males and 40% of females, respectively) and 200 mg/kg bw/day dose groups (100% of males and 67% of females, respectively).

. Reduction in the size of the thymus was observed in 10% of females (1/10) from each of the 40 and 200 mg/kg bw/day dose groups. Shrinkage of the thymus was further confirmed after histopathological examination in these two animals. Moreover, increased absolute and relative thymus weight was observed in males (9.8% and 15.3%, respectively) at high dose tested, compared with controls.

Systemic toxicity

Systemic toxicity was reported in two studies (Study ID Matrix: 50 and 52) with no effect reported on body weight and limited mortality in rats (1/10 male died at high dose group in the combined repeated and reproduction/developmental toxicity test, Study ID: 52).

WoE for T-mediated activity

In vitro mechanistic test guidelines for the T modality are currently not available as well as specific *in vivo* mechanistic tests on mammals. *In vitro* mechanistic data for thymol are available via the US EPA CompTox Chemicals Dashboard.

The EDSP21 tab in the CompTox Chemicals Dashboard includes 12 thyroid receptor bioassays for thymol. Negative results were obtained in all of them.

On the other hand, The Danish QSAR Database (OECD conceptual framework level 1) reports that thymol is within the applicability domain of the model for thyroid receptor binding when the "battery" prediction is made. However, when predictions were analysed individually through the three available models (CASE Ultra, Leadscope Predictive Data Miner and SciQSAR), only Case Ultra displayed that thymol was within the applicability domain. In addition, the Danish QSAR Database reports that thymol gave positive results in respect of thyroid peroxidase (TPO) inhibition in two respective Leadscope global, binary composite QSAR models: QSAR1 and QSAR2 (but only QSAR1 was within the applicability domain of these models). The highest ranking structural features associated with activity were versions of phenols, anisole and aniline whereas the most frequent structural features associated with inactivity included ethers, esters, aryl halides and a tertiary amine [22]. The structure of thymol only includes a phenol group, so the substance has one feature associated with TPO inhibition activity.

Overall, according to ECHA/EFSA ED guideline point 3.4.2, T-mediated activity has not been sufficiently investigated due to the absence of most thyroid parameters measures in mammals studies.

2.10.2.1.6. Selection of relevant scenario for the ED assessment of T-modality

[22] Rosenberg SA, Watt ED, Judson RS, Simmons SO, Paul Friedman K, Dybdahl M, Nikolov NG, Wedebye EB. QSAR models for thyroperoxidase inhibition and screening of U.S. and EU chemical inventories. *Computational Toxicology*. 2017, Vol 4,pp 11-21. https://doi.org/10.1016/j.comtox.2017.07.006.

According to the ECHA/EFSA ED GD, following the assessment of available ED evidence, relevant scenarios (see table below) should be identified to inform on the conclusion of the ED assessment for humans or to further steps of investigation that are required for the respective EATS-modalities.

Based on the lack of data regarding T-*in vivo* mechanistic and most T-mediated parameters, it can be concluded that T-adversity cannot be assessed, and T-activity has not sufficiently investigated.

The relevant scenario for the T-modality is identified as 2a (iii).

Table 2.10.2.1.6: Identification of relevant scenario for T-modality

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "T-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	x
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.2.1.7. MoA analysis for T-modality

According to the ECHA/EFSA guidance in cases of Scenario 2a(iii), a MoA analysis for T-modality is not required.

2.10.2.1.8. Conclusion of the assessment of T-modality

According to the ECHA/EFSA GD on ED, the relevant scenario proposed for the T-modality is 2a (iii). Based on the absence of following studies: OECD TG 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3, and the absence of data from most T-*in-vivo* mechanistic and T-mediated parameters in the provided studies, it has been concluded that T-mediated adversity and T-activity has not been sufficiently investigated. Moreover, *in vitro*-mechanistic data from Thyroid Bioactivity Model US EPA CompTox Chemicals Dashboard did not show positive/active results. However, equivocal evidences of thyroid activity were observed in guinea pigs after subcutaneous injections of thymol (ID: 51, from Möller *et al*., 1939), so further investigations are needed to clarify these findings and whether thymol exerts a potential role on T-modality.

2.10.2.2. ED assessment for EAS-modality

2.10.2.2.1. Analysis of non-experimental data

In accordance with the OECD Conceptual Framework and the ECHA/EFSA GD on ED, Level 1, EAS-related non-test information was gathered for thymol. Qualitative structural activity relationship (QSAR) data was obtained for thymol from the Danish QSAR database, and results are summarised below.

Table 2.10.2.2.1/1: Results of Danish QSAR database for thymol regarding EAS-modality

	Exp.	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)	NEG	NEG IN	NEG IN	NEG IN	NEG IN
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)	NEG	NEG IN	NEG IN	NEG IN	INC OUT

Estrogen Receptor α Activation (Human *in*		NEG IN	NEG IN	NEG IN	NEG OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)		NA	NA	INC_OUT	NA
Androgen Receptor Inhibition (Human *in vitro*)		NEG IN	NEG IN	NEG OUT	NEG IN
Androgen Receptor Binding, CoMPARA data (*in vitro*)	NEG	NA	NA	NEG IN	NA
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)	NEG	NA	NA	INC OUT	NA
Androgen Receptor Activation, CoMPARA data (*in vitro*)	NEG	NA	NA	NEG_IN	NA

Key: POS = Positive; NEG = Negative; IN = within the applicability domain of the model; OUT = outside of the applicability domain of the model

The Danish QSAR Database predictions report that thymol lacks the potential to interact with estrogen and androgen receptors.

OECD ToolBox
The results of the OECD QSAR Toolbox v.4.2 profilers for thymol in respect of E-related endpoints are shown below:

Table 2.10.2.2.1/2: Results of the OECD QSAR Toolbox v.4.2 for thymol

[1] Estrogen Receptor Binding, alerts in:	
parent only	Weak binder, OH group
metabolites from in vivo Rat metabolism simulator only	Moderate binder, OH group; Weak binder, OH group
metabolites from Rat liver S9 metabolism simulator only	Moderate binder, OH group; Weak binder, OH group
[2] rtER Expert System - USEPA, alerts in	
parent only	Alkoxyphenols
metabolites from in vivo Rat metabolism simulator only	No alert found
metabolites from Rat liver S9 metabolism simulator only	No alert found
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

[1] Estrogen receptor binding: Weak binder, OH group

Estrogen receptor (ER) binding is a molecular initiating event similar to protein binding that leads to a series of adverse outcomes, which are typically considered reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER-binding and possible endocrine disruption.

Since the ER-binding is a receptor mediated event, particular organic functional groups, size and shape are critical to binding potency. Chemicals with a single 5-or 6-member carbon ring structure with an unhindered hydroxyl-group (-OH) (a hydroxyl group in the para- or meta-position on the ring and without ortho substituents to the hydroxyl group) (5) are ER binders. Binding potency is related to the size and shape of non-hydroxylated-ring aspect of the molecule, which can be grossly measured by molecular weight.

The incorporated Toolbox ER binding profiling scheme is based on structural and parametric rules extracted from literature sources and supported by experimental data. The ER-binding profiler classifies chemicals as non-binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals:

1. Very strong binders: Chemicals with MW between 200 and 500 Da and two rings with a hydroxyl group connected to each of them.

2. Strong binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 200 and 500 Da.

3. Moderate binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 170 and 200 Da.

4. Weak binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW less than 170 Da.
If the target chemical does not meet some of the structural and parametric requirements listed above it is classified as Non binder:
- Non binder with impaired hydroxyl or amino group.
- Non binder, MW more than 500 Da.
- Non binders without hydroxyl or amino group.
- Non-binder, non-cyclic.

The OECD Toolbox v.4.2 predicts that thymol is not a potential ER binder.

[2] rtER Expert System USEPA: Alkoxyphenols

The rtER Expert System ver.1 – USEPA profiler consists of molecular definitions that mimic the structural criteria of chemical classes that are potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES) The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity. In the Toolbox, the rtER Expert System ver.1 – USEPA profiler is used for the purpose of categorization based on the structural definitions of the original ERES chemical classes. The rtER Expert System ver.1 – USEPA profiler is intended for categorization purpose and not for predicting relative binding affinity (RBA). rtER Expert System ver.1.
USEPA profiler predicts that thymol meets the criteria of chemical classes that are potential ER binders, on the basis that is an alkoxyphenol substance.

The rtER Expert System ver.1 – USEPA profiler consists of molecular definitions mimic the structural criteria of chemical classes potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES) The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity. The Estrogen Receptor Expert System (ERES) Profiler is an effects-based automated system used to predict estrogen receptor binding affinity

ToxCast: CERAPP Potency Level (ER-Related activity) and COMPARA (AR-related activity)

The ToxCast Model Dashboard includes predictions of the estrogen receptor activity of thymol, based on the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP[20]). The CERAPP is a large-scale modelling project which has investigated the efficacy of using predictive computational models trained on high-throughput screening data (e.g. from the EDSP21 initiative) to evaluate the ER-related activity of thousands of chemicals, and identify priorities for further testing.

On the other hand, the ToxCast Models Dashboard also includes predictions of the androgen receptor activity of thymol based on the COMPARA. COMPARA is a large scale collaboration between 35 international groups using QSAR models to predict androgen receptor activity using a common training set of 1746 compounds provided by the US EPA. The result is consensus model of AR agonist activity that is run against the DSSTox chemical library that aims to identify priorities for further testing. The CERAPP and COMPARA predictions for estrogen and androgen activity are summarised in the following table:

Table 2.10.2.2.1/3: Results of the CERAPP and COMPARA predictions for thymol

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0	0	-
ToxCast Pathway Model (AUC)	Estrogen	0.00465	0	-
COMPARA (Consensus)	Androgen	Inactive	Inactive	Inactive
CERAPP Potency Level (From Literature)	Estrogen	-	Inactive (Inactive)	-
CERAPP Potency Level (Consensus)	Estrogen	Inactive	Inactive (Inactive)	Inactive (Inactive)

As noted, thymol displayed inactive results for estrogen and androgen receptor activities.

2.10.2.2.2. US EPA CompTox Chemicals Dashboard

Estrogen receptor bioassays

The EDSP1 tab in the CompTox Chemicals Dashboard v4 includes 19 ER bioassays for thymol. The results were positive/active for only 2 assays (ATG_ERa_TRANS_up and OT_ER_ERbERb_0480, with AC_{50} values of 46.18 and 34.88, respectively) and negative for 17 assays. None of both assays presented flags. However, the cytotoxicity limit was 10 µM, so the AC_{50} values displayed in the assays was higher than the cytotoxicity limit, and therefore, the results were not reliable. The results are showed in table 2.10.2.2.2/1.

Table 2.10.2.2.2/1: Summary of US EPA ToxCast EDSP21- estrogenic bioactivity assays for thymol

Assay endpoint	Assay type	Organism	Result	Study ID Matrix
ACEA_ER_80hr	real-time cell-growth kinetics	human	Inactive	13
ACEA_ER_AUC_viability	real-time cell-growth kinetics	human	Inactive	14
ATG_ERa_TRANS_up	**mRNA induction**	**human**	**active**	**15**
ATG_ERE_CIS_up	mRNA induction	human	inactive	16
NVS_NR_hER	radioligand binding	human	inactive	17
OT_ERa_EREGFP_0120	fluorescent protein induction	human	Inactive	18
OT_ERa_EREGFP_0480	fluorescent protein induction	human	Inactive	19
OT_ER_ERaERa_0480	protein fragment complementation assay	human	Inactive	20
OT_ER_ERaERa_1440	protein fragment complementation assay	human	Inactive	21
OT_ER_ERaERb_0480	protein fragment complementation assay	human	Inactive	22
OT_ER_ERaERb_1440	protein fragment complementation assay	human	Inactive	23
OT_ER_ERbERb_0480	**protein fragment complementation assay**	**human**	**Active**	**24**
OT_ER_ERbERb_1440	protein fragment complementation assay	human		25
TOX21_ERa_BLA_Agonist_ratio	beta lactamase induction	human		26
TOX21_ERa_BLA_Antagonist_ratio	beta lactamase induction	human		27
TOX21_ERa_BLA_Antagonist_viability	ATP content	human		28
TOX21_ERa_LUC_VM7_Agonist	luciferase induction	human		29
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	luciferase induction	human		30
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability	ATP content	human		31

Androgen receptor bioassays

Thymol was tested in 14 assays included in the ToxCast AR Bioactivity Model. Negative results were obtained in all of them. The results are showed in table 2.10.2.2.2/2

Table 2.10.2.2.2/2: Summary of US EPA ToxCast EDSP21- androgenic bioactivity assays for thymol

Assay endpoint	Assay type	Organism	Result	Study ID Matrix
ATG_AR_TRANS_up	mRNA induction	human	Inactive	32
OT_AR_ARELUC_AG_1440	Luciferase induction	hamster	Inactive	33
OT_AR_ARSRC1_0480	protein fragment complementation assay	human	inactive	34
OT_AR_ARSRC1_0960	protein fragment complementation assay	human	inactive	35
TOX21_AR_BLA_Agonist_ratio	beta lactamase induction	human	inactive	36
TOX21_AR_BLA_Antagonist_ratio	beta lactamase induction	human	Inactive	37
TOX21_AR_BLA_Antagonist_viability	ATP-content	human	Inactive	38
TOX21_AR_LUC_MDAKB2_Agonist	Luciferase induction	human	Inactive	39
TOX21_AR_LUC_MDAKB2_Antagonist _0.5nM_R1881	Luciferase induction	human	Inactive	40
TOX21_AR_LUC_MDAKB2_Antagonist _0.5nM_R1881_viability	ATP-content	human	Inactive	41
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	Luciferase induction	human	Inactive	42
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881_viability	ATP-content	human	Inactive	43
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist	Protein-protein binding	human	Inactive	44
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	Protein-protein binding	human	Inactive	45

Steroidogenesis bioassays

The EDSP21 tab in the CompTox Chemicals Dashboard includes only 2 steroidogenesis bioassays for thymol. Negative results were displayed in these two assays. The results are showed in table 2.10.2.2.2/3

Table 2.10.2.2.2/3: Summary of US EPA ToxCast EDSP21- Steroidogenesis bioactivity assays for thymol.

Assay endpoint	Assay type	Organism	Result	Study ID Matrix
TOX21_Aromatase_Inhibition	Luciferase induction	human	Inactive	46
TOX21_Aromatase_Inhibition_viability	ATP-content	human	Inactive	47

2.10.2.2.3. Have EAS-mediated parameters been sufficiently investigated?

The available dataset of *in vivo* mammalian toxicology studies for thymol consists mainly in a combined repeated dose and reproduction/developmental toxicity study (ID: 52) conducted in rats (43 and 40 days for males and females, respectively), and a 19-week sub-chronic toxicity study conducted in rats (ID: 50, no guideline, checked for compliance with OECD TG 408). On the other hand, very short summaries of non-guideline published studies were also found within the documentation provided in the dossier [e.g. 8/9 and 16 days repeated subcutaneous injection study in guinea pigs (ID: 51), and a 14-days repeated toxicity study in rats (ID: 53)] Neither two-generation reproductive toxicity study (OECD TG 416), nor the extended one-generation reproductive toxicity study (OECD

TG 443) had been presented. Moreover, much of the available data pre-dates revisions that were made to the OECD Test Guidelines to include EAS-mediated parameters.

Table 14 of the ECHA/EFSA GD on ED provides a list of relevant EAS-mediated parameters that may be investigated in the OECD CF Level 4 and 5 *in vivo* OECD TG compliant mammalian toxicology studies. Using the currently available set of toxicological data for thymol, the table 2.10.2.2.3/1 summarises the available information on EAS-mediated parameters.

Therefore, based on the assessment provided in the table below, only data about oestrus cyclicity, organ weights and histopathology of the testis, prostate and epididymis, were found within the dossier documentation. No OECD TG 416 or 443 reproductive toxicity studies were provided in the renewal dossier for the active substance thymol.

Table 2.10.2.2.3/1: Summary of EAS-mediated parameters investigated in mammalian toxicology studies

EAS-mediated parameters	**OECD Test guideline**	**Sufficiently investigated?** **Overall conclusion: No (not sufficiently investigated)** Based on the lack of OECD 416 and 443 studies, and the absence of data regarding most EAS-mediated parameters in the studies provided.
Estradiol level	408 (optional)	No data
Follicle stimulating hormone (FSH) level	408 (optional)	No data
Luteinising hormone (LH) level	408 (optional)	No data
Testosterone level	408 (optional)	No data
Accessory sex organs histopathology	408, 421, 451-3	No data
Age at first oestrus	OPPTS 890.1450	No data
Age at balanopreputial separation	426, 416, 443	No data
Age at vaginal opening	426, 416, 443	No data
Anogenital distance (AGD)	414, 421, 426, 416, 443	No data
Cervix histopathology	407, 408, 415, 422, 451-3, 416, 443	No data
Coagulating gland histopathology	407, 408, 415, 422, 451-3, 416, 443	No data
Coagulating gland weight	407, 421, 422, 416, 443	No data
Cowper's gland weight	421 (optimal), 422 (optional)	No data
Epididymis histopathology	407, 408, 415 (optional) 421, 422, 451-3, 416, 443	Epididymis histopathology was performed in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422).
Epididymis weight	407, 408, 421, 422, 451-3, 416, 443	Epididymis weight was measured in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422).
Oestrus cyclicity	407 (optional), 408, 421, 422, 416, 443	Data of oestrus cyclicity was reported in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422).

EAS-mediated parameters	OECD Test guideline	**Sufficiently investigated?** **Overall conclusion: No (not sufficiently investigated)** Based on the lack of OECD 416 and 443 studies, and the absence of data regarding most EAS-mediated parameters in the studies provided.
Glans penis weight	421 (optimal), 422 (optional)	No data
Genital abnormalities	414, 415, 421, 422, 416, 443	No data
LABC weight	421 (optimal), 422 (optional), OPPTS 890.1500	No data
Mammary gland histopathology (male)	407 (optional), 408, 422, 443, 451-3 (optional)	No data
Mammary gland histopathology (female)	407, 408, 451-3, 443	No data
Nipple development	421, 422, 443	No data
Ovary histopathology	407, 408, 415 (optional) 421, 422, 426, 451-3, 416, 443	No data
Ovary weight	407 (optional), 408, 421 (optional), 422, 451-3, 416, 443	No data
Oviduct histopathology	408, 415 (optional), 443	No data
Prostate histopathology (with seminal vesicles and coagulating glands)	407, 408, 415 (optional) 421, 422, 426, 451-3, 416, 443	Prostate histopathology was performed in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422).
Prostate weight	407, 408, 421, 422, 416, 443	Prostate weight was measured in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422).
Seminal vesicles histopathology	407, 408, 415 (optional), 422, 451-3, 416, 443	No data
Seminal vesicles weight	407, 408, 421, 422, 416, 443	No data
Sperm morphology	408 (optional), 416, 443	No data
Sperm motility	408 (optional), 416, 443	No data
Sperm numbers	408 (optional), 416, 443	No data
Testis histopathology	407, 408, , 415 (optional) 421, 422, 451-3, 416, 443	Testis histopathology was performed in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422), and in the 19-weeks sub-chronic toxicity study in rats (checked for compliance with OECD TG 408).
Testis weight	407, 408, 421, 422, 451-3, 416, 443	Testes were weighted in the combined repeated dose and reproduction/developmental toxicity study conducted in rats (checked for compliance with OECD TG 422), and in the 19-weeks sub-chronic toxicity study in rats (checked for compliance with OECD TG 408).

EAS-mediated parameters	OECD Test guideline	Sufficiently investigated? **Overall conclusion: No (not sufficiently investigated)** Based on the lack of OECD 416 and 443 studies, and the absence of data regarding most EAS-mediated parameters in the studies provided.
Uterus histopathology (with cervix)	407, 408, 415 (optional), 421 (optional), 422, 451-3, 416, 443	No data
Uterus weight (with cervix)	407 (optional), 408, 414 (gravid uterus), 415 (optional), 421 (optional), 422, 451-3, 416, 443	No data
Vagina histopathology	407, 408, 415 (optional), 422, 451-3, 416, 443	No data
Vaginal smear	407 (optional), 408, 421, 422, 416, 443	No data

Table 2.10.2.2.3/2: Parameters related to EAS-modalities that have not been measured

OECD TG 422 EAS-mediated parameters not investigated
- Cervix histopathology - Coagulating gland histopathology - Coagulating gland weight - Mammary gland histopathology (male) -Nipple development -Ovary weight - Ovary histopathology - Seminal vesicles histopathology - Seminal vesicles weight. -Uterus weight (with cervix) - Uterus histopathology (with cervix) - Vaginal histopathology. - Vaginal smear - Anogenital distance measurement -Genital abnormalities
OECD TG 408 EAS-mediated parameters not investigated
- Accessory sex organs histopathology. - Cervix histopathology. - Coagulating gland histopathology - Epididymis weight - Epididymis histopathology - Oestrus cyclicity -Mammary gland histopathology (male and female) - Ovary weight - Ovary histopathology - Oviduct histopathology. - Prostate weight - Prostate histopathology - Seminal vesicles weight. - Seminal vesicles histopathology. - Uterus weight (with cervix) - Uterus histopathology (with cervix)

- Vaginal histopathology - Vaginal smear

Regarding to the EAS-mediated endocrine activity:

E-modality: It is considered sufficiently investigated based on the estrogenic activity output data from the US EPA ToxCast Bioactivity Model. However, equivocal positive results were obtained due to AC_{50} was higher than limit for cytotoxicity in the assays.

A-modality: It is not considered sufficiently investigated based on the lack of the "Stably transfected human androgen receptor transcriptional activation assay" (OECD TG 458).

S-modality: It is not considered sufficiently investigated based on the lack of the H295R Steroidogenesis assay (OECD TG 456) and/or a study in line with OPPTS 890.1200 (Aromatase assay).

Therefore, both EAS-mediated adversity and EAS-mediated endocrine activity have not been sufficiently investigated.

2.10.2.2.4. Lines of evidence for adverse effects and endocrine activity related to T-modality

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	32	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect	Negative results were obtained in ToxCast AR Bioactivity assays.	Negative results were obtained in ToxCast AR Bioactivity assays. However, antagonist activity was observed in two non-guideline assays models carried out in: Stably transfected human embryonic kidney cells (233Y) and in recombinant yeast assays.	A
	33	Androgen receptor	Hamster	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	34	Androgen receptor	Human	8 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	35	Androgen receptor	Human	16 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	36	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	37	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	38	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	39	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	40	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	41	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	42	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	43	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	44	Androgen receptor	Human	3 hour	Uptake from the medium (in vitro)	-	No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	45	Androgen receptor	Human	3 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	48	Androgen receptor	Human	16 hour	Uptake from the medium (in vitro)	-	No effect	No effect	Thymol exhibited anti-androgenic activity in cell-based human AR-mediated and in recombinant yeast assays.		
	48	Androgen receptor	Human	16 hour	Uptake from the medium (in vitro)	-	Change	Thymol inhibited hAR transcriptional activity induced by 0.125 nM testosterone (34% at 10 µM and by 11% at a concentration of 1.0 µM). Signs of anti-androgenic activity.			
	49	Androgen receptor	Human/ yeast	Yeast: 2.5 h, AIZ-AR cell line; 24 hour	Uptake from the medium (in vitro)	-	Change	In the androgenic recombinant yeast assay: thymol did not exhibit androgenic properties but was indicated as antagonist of the androgenic receptor. Thymol exhibited significant anti-androgenic effect in a concentration-dependent manner. The IC50 of the anti-androgenic effect was 73±9 µM. Thymol antagonistic effect was not observed in the case of the AIZ-AR human cell line, which is probably due to sensitivity of the assay when			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								higher applied concentrations were toxic to the cell line.			
	47	Cellular proliferation	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect	Negative results were obtained in ToxCast steroidogenesis Bioactivity assays.	Negative results were obtained in ToxCast steroidogenesis Bioactivity assays.	S
	46	CYP19A1	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	13	Estrogen receptor	Human	80 hour	Uptake from the medium (in vitro)	-	No effect	No effect	Two positive results were obtained in ToxCast ER Bioactivity assays. However, the cytotoxicity limit was 10 µM, so the AC50 values displayed in the assays was higher than the cytotoxicity limit, and therefore, the results were not reliable.	Positive results were obtained in ToxCast ER Bioactivity assays. However, the AC50 values were higher than cytotoxicity limit, so the results were not reliable. On the other hand, anti-estrogenic activity was observed in two non-guideline assays models carried out in: T47D breast carcinoma cell line and in recombinant yeast assays. However, the non-guideline study and the methodological deficiencies make the results of the study unreliable.	E
	14	Estrogen receptor	Human	80 hour	Uptake from the medium (in vitro)	46.18 µM	Change	Thymol is active for estrogen receptor activity assay. AC50 (hill model)= 46.18			
	15	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	16	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	17	Estrogen receptor	Human	18 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	18	Estrogen receptor	Human	2 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	19	Estrogen receptor	Human	8 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	20	Estrogen receptor	Human	8 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	21	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	22	Estrogen receptor	Human	8 hour	Uptake from the medium (in vitro)	-	No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	23	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	24	Estrogen receptor	Human	8 hour	Uptake from the medium (in vitro)	34.88 µM	Change	Thymol is active for estrogen receptor activity assay. AC50 (hill model)= 34.18			
	25	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	26	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	27	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	28	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	29	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	30	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	31	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)	-	No effect	No effect			
	49	Estrogen receptor	Human/ yeast	Yeast: 2.5 h, T47D cell line; 48 hour	Uptake from the medium (in vitro)	-	Change	Thymol did not trigger estrogenic effects even at the highest concentration levels used in the S. cerevisiae BMAEREluc/ERa yeast assay and in the T47D-CXCL12 test. On the other hand, thymol			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								exhibited significant anti-estrogenic effects in a concentration-dependent manner in both assays. The IC50 of the anti-estrogenic effect was 237±16 µM and 177±39 µM for the yeast and human cell line assays.			
EAS-mediated	52	Epididymis histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No effect	No effects were observed in oestrus cyclicity, and in the organ weights and histopathology of the testis, prostate and epididymis. Most of the EAS-mediated parameters were not measured.	EAS
	52	Epididymis weight	Rat	43 day	Oral	-	No effect	No effect	No effect		
	52	Estrus cyclicity	Rat	43 day	Oral	-	No effect	No effect	No effect		
	52	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No effect		
	52	Prostate weight	Rat	43 day	Oral	-	No effect	No effect	No effect		
	50	Testis histopathology	Rat	19 week	Oral	-	No effect	No effect	No effect		
	52	Testis histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No effect		
	50	Testis weight	Rat	19 week	Oral		No effect	No effect	No effect		
	52	Testis weight	Rat	43 day	Oral		No effect	No effect	No effect		
Sensitive to, but not diagnostic of, EATS	52	Adrenals histopathology	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Histopathological analysis showed an increase in lipid droplets in the fascicular zone of the adrenal gland in 10% (1/10) of females of high dose group.	Low incidence of lipid droplets was observed in adrenal at high dose tested.	No adverse effects were detected in sensitive EATS-related parameters.	N
	52	Adrenals weight	Rat	43 day	Oral	-	No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Brain histopathology examination	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		
	52	Dystocia	Rat	43 day	Oral	200 mg/kg bw/day	Increase	1 female failed to deliver in the high dose group. This finding was considered incidental.	No treatment related effect was observed.		
	52	Fertility (mammals)	Rat	43 day	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Number of implantations, corpora lutea	Rat	43 day	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Number of live births	Rat	43 day	Oral	-	No effect	No effect			
	52	Pituitary histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		
	52	Pituitary weight	Rat	43 day	Oral	-	No effect	No effect			
	52	Presence of anomalies (external, visceral, skeletal	Rat	43 day	Oral	40 mg/kg bw/day	Increase	One pup with missing tail and one pup with protrusion of the naval region were described in the low dose group.	No treatment related effect was observed.		
	53	Presence of anomalies (external, visceral, skeletal	Rat	14 day	Oral	-	No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	Rat			-	No effect	No effect			
	53	Presence of anomalies (external, visceral, skeletal	mice			-	No effect	No effect			
	53	Presence of anomalies	hamster			-	No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		(external, visceral, skeletal									
	53	Presence of anomalies (external, visceral, skeletal	rabbit			-	No effect	No effect			
	52	Sex ratio	Rat	43 day	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Time to mating	Rat	43 day	Oral	8 mg/kg bw/day	Increase	Time to mating was longer in the treated groups showing a non-statistically significant dose-increased trend (17, 30 and 56% for low, mid and high dose groups, compared with controls).	No treatment related effect was observed.		
Target organ toxicity	52	Adrenal gland necropsy	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Whitening of the adrenal gland was observed in 20% of females (2/10) in the high dose group; these findings were considered incidental due to their low incidence	No treatment related effect was observed.	Target organ toxicity was mainly observed in forestomach of both sexes at mid and high dose tested (hyperplasia of mucosa). Equivocal effects were noted in thymus (reduction in females at mid and high dose group, and increased weight on males at high dose).	
	50	Heart histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Heart histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Heart weight	Rat	19 week	Oral	-	No effect	No effect			
	50	Kidney histopathology	Rat	19 week	Oral	-	No effect	No effect	No treatment related effect was observed.		
	52	Kidney histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Kidney weight	Rat	19 week	Oral		No effect	No effect			
	52	Kidney weight	Rat	43 day	Oral		No effect	No effect			
	50	Liver histopathology	Rat	19 week	Oral		No effect	No effect	No treatment related effect was observed.		

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Liver histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	52	Lung histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect	No treatment related effect was observed.		
	50	Spleen histopathology	Rat	19 week	Oral		No effect	No effect	No treatment related effect was observed.		
	52	Spleen histopathology	Rat	43 day	Oral	200 mg/kg bw/day	No effect	No effect			
	50	Spleen weight	Rat	19 week	Oral	-	No effect	No effect			
	52	Stomach histopathology	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Histopathological examinations revealed hyperplasia of the mucosal epithelium and was observed in both sexes in 40 mg/kg bw/day (90% of males and 40% of females, respectively) and 200 mg/kg bw/day dose groups (100% of males and 67% of females, respectively).	A dose related increase of hyperplasia on the mucosa of forestomach was observed in both sexes.		
	52	Stomach necropsy	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Thickening of the forestomach wall (thickening of the proventricular wall) was observed in 100% of males (10/10) and in 11% of females (1/9) of the high dose group.			
	52	Thymus histopathology	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Shrinkage of the thymus was further confirmed after histopathological examination in 10% females of 40 and	Increased in the thymus weight (absolute and relative) was observed in males at high dose, whereas reduction of		

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								200 mg/kg bw/day dose groups.	the thymus was observed in females at mid and high dose groups (10% of females of each group).		
	52	Thymus necropsy	Rat	43 day	Oral	40 mg/kg bw/day	Increase	Reduction in the size of the thymus was observed in 10% of females (1/10) from each of the 40 and 200 mg/kg bw/day dose groups			
	52	Thymus weight	Rat	43 day	Oral	200 mg/kg bw/day	Increase	Increased absolute and relative thymus weight was observed in males (9.8% and 15.3%, respectively) at high dose tested.			
Systemic toxicity	52	Body weight	Rat	43 day	Oral	-	No effect	No effect	No treatment related effect was observed.	Signs of neurotoxicity (decrease motor activity and ataxia) were observed in females were observed at high dose tested. Both effects were mainly observed during the first 13 days of the treatment	
	52	Body weight	Rat	43 day	Oral	200 mg/kg bw/day	Decrease	Decrease on pups weights at birth (10.3% and 9.4% for males and females, respectively), and on Day 4 (10.2% and 8.8% for males and females, respectively) were found at the high dose level compared with controls, whereas body weight gains decreased up to 14.6% for males and 10.5% for females, compared with controls. However, these differences were not statistically significant.			
	50	Mortality	Rat	19 week	Oral	-	No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Mortality	Rat	43 day	Oral	200 mg/kg bw/day	Increase	1/10 male died	1/10 male died at high dose group		
	52	Mortality	Rat	43 day	Oral	-	No effect	No effect	No effect		
	52	Clinical signs	Rat	43 day	Oral	200 mg/kg bw/day	Decrease	At high dose group, 90% (9/10) of females showed decrease in spontaneous motor activity just after administration at least one day throughout the treatment. This effect was observed over 10 days in 2 females, over 3 days in 2 animals and only one day in 5 females, respectively			
	52	Clinical signs	Rat	43 day	Oral	200 mg/kg bw/day	Increase	At high dose group, 60% (6/10) females showed ataxic gait throughout the treatment, mostly accompanied by a decrease on spontaneous motor activity.			

2.10.2.2.5. Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

WoE for EAS-mediated adversity

This section provides the lines of evidence for the *in vivo* mammalian toxicology studies (Level 4) using test substance thymol in respect of the EAS-modality. The following sections provide an analysis of the integrated lines of evidence and report the weight of evidence in respect of EAS-mediated adversity.

Regarding EAS-mediated parameters

No effects were observed in oestrus cyclicity, and in the organ weights and histopathology of the testis, prostate and epididymis. These parameters were the only ones that were analysed in the combined repeated dose and reproduction/developmental toxicity study (ID: 52; no-guidelines stated, checked for compliance with OECD TG 422). Testis weight and histopathology was also performed in the 19-weeks study in rats (ID: 52, no guideline stated, checked for compliance with OECD TG 408), and no alterations were found.

Additional data regarding sensitive to, but not diagnostic of EATS and target organ toxicity parameters were extracted from short summaries of non-guideline published studies that were also found within the documentation provided in the dossier [e.g. 8/9 and 16 days repeated subcutaneous injection study in guinea pigs (ID: 51), and 14-days (ID: 53) repeated toxicity study in rats).

Regarding sensitive to, but not diagnostic of EATS parameters:

-Effects regarding sensitive to, but not diagnostic of EATS parameters were exclusively noted in the combined repeated dose and reproduction/developmental toxicity study (ID: 52):

- Time to mating was longer in the treated groups showing a not statistically significant dose-increased trend (17, 30 and 56% for low, mid and high dose groups, compared with controls).

- In the high dose group (200 mg/kg bw/day), the delivery rate was slightly lower than control group (12%). This reduction was not statistically significant and was caused by one female at high dose group that failed to delivery, in which 3 dead foetuses were found in the uterus at necropsy.

- At high dose group, 90% (9/10) of females showed decrease in spontaneous motor activity just after administration at least one day throughout the treatment. This effect was observed over 10 days in 2 females, over 3 days in 2 animals and only one day in 5 females, respectively. On the other hand, 60% (6/10) females showed ataxic gait throughout the treatment, mostly accompanied by a decrease on spontaneous motor activity. Both effects were mainly observed during the first 13 days of the treatment.

- Developmental parameters (e.g. skeletal and visceral abnormalities, pup weight, foetal development and post-implantation loss) are considered 'sensitive to, but not diagnostic of, EATS'. In the combined repeated dose toxicity study with the reproduction/developmental study there was no evidence for a treatment-related effect on development. Some minor effects on skeletal development and mating were observed with one pup with missing tail and one pup with protrusion of the naval region (confirmed at necropsy as naval hernia) at 40 mg/kg bw/day and 1 female failing to deliver at 200 mg/kg bw/day (however, since all other pairings in this group delivered a normal-sized litter this is considered to be a chance finding).

- Whitening of the adrenal gland was observed in 20% of females (2/10) in the high dose group; these findings were considered incidental due to their low incidence. Histopathological analysis showed an increase in lipid droplets in the fascicular zone in one female, whereas no abnormalities were found in the other affected adrenal gland.

Target organ toxicity

-Effects on target organ toxicity were exclusively noted in the combined repeated dose and reproduction/developmental toxicity study (ID: 52):

- Thickening of the forestomach wall (thickening of the proventricular wall) was observed in 100% of males (10/10) and in 11% of females (1/9) of the high dose group. The mucosa of the thickened forestomach mucosa was whitened and rough. Histopathological examinations revealed hyperplasia of the mucosal epithelium and was observed in both sexes in 40 mg/kg bw/day (90% of males and 40% of females, respectively) and 200 mg/kg bw/day dose groups (100% of males and 67% of females, respectively).

- Reduction in the size of the thymus was observed in 10% of females (1/10) from each of the 40 and 200 mg/kg bw/day dose groups. Shrinkage of the thymus was further confirmed after histopathological examination in these

two animals. Increased absolute and relative thymus weight was observed in males (9.8% and 15.3%, respectively) at high dose tested.

Systemic toxicity

Systemic toxicity was reported in two studies (Study ID Matrix: 50 and 52) with no effect reported on body weight and limited mortality in rats (1/10 male died at high dose group in the combined repeated and reproduction/developmental toxicity test, Study ID: 52).

WoE for EAS-mediated activity

-Toxcast ER Bioactivity model showed two active/positive assays (ID: 15 and 24). ATG_ERa_TRANS_up and OT_ER_ERbERb_0480. However, the AC_{50} value displayed in the assays (46.18 and 34.88 µM, respectively) was higher than the cytotoxicity limit (10 µM), and therefore, the results were not reliable. Moreover, the outcome from ToxCast Models Dashboard CERAPP (consensus) and COMPARA predictions showed inactive results for estrogen and androgen receptors activities.

On the other hand, neither Toxcast AR nor Steroidogenesis bioactivity models displayed active/positive results for thymol.

-On the other hand, an *in vitro* androgen/anti-androgen receptor binding assay (Chen *et al*, ID:48) showed a dose-dependent anti-androgenic activity in the mammalian stably-transfected cell line 2933Y, in which thymol inhibited in a dose-response pattern the hAR transcriptional activity induced by testosterone, without any evidence of cytotoxicity. Chen *et al.* used a modified version of the current AR-STTA (Stably transfected Human androgen receptor transactivation assay for detection of androgenic/anti-androgenic activity of chemicals) guideline (OCED TG 458). However, the highest concentration of 10 µM tested was approximately 100000-fold in excess of the T concentration used, so exceed several orders of magnitude a natural scenario. A study in line with OECD TG 458 should be conducted to confirm or rule out these findings.

-In addition, in another published study (Michalikova *et al*, ID: 49), thymol showed dose-dependent anti-estrogenic and anti-androgenic activity. The thymol IC_{50} value displayed in the androgen receptor inhibition assay (73µM) was low than the value in the estrogen inhibition tests systems (237 and 177, for recombinant yeast and TT47D-CXCL12 assays, respectively). These values showed that thymol was a weak competitive inhibitor of estrogen receptor compared with its potential role to inhibit androgen receptor. By contrast, no evidence of ER or AR agonist activity was shown in the assays. The anti-androgenic activity finding is in agreement with previous Chen *et al*. study. However, the non-guideline study and the methodological deficiencies make the results of this study unreliable.

Overall, further information regarding EAS-related activity are needed:

A modality: A study in line with OECD TG 458.

S modality: A study in line with OECD TG 456 (H295R Steroidogenesis Assay) and/or a study in line with OPPTS 890.1200 (Aromatase assay).

In case of OECD TG 458, 456 and OPPTS 890.1200 are negative, a study in line with OECD TG 441 (Hershberger Assay) is required.

2.10.2.2.6. Selection of relevant scenario for the ED assessment of EAS-modality

No OECD TG 443 or OECD TG 416 studies have been conducted with thymol, and there were no measurements of most EAS-mediated parameters in the studies provided. Overall, a comprehensive assessment of the EAS-adversity cannot be performed. On the other hand, not enough data was available regarding EAS-related endocrine activities. Only two positive results were obtained in the *in vitro* estrogen receptor mechanistic assays, however, the cytotoxicity limit was 10 µM, so the AC_{50} value displayed in the assays (46.18 and 34.88 µM) was higher than the cytotoxicity limit, and therefore, the results were not reliable. Equivocal evidences of anti-androgenic activity were observed in two non-guidelines studies, which presented methodological deficiencies. Hence, studies in line with OECD-guidelines are required to proper assessment EAS-mediated activity. Overall, according to the EFSA/ECHA ED guidance, it corresponds to the scenario 2a (iii).

The relevant scenario for the EAS-modality is identified as 2a (iii).

Table 2.10.2.2.6: Identification of relevant scenario for EAS-modality

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "EAS-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EAS-mediated" parameters. Depending on the outcome move to corresponding scenario	x
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.2.2.7. MoA analysis for EAS-modalities

According to the ED EFSA/ECHA guidance (2018), in cases of Scenario 2a (iii), a MoA analysis for EAS-modalities is not required.

2.10.2.2.8. Conclusion on the assessment of EAS-modalities

Based on the available data, it was concluded that endocrine activity has not been sufficiently investigated and the absence of most EAS-parameters measurements does not enable conclusions to be drawn about EAS adversity. Therefore, a scenario 2a (iii) has been established. Additional studies in line with the ED EFSA/ECHA guidance are required:

A modality: A study in line with OECD TG 458.

S modality: A study in line with OECD TG 456 (H295R Steroidogenesis Assay) and/or a study in line with OPPTS 890.1200 (Aromatase assay).

In case of OECD TG 458, 456 and OPPTS 890.1200 are negative, a study in line with OECD TG 441 (Hershberger Assay) is required.

To have the EAS-mediated adversity with regard to humans and mammals sufficiently investigated, all the data requirements of the specific Regulations must be fulfilled. This should include all the "EAS-mediated" parameters foreseen to be investigated in an Extended one generation reproductive toxicity study (OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation) or a two-generation reproductive toxicity study (OECD TG 416).

2.10.2.2.9. Overall conclusion on the ED assessment for humans

T-modality: It was concluded that T-mediated adversity and T-mediated activity has not sufficiently investigated due to most T-*in vivo* mechanistic and T-mediated parameters were not available within the dossier documentation (only liver weight data was assessable), and the absence of TG 407, 408, 409, 416 (or 443) and 451-3 studies. Additionally, no positive results were obtained from the EDSP21 Thyroid Bioactivity Model. On the other hand, equivocal evidences of thyroid activity were observed in guinea pigs after subcutaneous injections of thymol, so further investigations are needed to clarify these findings and whether thymol exerts a potential role on T-activity. Therefore, based on the available information a comprehensive evaluation of T-modality cannot be performed.

EAS-modality: Based on the available information, it was concluded that EAS-mediated adversity has not been sufficiently investigated due to the absence of most EAS-mediated parameters in the studies provided, and the non-availability of the OECD TG 416 and OECD TG 443 studies with the active substance thymol. On the other hand, EAS-mediated activity has not been sufficiently investigated due to the OECD TG 458 assay was not conducted for A-modality, whereas OECD TG 456 assay and/or a study in line with OPPTS 890.1200 (Aromatase assay) were not conducted for S-modality. In case of OECD TG 458, 456 and OPPTS 890.1200 are negative, a study in line with OECD TG 441 (Hershberger Assay) is required.

Overall, the lack of EAS-parameters in the documentation provided precludes a reliable assessment of EAS-modalities.

There was disagreement between RMS and co-RMS in the conclusion to this section. The opinion of the co-RMS was the following: "*Acknowledging the corrosive properties of thymol the co-RMS is of the opinion that further testing should not be performed, and it is not required. Reference is made to Figure 1, Note b of the ECHA/EFSA Guidance for the identification of endocrine disruptors in the con-text of Regulations (EU) No 528/2012 and (EC) No 1107/2009, where it is stated that some substances may not need to be assessed for ED properties. Further justification is provided in Section 3.1 of the guidance noting that due to the knowledge on the physicochemical and/or toxicological properties of a substance further testing does not appear scientifically necessary or technical possible". The RMS disagrees with the co-RMS in the waiving for ED properties".* In the opinion of the RMS, further testing is needed because evidences of toxicity were observed across the RAR and further testing is technically possible because dilution can avoid impediments due to the corrosive effect of the substance.

2.10.1 ED assessment for non-target organims

For the assessment of the endocrine disrupting properties of Thymol, the applicant has provided a document ("Thymol: Assessment of endocrine properties", by Staphyt Ltd. and Eden Research, 2021, KCA 5.8.3/01a), which has been taken into account by the RMS.

For non-target organisms, there are no available studies with Thymol investigating endpoints for EATS-mediated activity or endpoints that are sensitive to, but not diagnostic of, EATS modalities

A invertebrate study, a *Daphnia magna* reproduction test is available (Vol. 3 CA B.9.2.5.1/01). These invertebrate assays are considered as OECD Conceptual Framework Level 4 studies (OECD GD 150, 2018), however, invertebrates studies are not considered in this assessment since invertebrate organisms are out of the scope of the ED assessment as per the EFSA/ECHA Guidance due to the scarce knowledge on the endocrinology for non-target invertebrates and the limited amount of information provided in these studies for the identification of potential ED-related effects.

Furthermore, for non-target organisms no literature papers were considered of possible relevance to the assessment of endocrine disrupting properties of Thymol (Vol. 3 CA B.9.11.1). One paper (Vol. 3 CA B.9, Study B.9.1.1.2/01) may be of potential interest as supporting information, in which dietary supplementation of Coturnix coturnix (Japanese quail) with thymol showed no significant effects on body weight change, but no endocrine-specific parameters were assessed in this study. A further three papers (Vol. 3 CA B.9 Appendix I, CA 9.6.3.4/05; CA 9.6.3.4/28; and CA 9.6.3.4/29) assessed dietary supplementation of various fish species with thymol, but again no endocrine-specific parameters were assessed in these studies and they are considered of limited reliability as analytical verification of thymol in the fish diets was not reported. All these papers are considered of limited relevance and reliability for use in the assessment of endocrine disrupting properties of thymol and have therefore not been discussed further in this document.

It should be noted that in Section 3.1 of the EFSA/ECHA (2018) guidance, there may be cases in which due to the knowledge on the physico-chemical and (eco)toxicological properties of the substance an ED assessment does not appear scientifically necessary. In such cases, it swould be justified.

The applicant has included the following weight of evidence (WoE) ED assessment for non-target organisms to justifiy that additional testing on terrestrial or aquatic vertebrate non-target organisms is not scientifically justified and no further data are therefore presented :

Thymol is a naturally occurring phenol compound 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 and Vol. 3 CA B.9.1.3, 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. Following field application of the representative formulated product, Mevalone, initial environmental exposure of thymol will decline rapidly in relation to the applied dose due to volatilisation and degradation. It is observed that the DT50 in soil for thymol is less than one day and the DT50 in air obtained from the Atkinson model is 1.197 hours (please see Vol. 3 CA B.8 for details). Consequently, the duration of exposure under typical conditions will be very limited, particularly in relation to background levels of thymol in the environment. This is also confirmed by the results of the residue trials conducted with Mevalone on grapevines and apples ((Vol. 3 CA B.7.3). A total of 11 trials in grapes were conducted in Northern EU countries (Austria, Germany and Northern France) and in Southern EU countries (Spain, Portugal and Italy) in 2006 and 2020. All 2020 trials were conducted according to the critical GAP for the renewal and are therefore relevant to support the use of Mevalone in the EU. 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. Furthermore, a total of 6 trials in apples were conducted in Northern EU countries (Austria, Germany and Northern France) and in Southern EU countries (Spain, Southern France and Italy) in 2020 with an LOQ of 0.01 mg/kg. All trials were conducted according to the critical GAP for the renewal and are therefore relevant to support the use of Mevalone in the EU. 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.

Furthermore, thymol is of low acute toxicity to birds (acute oral avian LD50 > 10000 mg Mevalone/kg bw (corresponding to >640 mg thymol/kg bw based on the nominal thymol content of 6.4% w/w); Vol. 3 CP B.9.1.1, Table 9.1.1-1.). In an 8-day dietary toxicity study (please see 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 > 375.4 mg thymol/kg bw based on the nominal thymol content of 6.4% w/w). An open literature paper assessing effects of dietary supplementation of thymol for up to 15 days in Japanese quail (Coturnix coturnix) is also summarised in Vol. 3 CA B.8.1.1.2/01 as supporting information, supporting that dietary supplementation with 2 g thymol/kg feed during 2 to 15 days showed no negative effects on feed intake, body weight gain or ambulatory activity. However, no endocrine-specific parameters were assessed in this study

Furthermore, thymol is recognised as a veterinary medicine product for use in all food producing species[23] . In the interests of minimising vertebrate testing, it is not justified to conduct a new reproductive avian toxicity study for an active substance that is ubiquitous in the environment, degrades rapidly following application as a plant protection product and is of known low acute oral avian toxicity. Essentially, minimal long-term exposure to birds is expected, including during the reproductive period. Coupled with the lack of EATS-mediated activity observed in a range of in vitro assays and no endocrine related effects observed in available in vivo mammalian studies, no endocrine population effects on birds are expected. Similarly, additional vertebrate testing of aquatic organisms, including amphibians (e.g. Xenopus) or fish, is not justified based on minimal chronic exposure (thymol is rapidly degradable, volatile and shows low potential for bioconcentration) and lack of expected toxicity based on the available vertebrate (mammalian) data package. Low toxicity to fish is confirmed based on the acute fish toxicity (96-hour LC50 3 mg thymol/L for rainbow trout; Vol.3 CA B.9.2.1/01). As discussed in Section 2 above, the ED criteria are not met based on the available mammalian data. Given the high level of conservation of the endocrine system across taxonomic groups of vertebrates (EFSA/ECHA (2018) guidance, Section 3.1 and Section 4.2), natural occurrence of thymol, as well as the favourable physico-chemical and (eco)toxicological characteristics of thymol, it is highly unlikely that the rapidly degradable substance would result in any effects on EATS-mediated activity in other terrestrial or aquatic non-target organisms.

Information on natural background levels of Thymol was provided as confirmatory data (Thymol Addendum – Confirmatory Data, August 2016). The RMS (UK) concluded that *«... the confirmatory data requirement are not met, as it cannot be established that background exposure is greater or similar to predicted exposures...».* EFSA agreed with RMS conclusion (EFSA Supporting publication 2017:EN-1162). No new data has been submitted to enable a comparison between the natural background exposure and the exposure due to the use of the plant protection product. Therefore, a negligible exposure has not been demonstrated.

Additionally, the data of residues of Thymol in plants other than grape or pome (1 – 111000 ,g/kg, in plant tissue) were included as confirmatory data information on natural background levels of Thymol (Thymol Addendum – Confirmatory Data, August 2016). It was concluded that there is insufficient information to establish a background exposure concentration of Thymol on the basis of this data since there are significant uncertainties regarding the reliability of these data. Furthermore, under some circumstances background exposure could exceed the predicted based on the proposed use (please, see Vol. 3 CA B.9.1.1.3 for further justification).

Moreover, considering the Table 9 of EFSA/Echa GD on ED (EFSA Journal 2018 :16(6)-5311) no studies are available included in the « OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals » with the active substance Thymol.

Furthermore, the available toxicology (mammalian) data for Thymol showed that EATS-mediated adversity and EATS-activity have not been sufficiently investigated, therefore, further data need to be generated before a conclusion on whether or not the ED criteria are met for the EATS-modalities can be drawn (see point 2.10.2.2.9).

[23] The European Agency for the Evaluation of Medicinal Products. EMEA/MRL/075/96-FINAL March 1996. Committee for veterinary medicinal products: Thymol: Summary Report. https://www.ema.europa.eu/en/documents/mrl-report/thymol-summary-report-committee-veterinary-medicinal-products_en-0.pdf

2.10.1.1 ED assessment for T-modality

2.10.1.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

There are no available guideline studies with Thymol investigating endpoints for adverse effects and endocrine activity related to T-modality in non-target organisms.

2.10.1.1.1.1 Assessment of the integrated lines of evidence and weight of evidence

The assessment of the available mammalian studies (toxicology) concluded that based on the absence of guideline studies, and the absence of data from most T-*in-vivo* mechanistic and T-mediated parameters in the provided studies, it has been concluded that T-mediated adversity and T-activity has not been sufficiently investigated. Moreover, *in vitro*-mechanistic data from Thyroid Bioactivity Model US EPA CompTox Chemicals Dashboard (12 assays) did not show positive/active results. However, equivocal evidences of thyroid activity were observed in guinea pigs after subcutaneous injections of thymol (Möller *et al*., 1939), so further investigations are needed to clarify these findings and whether thymol exerts a potential role on T-modality.

No evidence for T-mediated activity or adversity was found for non-target organisms other than mammals, however, considering that amphibians' studies are not available in the dossier; T-mediated activity cannot considered as sufficiently investigated. Therefore, further information should be submitted for addressing the potential T-mediated adversity in non-target organisms.

According to EFSA/ECHA GD on ED (2018).

- *To have the **T-mediated adversity** with regard to other non-target organisms sufficiently investigated the results from all the 'T-mediated' parameters foreseen to be investigated in the Larval Amphibian Growth and Development Assay (LAGDA; OECD TG 241) would be needed. However, if the T-mediated parameters foreseen to be investigated in an Amphibian Metamorphosis Assay (AMA, OECD TG 231) are negative, this would be sufficient to support that T-mediated adversity is unlikely because no T-related endocrine activity has been observed".*

Amphibians' studies are not available in the dossier; consequently, RMS considers that further information should be needed for addressing the potential T-mediated adversity in non-target organisms. In particular, and following the EFSA/ECHA GD on ED (2018), an Amphibian Metamorphosis Assay (AMA, OECD TG 231) should be submitted by applicant to address T-modality endocrine activity of Thymol.

However, considering the information in the mammalian toxicity section, and in line with the XETA Annex of the EFSA/ECHA ED Guidance, the XETA (Xenopus Eleutheroembryo Thyroid Assay, OECD 248, 2019) could also be appropiate to investigate the potential T-mediated mediated adversity in non-target organisms

2.10.1.1.2 Initial analyisis fo the evidence and identification of relevant scenario

For non-target organisms, there are no available studies with Thymol investigating endpoints for adverse effects and endocrine activity related to T-modality. According to the ECHA/EFSA ED guidance (EFSA Journal 2018;16(6):5311), Thymol falls into the 2a (iii) scenario where T modalities have not been sufficiently investigated in non-target organisms. "To consider the T-modality sufficiently investigated, a Leverl 3 Study : 'Amphibian metamorphosis assay' (AMA; OECD TG 231 (OECD, 2009c)) should be conducted" (ECHA/EFSA ED guidance (EFSA Journal 2018;16(6):5311)).

The relevant scenario for the T-modality is identifiedd as 2a (iii)

Table 2.10.3.1.2-1. Identification of relevant scenario for T-modality

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (cd)	Yes/No	1a	Conclude: ED criteria not met because there is not "T-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	**No (not sufficiently investigated)**	**2a (iii)**	**Generate missing level 2 and 3 information. Alternatively, generate missing "T-mediated" parameters. Depending on the outcome move to corresponding scenario**	**X**
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.1.1.3 MoA analysis for T-modality

According to the ED EFSA/ECHA guidance (2018), in cases of Scenario 2a (iii), a MoA analysis for EAS-modalities is not required.

2.10.1.1.4 Conclusion on the ED assessment for T-modality

The available toxicology (mammalian) data for Thymol showed that T-mediated adversity and T-activity have not been sufficiently investigated, therefore, further data need to be generated before a conclusion on whether or not the ED criteria are met for the T-modality can be drawn (see point 2.10.2.2.9).

T-mediated adversity and T-activity are not considered sufficiently investigated on non target organisms other than mammals, because Amphibians' studies are not available. A level 3 study Amphibian Metamorphosis Assay (AMA, OECD TG 231) or a Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD TG 248) should be submitted before a conclusion on whether or not the ED criteria are met for the T-modality can be drawn. Once provided, the need of further data will be triggered by the following scenarios:

1. If the above study is negative, the scenario 2a(ii) applies and ED criteria are not met for T modality.
2. If positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis, i.e. a Level 4 study following OECD TG 241 (LAGDA, Larval Amphibian Growth and Development Assay).

2.10.1.2 ED assessment for EAS-modality

2.10.1.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

There are no available guideline studies with Thymol investigating endpoints for adverse effects and endocrine activity related to EAS-modalities in non-target organisms.

2.10.1.2.1.1 Assessment of the integrated lines of evidence and weight of evidence

The assessment of available in vivo toxicity data to study EAS-mediated adversity and EAS-activity on mammals (toxicology) have not been sufficiently investigated due to the absence of most EAS-mediated parameters in the studies provided, and the non-availability of the OECD TG 416 and OECD TG 443 studies with the active substance thymol. On the other hand, EAS-mediated activity has not been sufficiently investigated due to the OECD TG 458 assay was not conducted for A-modality, whereas OECD TG 456 assay and/or a study in line with OPPTS 890.1200 (Aromatase assay) were not conducted for S-modality. Overall, the lack of EAS-parameters in the documentation provided precludes a reliable assessment of EAS-modalities.

The available dataset of *in vitro* mechanistic assays showed the following results in each of the EAS-mediated parameter:

- Toxcast ER bioactivity model showed two active/positive assays (ATG_ERa_TRANS_up and OT_ER_ERbERb_0480, with AC50 values of 46.18 and 34.88, respectively) and negative for 17 assays. None of both assays presented flags. However, the cytotoxicity limit was 10 µM, so the AC50 values displayed in the assays was higher than the cytotoxicity limit, and therefore, the results were not reliable.
- Toxcast AR bioactivity model displayed negative results in the 14 tested assays.
- Toxcast Steroidogenesis bioactivity model includes only 2 steroidogenesis bioassays for thymol. Negative results were displayed in these two assays.

No evidence for EAS-mediated activity or adversity was found for non-target organisms other than mammals, however, considering that studies with Thymol investigating endpoints for adverse effects and endocrine activity related to EAS-modalities in non-target organisms are not available in the dossier; EAS-mediated adversity cannot considered as sufficiently investigated. Therefore, further information should be submitted for addressing the potential EAS-mediated adversity in non-target organisms.

According to EFSA/ECHA GD on ED (2018).

- "*To consider the **E, A, S modalities** for non-target organisms other than mammals sufficiently investigated, preferably the 'Fish short term reproduction assay' (FSTRA; OECD TG 229) should have been conducted; however the 21-day fish assay OECD TG 230 (OECD, 2009b) is acceptable as well…*"

In order to make sufficient data available to reach a conclusion on EAS modalities in non-target organisms RMS considers that a "Fish short-term reproduction assay (FSTRA, OECD 229)" included gonad histhopatology should be submitted to address the E, A, S-modalities endocrine activity of Thymol.

2.10.1.2.2 Initial analysis of the evidence and identification of the relevant scenario

For non-target organisms, there are no available studies with Thymol investigating endpoints for adverse effects and endocrine activity related to EAS-modalities. According to the ECHA/EFSA ED guidance (EFSA Journal 2018;16(6):5311), Thymol falls into the 2a (iii) scenario EAS-modalities, where EAS modalities have not been sufficiently investigated, a Level 3 study : Fish short term reproduction assay' (FSTRA; OECD TG 231 (OECD, 2009c)), included gonad histopathology, should be submitted before a conclusion on whether or not the ED criteria are met for the EAS-modalities can be drawn.

The relevant scenario for the EAS-modality is identified as 2a (iii).

Table 2.10.3.2.2-2: Identification of relevant scenario for EAS-modality

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "EAS-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	
No (not sufficiently investigated)	**No (not sufficiently investigated)**	**2a (iii)**	**Generate missing level 2 and 3 information. Alternatively, generate missing "EAS-mediated" parameters. Depending on the outcome move to corresponding scenario**	**X**
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.1.2.3 MoA analysis for EAS-modalities

According to the ED EFSA/ECHA guidance (2018), in cases of Scenario 2a (iii), a MoA analysis for EAS-modalities is not required.

2.10.1.2.4 Conclusion on the ED assessment for EAS-modalities

The available toxicology (mammalian) data for Thymol showed that EAS-mediated adversity and EAS-activity have not been sufficiently investigated, therefore, further data need to be generated before a conclusion on whether or not the ED criteria are met for the EAS-modalities can be drawn (see point 2.10.2.2.9)..

EAS-mediated adversity and EAS-activity are not considered sufficiently investigated on non-target organisms other than mammals, because no studies with Thymol investigating endpoints for adverse effects and endocrine activity related to EAS-modalities are available. A level 3 study Fish Short Term Reproduction Assay (FSTRA, OECD TG 229), including histopathology assessment, should be submitted before a conclusion on whether or not the ED criteria are met for the EAS-modalities can be drawn. Once provided, the need of further data will be triggered by the following scenarios:

1. If the above study is negative, the scenario 2a(ii) applies and ED criteria are not met for EAS modalities.
2. If positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis, i.e. a level 5 study following OECD TG 240 (MEOGRT, Medaka Extended One Generation Reproduction Test) would be necessary.

2.10.1.2.5 Overall conclusions on the ED assessment for non-target organisms

The ED-assessment for **wild mammals** is based on the same dataset as used for the human health assessment, but with additional consideration for the population relevant of any adverse effects observed for the EATS-modalities. Given that in the human health assessment, it was concluded that EATS-mediated adversity and EATS-activity have not been sufficiently investigated and further data should be generated before a conclusion on whether or not the ED criteria are met for the EAS-modalities on wild mammals can be drawn (see point 2.10.2.2.9).

It was concluded that EATS-mediated adversity and EATS-activities for non-target organisms other than mammals have not been sufficiently investigated. Consequently, the scenario 2a (iii) applies.

Therefore, level 3 tests should be conducted as follows:

- A test according to OECD TG 229 (Fish Short Term Reproduction Assay, FSTRA), including histophatology assessment.
- A test according to OECD TG 231 (Amphibian Metamorphosis Assay ; AMA) ; a OECD TG 248 (Xenopus Eleutheroembryo Thyroid Assay ; XETA) would also be considered suitable in this case.

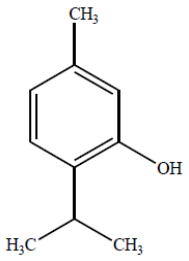
If the mentioned studies are negative, the scenario 2a(ii) applies and ED criteria are not met for EATS modalities. However, if these studies are positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis: a study in line with OECD TG 240 (MEOGRT, Medaka Extended One Generation Reproduction Test) and/or with OECD TG 241 (LAGDA, Larval Amphibian Growth and Development Assay).

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 83: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	thymol; 5-methyl-2-(propan-2-yl)phenol
Other names (usual name, trade name, abbreviation)	thymol
ISO common name (if available and appropriate)	No ISO common name
EC number (if available and appropriate)	201-944-8
EC name (if available and appropriate)	thymol
CAS number (if available)	86-83-8
Other identity code (if available)	CIPAC 969
Molecular formula	$C_{10}H_{14}O$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	150.22 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The active substance is not a mixture of isomers. Therefore, consideration of isomeric composition is not relevant.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not a UVCB substance CONFIDENTIAL information - data provided separately (Volume 4)
Degree of purity (%) (if relevant for the entry in Annex VI)	≥990 g/kg (thymol)

2.11.1.2 Composition of the substance

Table 84: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Thymol; 5-methyl-2-propan-2-yl-phenol CAS Number: 89-83-8	990 g/kg minimum	Acute Tox. 4 Skin Corr. 1B Aquatic Chronic 2	Acute Tox. 4 Skin Corr. 1B Skin Corr. 1C Aquatic Chronic 2 Eye Dam. 1 Skin Irrit. 2
The remaining components of thymol are confidential			

Table 85: Impurities (non-confidential information) if relevant for the classification of the substance

Thymol does not contain relevant impurities.

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
No relevant impurities				

Table 86: Additives (non-confidential information) if relevant for the classification of the substance

Thymol does not contain additives.

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
Thymol does not contain additives					

Table 87: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Thymol	≥ 99 %	No relevant impurities		Physicochemical tests, CA, B2.

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 88: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	604-032-00-1	thymol	201-944-8	89-83-8	Acute Tox. 4* Skin Corr. 1B Aquatic Chronic 2	H302 H314 H411	GHS05 GHS07 GHS09 Dgr	H302 H314 H411			
Dossier submitters proposal	604-032-00-1	thymol; 5-methyl-2-(propan-2-yl)phenol	201-944-8	89-83-8	**Add** Skin Sens. 1 Eye Dam. 1 STOT SE 3 **Modify** Skin Corr. 1 Aquatic Chronic 3 **Retain** Acute Tox. 4	**Add** H317 H318 H336 **Modify** H412 **Retain** H302 H314	**Retain** GHS05 GHS07 Dgr **Remove** GHS09	**Add** H317 H336 **Modify** H412 **Retain** H302 H314	**Add** EUH071	**Add** ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	604-032-00-1	thymol; 5-methyl-2-(propan-2-yl)phenol	201-944-8	89-83-8	Acute Tox. 4 Skin Corr. 1 Skin Sens. 1 Eye Dam. 1 STOT SE 3 Aquatic Chronic 3	H302 H314 H317 H318 H336 H412	GHS05 GHS07 Dgr	H302 H314 H317 H336 H412	EUH071	ATE = 500 mg/kg bw	

2.11.2.2 Additional hazard statements / labelling

Table 89: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification.	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification.	Yes
Acute toxicity via oral route	**Harmonised classification proposed: Acute Tox. 4 (H302)**	Yes
Acute toxicity via dermal route	Hazard class not applicable (corrosive)	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	**Harmonised classification proposed: Skin Corr. 1 (H314)**	Yes
Serious eye damage/eye irritation	**Harmonised classification proposed: Eye Dam. 1 (H318)**	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	**Harmonised classification proposed: Skin Sens. 1 (H317)**	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data lacking	Yes
Reproductive toxicity	Data lacking	Yes
Specific target organ toxicity-single exposure	**Harmonised classification proposed: STOT SE 3 (H336)**	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not applicable	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Hazardous to the aquatic environment	Aquatic Chronic 3	Yes
Hazardous to the ozone layer	Not Classified	Yes

2.11.3 History of the previous classification and labelling

Thymol is an active substance for plant protection products (PPP) approved under Regulation (EC) 1107/2009 by Commission Implementing Regulation (EU) no. 568/2013.

The current harmonized classification for thymol (CAS 89-83-8) in Annex VI of Regulation (EC) 1272/2008 corresponds to Acute Tox. 4* (H302), Skin Corr. 1B (H314) and Aquatic Chronic 2 (H411). This classification has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC (DSD) where it was classified as R22, R34 and R51/53 as presented in Commission Directive 98/73/EC. A new proposal for harmonized classification and labelling (CLH Report) in accordance to Regulation (EC) no. 1272/2008 has not been presented and consequently there is not a Risk Assessment Committee (RAC) opinion for thymol.

2.11.4 Identified uses

2.11.5 Data sources

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

There are no metabolites of relevance.

2.12.1 STEP 1: Exclusion of degradation products of no concern
2.12.2 STEP 2: Quantification of potential groundwater contamination
2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

2.12.3.3 STEP 3, Stage 3: screening for toxicity

2.12.4 STEP 4: Exposure assessment – threshold of concern approach
2.12.5 STEP 5: Refined risk assessment
2.12.6 Overall conclusion

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

Thymol does not containg isomers

2.13.1 Identity and physical chemical properties

Thymol does not containg isomers

2.13.2 Methods of analysis

Thymol does not containg isomers

2.13.3 Mammalian toxicity

Thymol does not containg isomers

2.13.4 Operator, Worker, Bystander and Resident exposure

Thymol does not containg isomers

2.13.5 Residues and Consumer risk assessment

Thymol does not containg isomers

2.13.6 Environmental fate

Thymol does not containg isomers

2.13.7 Ecotoxicology

Thymol does not containg isomers

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: Thymol. However, it is proposed thymol to be included into Annex IV to Regulation (EC) No. 396/2005 and so a residue definition would not be required.

Food of animal origin: A residue definition in animal matrices is not required

Soil: Thymol

Groundwater: Thymol

Surface water: Thymol

Sediment: Thymol

Air: Thymol

2.14.2 Definition of residues for monitoring

Food of plant origin: Thymol. However, it is proposed thymol to be included into Annex IV to Regulation (EC) No. 396/2005 and so a residue definition would not be required.

Food of animal origin: A residue definition in animal matrices is not required

Soil: Thymol

Groundwater: Thymol

Surface water: Thymol

Sediment: Thymol

Air: Thymol

Level 3

THYMOL

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4

		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.			*Assessment not finalised* Thymol The CS formulation, MEVALONE (3AEY), containing 33 g/L eugenol, 66 g/L geraniol and 66 g/L thymol as active substances is the representative formulation for the renewal of approval. The CS formulation MEVALONE (3AEY) is a Capsule Suspension formulation. Thymol as one of its three active substances has a minimum concentration of 990 g/kg (99% w/w). The representative formulation Mevalone, is a CS formulation containing 33 g/L eugenol, 66 g/L geraniol and 66 g/L thymol. Mevalone is the common representative product of the three active substances eugenol, geraniol and thymol intended to be renewed at the same time. Representative uses in this submission are spray applications to vines (BBCH 60-89, field use) and apples (BBCH 75-87). **Toxicology:** Applicant should submit further information on: • Data to conclude on reproduction toxicity • Data required for endocrine disruptor assessment (EATS-modalities) **Ecotoxicology** • **Birds:** No acute risk. No data or a strong justification are available to address the reproductive risk on birds. Therefore, further information should be submitted.

				• **Mammals:** No acute risk. No data or a strong justification are available to address long-term risk on mammals. Therefore, further information should be submitted. • **Fish:** No acute risk. No data is available to address the long-term risk on fish. Therefore, further information should be submitted. No risk is expected in bees, non-target arthropods other than bees, non-target soil meso- and macrofauna, Soil nitrogen transformation or non-target terrestrial plants.

3.1.1.2 Submission of further information

		Yes	No	
i)	It is considered that a complete dossier has been submitted		X	See data gaps in the list of studies to be generated, still ongoing or available but not peer reviewed in point 3.1.4.
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			*Assessment not finalised* Please refer to Point 3.1.4. List of studies to be generated, still ongoing or available but not peer reviewed. ***Identity:*** *Applicant should submit further information on:* • LoS shall be provided for the manufacturing site(s) of the Plant Protection Product. ***Further information:*** *Applicant should submit further information on:* • Updated set of SDS for all technical materials (eugenol, thymol, geraniol) of the formulation (See C.1.3.2, Confidential) according to most updated REACH Regulation (2015/830) and CLP Regulation. • The information on the potential impact of water treatment processes on the active substance and its metabolites in water for drinking water uses is required for the consumer risk assessment performed in the residue section of the DRAR. ***Toxicology:*** *Applicant should submit further information on:* • Data to conclude on reproduction toxicity

				• Data required for endocrine disruptor assessment (EATS-modalities) ***Fate and behaviour in the environment*** *Applicant should submit further information about:* • A data gap has been identified to address the route and rate of degradation in soil under irradiated conditions. • A new direct photochemical study with radio-labelled thymol is requested in order to address the route of degradation in water. • A new aerobic mineralization study using radio-labelled thymol is required. • A data gap has been identifed to address the route and rate of degradation under natural water/sediment system. • An analysis of the short-range transport in air should be included. • A data gap has been proposed for information to address the effect of water treatment processes on the nature of residues of the active substance when surface water is abstracted for drinking water to address Article 4 (approval criteria for active substances) 3(b) of Regulation (EC) No 1107/2009. ***Ecotoxicology section:*** *Applicant should submit further information on:* • Further information should be submitted to address the chronic risk tobirds and mammals. • A new chronic toxicity study on fish.
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	

	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		The following values are proposed: ADI = 0.07 mg/kg bw/day AOEL = 0.4 mg/kg bw/day ARfD = 0.4 mg/kg bw
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		Thymol. However, it is proposed thymol to be included into Annex IV to Regulation (EC) No. 396/2005 and so a residue definition would not be required. Highest TMDI: 2 % of ADI (NL, toddler) IESTI : **For children** Potatoes: 0.4 % of ARfD Melons: 0.4 % of ARfD Pears: 0.4 % of ARfD **For adults** Head cabbages: 0.1 % of ARfD Watermelons: 0.1 % of ARfD Melons: 0.1 % of ARfD
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.			*[Explain if this applies to all or some of the representative uses/use scenarios/products]*
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Terpene compounds such as eugenol, geraniol and thymol generally possess antifungal activity, having effects on spore germination, hyphal penetration, mycelial growth and hyphal growth. For the uses grapes/BOTRCI and UNCINE, the representative formulation, MEVALONE, is currently commercially available and supported by efficacy data evaluated under Uniform Principles for national registrations. For pome fruits/postharvest storage diseases (PHYTSP, ALTESP and BOTRCI), currently, this use is not registered, however, it is considered that the GAP is realistic from an efficacy point of view considering the studies provided by the applicant (studies submitted for new registration in Central Zone in July 2021).

				Thymol is a contact action fungicide. It prevents the development of fungal mycelium from spores or destroys existing mycelium by a direct action on the cell membranes. Due to the mode of action, no problems with resistance or cross-resistance are expected. Thymol, is a plant extract included in the terpene alcohols chemical group, classified by FRAC into plant oils, FRAC codes F7: cell membrane disruption /46, with resistance not known. MEVALONE formulation has been applied in various EU member states for many years without reports of adverse effects on treated crops. Available efficacy used to obtain registration of the representative formulation in various countries shows the absence of phytotoxicity when the product is used according to the GAP. Consequently, no negative impact is expected on treated crops when used according to recommendations." There is no evidence of any undesirable or unintended side-effects.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.			*[Explain if this applies to all or some of the representative uses/use scenarios/products]*
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		Thymol has a minimum purity of 990 g/kg (99% w/w). Thymol does not contain relevant impurities.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		Min 98% (Specifications for Flavourings, Session 55, JECFA 2000)
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted		X	Not required.
Methods of analysis				
		Yes	Yes	No
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which	X		Thymol does not contain relevant impurities. Refer to Volume 4, confidential for further details on composition.

	are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.			
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		Suitable validated methods are available for residue analysis for the active substance thymol.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The following values are proposed: ADI = 0.07 mg/kg bw/day AOEL = 0.4 mg/kg bw/day ARfD = 0.4 mg/kg bw
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Most of the studies provided were also presented in the DAR and six additional studies were submitted for the renewal process. Thymol was negative in bacterial gene mutation tests and in in vitro mammalian gene mutation assays. In the weight of evidence, the gene mutation endpoint was considered as fully investigated. Most of the in vitro studies to investigate the potential of thymol to induce chromosome aberrations were positive: three of the chromosome aberration tests and one micronucleus test were positive, and only one chromosome aberration test and one micronucleus test were negative. Furthermore, one positive SCE assay was reported and there were two comet assays in vitro, being one of them negative and the other one positive. To follow up the chromosome aberrations observed in vitro, three in vivo studies were provided: one acceptable MN test with a negative result, one supportive chromosome aberration test with a positive result performed by intraperitoneal route (not a relevant route) and one acceptable chromosome aberration test with a negative result.

				Based on the information available, no classification as mutagen is proposed for thymol.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	No studies about carcinogenicity or long-term toxicity were provided for the renewal process. In the absence of any alerts regarding carcinogenicity with the available information, considering the extended use of this active substance as food flavouring, a waiving could be justified for this data requirement. Therefore, classification as carcinogenic is not proposed for thymol.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*[if no provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			During the assessment, the main study provided by the applicant on reproductive toxicity of thymol was a repeated dose and reproductive/developmental toxicity study in rats ([redacted], 1996), comparable to OECD TG 422 (1996), which showed methodological deficits leading to the consideration of the study as supportive information only. No adverse effects on fertility were observed in this study up to 200 mg/kg bw/day (the highest dose tested). A NOAEL of 40 mg/kg bw/day was established for offspring, based on the reduction of pup weights and weight gains at 200 mg/kg bw/day. As indicated in the OECD test guideline 422 (2016), this test could be used as a preliminary study to a long-term or a full-generation study, since, this guideline is designed to generate limited information, but it is not an alternative to, nor does it replace the existing test guidelines 414 (Prenatal Developmental Toxicity Study), 415 (One-Generation Reproduction Toxicity Study), 416 (Two-Generation Reproduction Toxicity) or 443 (Extended One-Generation Reproductive Toxicity Study).

				Therefore, a conclusion about the classification as toxic for reproduction cannot be reached. The available information cannot be considered as enough in view of the effects on pups´growth.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*[if yes provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009.			Assessment on thymol endocrine disrupting properties for humans: - T-modality: T-mediated adversity and T-mediated activity have not sufficiently investigated due to most T-*in vivo* mechanistic and T-mediated parameters were not available within the dossier documentation and the absence of TG 407, 408, 409, 416 (or 443) and 451-3 studies. Additionally, no positive results were obtained from the EDSP21 Thyroid Bioactivity Model. On the other hand, equivocal evidences of thyroid activity were observed in guinea pigs after subcutaneous injections of thymol, so further investigations are needed to clarify these findings and whether thymol exerts a potential role on T-activity. Therefore, based on the available information a comprehensive evaluation of T-modality cannot be performed. - EAS-modality: Based on the available information, it was concluded that EAS-mediated adversity has not been sufficiently investigated due to the absence of most EAS-mediated parameters in the studies provided, and the non-availability of the OECD TG 416 and OECD TG 443 studies with the active substance thymol. On the other hand, AS-mediated activity has not been sufficiently investigated due to the OECD TG 458 assay was not conducted for A-modality, whereas OECD TG 456 assay and/or a study in line with OPPTS 890.1200 (Aromatase assay) were not conducted for S-modality. Overall, the lack of EAS-parameters in the documentation provided precludes a reliable assessment of EAS-modalities. Thus, further data need to be generated or submitted, before a conclusion on whether or not the ED criteria are met can be drawn for thymol.

ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*[if yes provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	***1.- Persistence criterion*** **Soil:** The route and rate of degradation of thymol were studied in four soils in the laboratory under aerobic conditions. Thymol declined rapidly in each soil type. Modelled DT_{50} and DT_{90} values ranged from 0.6 – 0.8 days and 1.8 – 2.6 days, respectively. Based on the laboratory DT_{90} of < 3 days, field studies are not triggered for thymol and therefore no data is submitted. No studies on anaerobic degradation have been performed. Based on the fast aerobic degradation and the application timing of Mevalone, it is unlikely that thymol residues would be found in anaerobic conditions. Overall, thymol does not fulfil the persistence criterion in soil set out in points 3.7.1.1 (POP criteria), 3.7.2.1 (PBT criteria), 3.7.3.1 (vPvB criteria) of annex II of the regulation 1107/200 ***Aquatic system:*** The ready biodegradability of thymol has been previously evaluated, and the study remains valid. Thymol can be classified as readily biodegradable under the test conditions. Studies on degradation water/sediment systems have not been provided. Thymol was shown to be hydrolytically stable at environmental temperatures.

				Overall, thymol does not fulfills the persistence criterion in aquatic systems set out in point 3.7.2.1 (PBT criteria) ***2.- Bioaccumulation criterion*** The log P_{ow} values for thymol are 3.43, 3.44 and 3.41 at pH values 4,7 and 9, respectively and thus just exceed the trigger value of 3, 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. 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", and therefore no studies are required. Furthermore, in line with Annex I, Section 4.1.2.8.1 of the CLP Regulation[24], these log P_{ow} values are less than the CLP cut-off criteria of 4, indicating thymol does not show potential to bioaccumulate. ***3.- Toxicity criterion*** Based on the chronic toxicity on algae and aquatic invertebrates, it can be concluded that thymol does not fulfill the criterion of toxicity to aquatic organisms set out in the Annex II of the regulation 1107/2009. ***4.- Atmospheric Long range transport*** The Atkinson calculation outputs a DT50 of thymol in air of 1.197 hours and therefore it is considered that thymol is not of potential concern for LRT.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	See previous paragraph
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	

[24] Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	See previous paragraph
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.			**Ecotoxicology** • **Birds:** No acute risk. No data or a strong justification are available to address the reproductive risk on birds. Therefore, further information should be submitted. • **Mammals:** No acute risk. No data or a strong justification are available to address the reproductive risk on mammals. Therefore, further information should be submitted. • **Fish:** No acute risk. No data is available to address the long-term risk on fish. Therefore, further information should be submitted. No risk is expected in bees, non-target arthropods other than bees, non-target soil meso- and macrofauna, Soil nitrogen transformation or non-target terrestrial plants.
ii	It is considered that, the substance **SHOULD BE identified as having endocrine disrupting properties** that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.		X	No ED adversity for eugenol has been observed on NTO. However, the EATS-mediated adversity was not considered sufficiently investigated. Therefore, it is necessary to generate further information (Level 3 studies): • A study according to OECD TG 231 (AMA) • A study according to OECD 229 (FSTRA).
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			**Ecotoxicology** • **Birds:** No acute risk. No data or a strong justification are available to address the reproductive risk on birds. Therefore, further information should be submitted. • **Fish:** No acute risk. No data is available to address the long-term risk on fish. Therefore, further information should be submitted. No risk is expected in, bees, non-target arthropods other than bees, non-target soil meso- and macrofauna, Soil nitrogen transformation or non-target terrestrial plants.

iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			The risks to bees is considered acceptable following the proposed representative uses in vineyards and pome fruit without the need for mitigation,.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		Thymol. However, it is proposed thymol to be included into Annex IV to Regulation (EC) No. 396/2005 and so a residue definition would not be required. Soil; water, air; Thymol
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		The representative product Mevalone is a CS formulation containing 33 g/L eugenol, 66 g/L geraniol and 66 g/L thymol. Mevalone is the common representative product of the three active substances eugenol, geraniol and thymol intended to be renewed at the same time. The active substances are for use as a fungicide, for application on grapes and pome fruits. Relevant predefined scenarios for the respective crops were chosen. Application timing was chosen under consideration of the appropriate growth stages for the different FOCUS crops and scenarios. PEC_{gw} values were below the 0.1 µg/L limit for eugenol using all models. The risk to groundwater was determined to be acceptable for all uses of Mevalone containing thymol.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution			*[If yes identify the criteria considered met by the substance* *i.e.* *its ADI, ARfD or AOEL is significantly lower than those of the majority of the approved active substances within groups of substances/use categories,* *— it meets two of the criteria to be considered as a PBT substance* *— there are reasons for concern linked to the nature of the critical effects (such as developmental neurotoxic or immunotoxic effects) which, in combination with the use/exposure patterns, amount to situations of use that could still cause concern, for example, high potential of risk to groundwater; even with very restrictive risk management measures (such as extensive personal protective equipment or very large buffer zones),* *— it contains a significant proportion of non-active isomers,* *— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B, if the substance has not been excluded in accordance with the criteria laid down in point 3.6.3,* *— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B if the substance has not been excluded in accordance with the criteria laid down in point 3.6.4,* *— if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, reviewed by the Authority, it is considered to have endocrine disrupting properties that may cause adverse effects in humans if the substance has not been excluded in accordance with the criteria laid down in point 3.6.5.]*

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	Thymol is currently classified as: - Skin corrosive Cat.1B (H314) Moreover, in accordance to Regulation (EC) No. 1272/2008, the RMS proposes the classification of thymol as: - Skin sensitiser Cat.1 (H317) - Eye damage Cat. 1 (H318)

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Updated set of SDS for all technical materials (eugenol, thymol, geraniol) of the formulation (See C.1.3.2, Confidential) according to most updated REACH Regulation (2015/830) and CLP Regulation.	Not relevant	X		
LoS shall be provided for the manufacturing site(s) of the Plant Protection Product.	Relevant	X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
No data required				
3.1.4.3 Data on uses and efficacy				
No data required				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
No data required				

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.5 Methods of analysis				
No data required				
3.1.4.6 Toxicology and metabolism				
Data required for endocrine disruption assessment (AST-modalities)	All representative uses	X		
3.1.4.7 Residue data				
No data required				
3.1.4.8 Environmental fate and behaviour				
A data gap has been identified to address the route and rate of degradation in soil under irradiated conditions.	All intended uses	X		
A new direct photochemical study with radio-labelled thymol is requested in order to address the route of degradation in water.	All intended uses	X		
A new aerobic mineralization study using radio-labelled thymol is required.	All intended uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
A data gap has been identifed to address the route and rate of degradation under natural water/sediment system.	All intended uses	X		
An analysis of the short-range transport in air should be included.	All intended uses	X		
A data gap has been proposed for information to address the effect of water treatment processes on the nature of residues of the active substance when surface water is abstracted for drinking water to address Article 4 (approval criteria for active substances) 3(b) of Regulation (EC) No 1107/2009.	All intended uses	X		
3.1.4.9 Ecotoxicology				
The applicant is called to submit further information to assess the chronic risk on birds.	All intended uses	X		
The applicant is called to submit further information to assess the chronic risk on mammals	All intended uses	X		
The applicant is called to submit a new chronic toxicity study on fish	All intended uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
The applicant is called to submit further information/data for addressing the potential ED activity of eugenol on non-target organisms. In particular, level 3 tests should be conducted as follows: • A test according to OECD TG 229 (Test No. 229: Fish Short Term Reproduction Assay). • A test according to OECD TG 231 (Test No. 231: Amphibian Metamorphosis Assay) In case of positive result/s based on the level 3 test, additional testing (OECD TG 241 and/or OECD TG 240) might be needed in order to further investigate the adversity.	All intended uses	X		

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

	Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
1	Reproduction	All representative uses
2	Endocrine disruption properties (humans)	All representative uses
3	Impact of water treatment process on eugenol when water is abstracted for drinking water	All representative uses
4	The characterization of photoproducts in soil.	All representative uses
5	The characterization of photoproducts in aquatic systems.	All representative uses
6	A data gap has been identifed to address the route and rate of degradation under natural water/sediment system.	All representative uses
7	The analysis of the short-range transport in air for thymol.	All representative uses
8	Chronic risk to birds	All representative uses
9	Chronic risk to mammals	All representative uses
10	Chronic risk to fish	All representative uses
11	EAST mediated adversity of eugenol with regard to non-target organisms	All representative uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or

(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
8. Chronic risk to birds	All representative uses
9. Chronic risk to mammals	All representative uses
10. Chronic risk to fish	All representative uses

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use "Vineyards" (X^1)	Use "Apples" (X^1)
Operator risk	Risk identified		
	Assessment not finalised	X^1, X^2	X^1, X^2
Worker risk	Risk identified		
	Assessment not finalised	X^1, X^2	X^1, X^2
Bystander risk	Risk identified		
	Assessment not finalised	X^1, X^2	X^1, X^2
Consumer risk	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised	$X^{8,9}$	$X^{8,9}$
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified		
	Assessment not finalised		
Risk to aquatic organisms	Risk identified		
	Assessment not finalised	X^{10}	X^{10}
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10µg/L[(a)] breached		
	Assessment not finalised		
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
	[specify the reasons why expert consultation is considered necessary]

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Oral absorption	50%	100%
Endocrine disruption	Further testing not required	Further testing required

3.2 PROPOSED DECISION

It is proposed that:

[redacted]

[redacted]

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

[redacted]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

- COMMISSION IMPLEMENTING REGULATION (EU) No. 844/2012 of 18 September 2012; setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.
- COMMISSION REGULATION (EU) No. 283/2013 of 1 March 2013; setting out the data requirements for active substances, in accordance with Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products in the market.
- COMMISSION REGULATION (EU) No. 544/2011 of 10 June 2011, implementing Regulation (EC) No. 1107/2009 of the European Parliament and of the Council as regards of data requirements for active substances.
- REGULATION (EC) No. 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006. Concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No. 793/93 and Commission Regulation (EC) No. 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
- REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008; on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006.

Section identity, physical chemical and analytical methods

Section physico chemical properties

- Manual on development and use of FAO and WHO specifications for pesticides: PLANT PRODUCTION AND PROTECTION PAPER 228; FAO/WHO Joint Meeting on Pesticide Specifications (JMPS); First edition-third revision; 2016.
- CIPAC MT 31: Free acidity or alkalinity, (Handbook F, p. 96), (2007)
- CIPAC MT 39.3: Low temperature stability of liquid formulations, (Handbook J, p. 126), (2007)
- CIPAC MT 46: Accelerated storage procedure, (Handbook J, p. 148), (2007)
- CIPAC MT 46.3: Accelerated storage procedure, (Handbook F, p. 128), (2000)
- CIPAC MT 47: Persistent foaming, (Handbook F, p. 152), (2007)
- CIPAC MT 59.3: Sieve analysis, wet sieving, (Handbook F, p. 179), (2007)
- CIPAC MT 75: Determination of pH values, (Handbook F, p. 205) ,(1994)
- CIPAC MT 75.3: Determination of pH values, (Handbook J, p. 131) ,(2000)
- CIPAC MT 157: Water solubility, (Handbook F, p. 379), (2007)
- CIPAC MT 160: Spontaneity of dispersion, of suspension concentrates, (Handbook F, p. 391), (2007)
- CIPAC MT 161: Suspensibility of aqueous suspension concentrates, (Handbook F, p. 394), (2007)
- CIPAC MT 181: Solubility in Organic Solvents, (Handbook H, p. 314), (2007)
- COUNCIL REGULATION (EC) No. 440/2008 of 30 May 2008
  Laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
- OECD Test Guideline 69: Guidance Document on the Validation of (Q)SAR Models, (2007)
- OECD Test Guideline 101: UV-VIS Absorption Spectra (Spectrometric Method), (1981)
- OECD Test Guideline 102: Melting Point/Melting Range, (1995)
- OECD Test Guideline 103: Boiling Point, (1995)
- OECD Test Guideline 104: Vapour Pressure, (2006)
- OECD Test Guideline 105: Water Solubility, (1995)
- OECD Test Guideline 107: Partition Coefficient (n-octanol/water): Shake Flask Method, (1995)

- OECD Test Guideline 109: Density of Liquids and Solids, (2012)
- OECD Test Guideline 112: Dissociation Constants in Water (Titration Method), (1981)
- OECD Test Guideline 114: Viscosity of liquids, (2012).
- OECD Test Guideline 115: Surface Tension of Aqueous Solutions, (1995).
- OPPTS 830.6314 Oxidation/Reduction: Chemical Incompatibility, (1996).
- UN Test N.4: Test method for self-heating substances. (UNECE, 2009).
- Estimation of volatile emission potential method of pesticides by thermogravimetry, Department of Pesticide Regulation, United State of California, Attachment B, Method date: 2-9-05

Section analytical methods

- SANCO 3030/99 rev. 5 of 22 March 2019: Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No. 284/2013.
- SANCO 3029/ 99 rev. 4 of 11/07/00. Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.
- SANCO /825/00 rev. 8.1 of 16/11/2010. Guidance document on pesticide residue analytical methods.
- SANTE 2017/10632 Rev. 3 of 22 November 2017: Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods.
- OECD 501 of 8 January 2007. Metabolism in crops.
- EU guideline for residue data 7028/VI/95 rev.3, APPENDIX A of 22/07/1997. Metabolism and distribution in plants.

Section Data on application and efficacy

Section Toxicology

- ECHA Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 July 2017.
- Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, EFSA/ECHA (2018), Adopted on 5 June 2018.
- Guidance on dermal absorption. 2017. Buist H, Craig P, Dewhurst I, HougaardBennekou S, Kneuer C, Machera K, Pieper C, Court Marques D, Guillot G, Ruffo F and Chiusolo A. EFSA Journal 2017; 15(6): 4873. doi: 10.2903/j.efsa.2017.4873
- SANTE/2018/10591 rev.1. Note for agreement by Member States's Competent Authorities in the SCoPAFF: Phytopharmaceutical legislation section. Guidance on dermal absorption.
- Guidance of EFSA Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/20091. EFSA Journal 2011; 9(2): 2092.
- Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012; 10 (3): 2579.
- Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA Supporting publication 2016: EN-1074.
- Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011; 9(9): 2379.
- Scientific opinion: clarification of some aspects related to genotoxicity assessment. November 2017. EFSA Journal. doi: 10.2903/j.efsa.2017.5113

- Retrospective analysis of the immunotoxic effects of plant protection products as reported in the Draft Assessment Reports for their peer review at EU level (Dewhurst, I., Koshy, L, Samuel, S. and Shillaker, D., 2015, EFSA supporting publication 2015:EN-782).
- Guidance for immunotoxicity risk assessment for chemicals. IPCS harmonization project document; no. 10. World Health Organization and International Programme on Chemical Safety (2012).
- SANCO 7531 – rev.10 Draft Guidance for the setting and application of acceptable operator exposure levels (AOELs) 7 July 2006.
- SANCO 7199/VI/99 rev. 5 Draft Guidance document Guidance for the setting of an acute reference dose (ARfD) 05/07/2001.
- Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests. OECD series on Testing & Assessment No. 237 (2016).
- OECD Test Guideline 404. Acute Dermal Irritation/Corrosion (2015).
- OECD Test Guideline 405 Acute Eye Irritation/Corrosion (2021)
- OECD Test Guideline 406: Skin Sensitisation (2021).
- OECD Test Guideline 408: Repeated Dose 90-day Oral Toxicity Study in Rodents (2018).
- OECD Test Guideline 417: Toxicokinetics (2010).
- OECD Test Guideline 420: Acute Oral Toxicity- Fixed Dose Method (2001).
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (2016)
- OECD Test Guideline 471: Bacterial Reverse Mutation Test (2020).
- OECD Test Guideline 473: *In Vitro* Mammalian Chromosomal Aberration Test (2016).
- OECD Test Guideline 474: Mammalian Erythrocyte Micronucleus Test (2016).
- OECD Test Guideline 475: Mammalian Bone Marrow Chromosomal Aberration Test (2016).
- OECD Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes (2016)
- OECD Test Guideline 479: Genetic Toxicology: In vitro Sister Chromatid Exchange Assay in Mammalian Cells (1986, deleted 2014).
- OECD Test Guideline 487: *In Vitro* Mammalian Cell Micronucleus Test (2016)
- OECD Test Guideline 490: *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene (2016).

Section Residue and consumer risk assessment

Section fate and behavior in environment

- EFSA (European Food Safety Authority), 2007. Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32.
- EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. 49 pp. doi:10.2903/j.efsa.2011.2092.
- EFSA (European Food Safety Authority) 2014b. EFSA Guidance Document for evaluating

laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662.

- EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.
- FOCUS 1997. Soil persistence models and EU registration. The final report of the work of the Soil Modelling Work group of FOCUS. 77 pp.
- FOCUS 2000. FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000 rev.2, 202 pp.
- FOCUS 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp., as updated by the Generic Guidance for FOCUS surface water scenarios, version 1.1 dated March 2012
- FOCUS 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp. June 2006.
- FOCUS 2008. "Pesticides in Air: Considerations for Exposure Assessment". Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.
- FOCUS 2011. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Version 1.0 (23/11/2011).
- FOCUS (2014a) : Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. version 1.1 (18/12/2014). 440 pp.
- FOCUS 2014b. Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU. The Final Report of the Ground Water Work Group of FOCUS, SANCO/13144/2010 version 3, 613 pp., 10 October 2014.
- FOCUS 2014c. Generic guidance for Tier 1 FOCUS ground water assessments. Technical Report Version 2.2, FOrum for the Co-ordination of pesticide fate models and their Use
- FOCUS 2015. Generic guidance for FOCUS surface water scenarios. Technical Report Version 1.4, FOrum for the Co-ordination of pesticide fate models and their USe. May 2015.
- SANCO 221/2000 rev 10 : European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000-rev. 10 - final, 25 February 2003.
- OECD 307. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Aerobic and Anaerobic Transformation in Soil, 2002.
- OECD "Phototransformation of Chemicals on Soil Surfaces" (Draft, January 2002).
- OECD 106. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Adsorption - Desorption Using a Batch Equilibrium Method, 2000.
- OECD 111 (2002); OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Hydrolysis as a Function of pH, 2004.
- OECD 301 D. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Ready Biodegradability, 1992.
- OECD Guideline 309. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test, 2004.

- OECD 308. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, 2002.

Section ecotoxicology

- EFSA GD for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018; 16(6): 5311, 135 pp.
- Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009.
- Blümer et al., 2000. Laboratory residual contact test with the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. M.P. CANDOLFI et al. (2000): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative.
- ECHA Guidance to Regulation (EC) no 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5 July 2017.
- EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA Journal 2009;7(12):1438, 358 pp. doi:10.2903/j.efsa.2009.1438.
- EFSA (European Food Safety Authority), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290
- EFSA (European Food Safety Authority), 2013. 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, 268 pp., doi:10.2903/j.efsa.2013.3295.
- European Commission, 2002a. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2 final, 17 October 2002
- Mead-Briggs et al., 2000. A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera: Braconidae). M.P. CANDOLFI et al. (2000): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative.
- OECD 201 (1984, update 2002). OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Freshwater Alga and Cyanobacteria, Growth Inhibition Test, 2011.
- OECD 202 (1984). OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Daphnia sp., Acute Immobilisation Test, 2004.
- OECD 203 (1992). OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Fish, Acute Toxicity Test, 1992.
- OECD 204 (1984). OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Fish, Prolonged Toxicity Test: 14-day Study, 1984.
- OECD 205. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Avian Dietary Toxicity Test, 1984.
- OECD 206. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Avian Reproduction Test, 1984.
- OECD 207 (1984) and ISO 11268-1 (1993). OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Earthworm, Acute Toxicity Tests, 1984.
- OECD 209. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Activated Sludge,

Respiration Inhibition Test (Carbon and Ammonium Oxidation), 2010.
- OECD 210. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Fish, Early-life Stage Toxicity Test, 2013.
- OECD 211. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Daphnia magna Reproduction Test, 2012.
- OECD 211. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Daphnia magna Reproduction Test.
- OECD 213. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Honeybees, Acute Oral Toxicity Test, 1998.
- OECD 214. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Honeybees, Acute Contact Toxicity Test, 1998.
- OECD 215. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Fish, Juvenile Growth Test, 2000.
- OECD 216. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Soil Microorganisms: Nitrogen Transformation Test, 2000.
- OECD 217. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Soil Microorganisms: Carbon Transformation Test, 2000.
- OECD 218. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Sediment-Water Chironomid Toxicity Test Using Spiked Sediment, 2004.
- OECD 222. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Earthworm Reproduction Test (Eisenia fetida/ Eisenia andrei, 2016.
- OECD 223. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Avian Acute Oral Toxicity Test, 2016.
- OECD 226. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, 2016.
- OECD 232. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Collembolan Reproduction Test in Soil, 2016.
- SETAC (Society of Environmental Toxicology and Chemistry), 2001. Guidance Document on Regulatory Testing and Risk Assessment procedures for Plant Protection Products with Non-Target Arthropods. ESCORT 2.

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

Section data on application and efficacy

Section toxicology

- Draft Assessment Report (DAR) on the active substance thymol, RMS: UK (2011).
- Final Addendum to Draft Assessment Report (DAR) on thymol, RMS: UK (June 2012).
- Peer review report on thymol (October 2012).
- Conclusion on the peer review of the pesticide risk assessment of the active substance Thymol. EFSA Journal 2012; 10(11):2916.
- Final Review report for the active substance thymol. SANCO/10581/2013 rev 3 17 May 2013
- Thymol Addendum _ Confirmatory Data August 2016
- Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for thymol in light of confirmatory data. 12 January 2017 doi:10.2903/sp.efsa.2017.EN-1162.

Section residue and consumer risk assessment

Section fate and behavior in environment

Section ecotoxicology