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

Tea Tree Oil (TTO)

Volume 1

Rapporteur Member State: Poland
Co-Rapporteur Member State: Bulgaria

August 2022

Version History

When	What
August 2022	Initial RAR

The notified active substance shall be identified with the names and identifiers: Melaleuca alternifolia, essential oil; tea tree oil; CAS 68647-73-4 and Melaleuca alternifolia, ext. CAS 85085-48-9; EC 285-377-1. The name "extract of tea tree" is not appropriate to identify the notified active substance. The RMS will make all possible efforts to amend the name and identifiers of the notified substance in the whole RAR at the later stages of the process.

Table of contents

Level 1

Tea Tree Oil (TTO)

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 active ingredient Tea Tree Oil (TTO) is due to re-approval according to Commission Regulation (EU) No. 823/2012 of 14.09.2012.

A dossier has been submitted by 28 February 2018 (SANTE-2016-10616–rev 4 of October 2016). This dossier is submitted by Stockton Europe Ltd. in order to support the renewal (re-approval) of Tea Tree Oil, an existing active substance, according to the Commission Regulation (EC) 1107/2009 of 21 October 2009.

The information submitted includes new data and new risk assessments to reflect the changes in data requirements and the changes in scientific or technical knowledge since Tea Tree Oil was first included in Annex I to Directive 91/414/EC on 01.09.2009 (Commission Directive 2008/127/EC).

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

Poland – as Rapporteur Member State (RMS) – conducted the full evaluation and prepared the RAR for Tea Tree Oil. There was no co-RMS involved.

1.1.3 EU Regulatory history for use in Plant Protection Products

The dossier to support the first approval of Tee Tree Oil (under the name “Extract from tea tree”) in the EU was submitted by the notifier SIA ‘Biomor Latvija to Latvia (RMS) in 2005. Latvia being the designated rapporteur Member State submitted the DAR on extract from tea tree (Tea Tree Oil) in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 25 October 2007. In 2010 the notification ownership was transferred to the UK daughter company “Biomor Europe Ltd.” and in 2012 Biomor Company and its affiliates was bought by its mother company Stockton Israel Ltd, therefore Stockton Europe Ltd. became the notifier for Tea Tree Oil (TTO) in EU.

Stockton Europe Ltd has submitted a dossier to Poland (RMS) in the framework of AIR 4 substance renewal on 28 February 2018.

1.1.4 Evaluations carried out under other regulatory contexts

Not applicable.

1.2 APPLICANT INFORMATION

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

Stockton Europe Ltd.
Bahnhofstrasse 11
CH-6301 Zug
Switzerland

Primary contact:

Contact 2:

1.2.2 Producer or producers of the active substance

Stockton Europe Ltd.
Bahnhofstrasse 11
CH-6301 Zug
Switzerland

Contact:

Production site:
Please refer to Volume 4, confidential document

1.2.3 Information relating to the collective provision of dossiers

Not applicable

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Tea Tree Oil [1] Synonyms: Oil of *Melaleuca alternifolia* (Terpinen-4-ol Type) Essential oil of *Melaleuca alternifolia*
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	No chemical denomination can be assigned to Tea Tree Oil (TTO) because it is a complex composition[2]. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The specified components according to ISO 4730:2004 are:

[1] TTO does not have an ISO common name
[2] RMS note: TTO is Substance of Unknown or Variable composition, Biological material (UVCB) according to definition in ECHA 'Guidance for identification and naming of substances under REACH and CLP' Version 2.1 - May 2017.

	Name / **Chemical Name** Terpinen-4-ol: 1-Methyl-4-isopropyl-1-cyclohexen-4-ol (±) Enantiomeric Ratio γ-Terpinene: 1-Methyl-4-isopropyl-1,4-cycloheyadiene α-Terpinene: 1-Methyl-4-isopropyl-1,3-cyclohexadiene α-Terpineol: 2-[(4-methyl-1-cyclohex-3-enyl)]propan-2-ol α-Terpinolene: 4-Isopropylidene-1-methylcyclohexene α-Pinene: 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene p-Cymene: 1-Methyl-4-isopropylbenzene 1,8-Cineole (Eucalyptol): 1,33-trimethyl-2-oxabicyclo[2.2.2]octane Limonene: 1-Methyl-4-1(1-methylethenyl) cyclohexene Aromaden-drene: 1H-Cycloprop[e]azulene, decahydro-1,17-trimethyl-4-methylene-,[1 ar- 1aalpha,4aalpha, 7alpha, 7beta, 7baplha]- δ-Cadinene: 1,2,3,5,6,8a-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene Sabinene: 1-Isopropyl-4-methylenebicyclo[3.1.0] hexane Globulol: 1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-(1aR,4R,4aR,7R,7aS,7bS)-(8cl,9Cl) Viridiflorol: (±)-viridiflorol, δ-viridiflorol,decahydro-1,1,4,7-tetra-methyl-(1aR,4S,4aS,7R,7aS,7bS)-1H-Cycloprop[e] azulen-4-ol,himbaccol Ledene: 1,1,4,7-Tetramethyl-1a,2,3,5,6,7,7a,7b-octahydro-1H-cyclopropa[e]azulene
CA	See above
1.3.3 Producer's development code number	-
1.3.4 CAS, EEC and CIPAC numbers	
CAS	68647-73-4 Tea Tree Oil (TTO) is naturally occurring substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. For the components please refer to following table:

Name	CAS No.	EC No.
Terpinen-4-ol	562-74-3	209-235-5
γ-Terpinene	99-85-4	202-794-6
α-Terpinene	99-86-5	202-795-1
α-Terpineol	98-55-5	202-680-6
α-Terpinolene	586-62-9	209-578-0
α-Pinene	80-56-8	201-291-9
p-Cymene	99-87-6	202-796-7
1,8-Cineole (Eucalyptol)	470-82-6	207-431-5
Limonene	138-86-3	205-341-0
Aromadendrene	489-39-4	207-694-6
δ-Cadinene	483-76-1	--
Sabinene	3387-41-5	222-212-4
Globulol	489-41-8	207-696-7
Viridiflorol	552-02-3	209-003-3

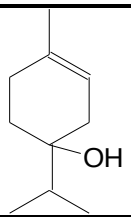
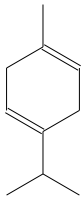
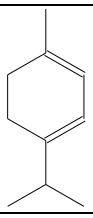
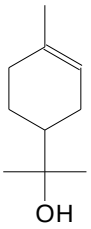
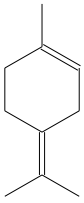
	Ledene	21747-46-6	244-565-3
EEC	-[3]		
CIPAC	Not assigned		

1.3.5 Molecular and structural formula, molecular mass

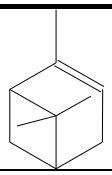
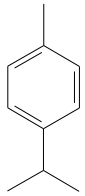
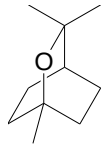
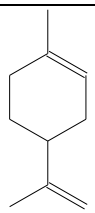
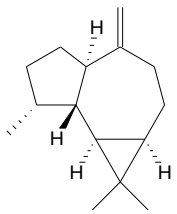
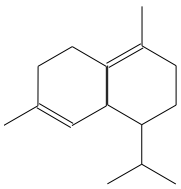
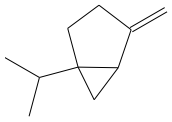
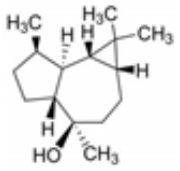
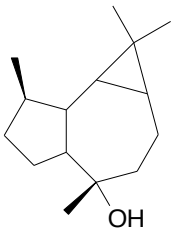
Molecular formula

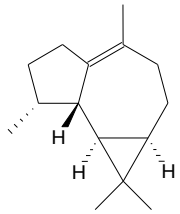
No molecular formula and mass can be assigned to Tea Tree Oil because it is a substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The specified components according to ISO 4730:2004 are displayed in the following table. Furthermore, the components contained in Tea Tree Oil can be grouped according to their chemical structure as several components have very similar structures. Consequently, their chemical properties within the assigned groups are comparable.

The specified components according to ISO 4730:2004 are:

Name	Mole-cular weight	Molecular formula	Structural formula
Terpinen-4-ol	154.25	$C_{10}H_{18}O$	
γ-Terpinene	136.24	$C_{10}H_{16}$	
α-Terpinene	136.24	$C_{10}H_{16}$	
α-Terpineol	154.25	$C_{10}H_{18}O$	
α-Terpinolene	136.24	$C_{10}H_{16}$	

[3] RMS note: EC no 285-377-1 corresponds to substance Melaleuca alternifolia, ext., registered under REACH (https://echa.europa.eu/registration-dossier/-/registered-dossier/20921) described by CAS no 85085-48-9

α-Pinene	136.24	$C_{10}H_{16}$	
p-Cymene	134.22	$C_{10}H_{14}$	
1,8-Cineole (Eucalyptol)	154.25	$C_{10}H_{18}O$	
Limonene	136.24	$C_{10}H_{16}$	
Aromaden-drene	204.35	$C_{15}H_{24}$	
δ-Cadinene	204.35	$C_{15}H_{24}$	
Sabinene	136.24	$C_{10}H_{16}$	
Globulol	222.37	$C_{15}H_{26}O$	
Viridiflorol	222.37	$C_{15}H_{26}O$	

	Ledene (Viridiflorene) | 204.35 | $C_{15}H_{24}$ 
Structural formula	See above
Molecular mass	See above
1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately in Volume 4
1.3.7 Specification of purity of the active substance in g/kg	No FAO specification available ISO 4730:2004:

Name	CAS No.	EC No.	Min. %	Max. %
Terpinen-4-ol	562-74-3	209-235-5	30	48
γ-Terpinene	99-85-4	202-794-6	10	28
α-Terpinene	99-86-5	202-795-1	5	13
α-Terpineol	98-55-5	202-680-6	1.5	8
α-Terpinolene	586-62-9	209-578-0	1.5	5
α-Pinene	80-56-8	201-291-9	1	6
p-Cymene	99-87-6	202-796-7	0.5	8
1,8-Cineole (Eucalyptol)	470-82-6	207-431-5	trace	15
Limonene	138-86-3	205-341-0	0.5	1.5
Aromadendrene	489-39-4	207-694-6	0.5	3
δ-Cadinene	483-76-1	--	trace	3
Sabinene	3387-41-5	222-212-4	trace	3.5
Globulol	489-41-8	207-696-7	trace	1
Viridiflorol	552-02-3	209-003-3	trace	1
Ledene	--	--	trace	3

1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	CONFIDENTIAL information - data provided separately in Volume 4
1.3.8.2 Significant impurities	CONFIDENTIAL information - data provided separately in Volume 4
1.3.8.3 Relevant impurities	CONFIDENTIAL information - data provided separately in Volume 4
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately in Volume 4

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	**Stockton Europe Ltd.** Bahnhofstrasse 11 CH-6301 Zug Switzerland Primary contact: [redacted] Contact 2: [redacted]
1.4.2. Producer of the plant protection product	**Stockton Europe Ltd.** Bahnhofstrasse 11 CH-6301 Zug Switzerland Contact: [redacted] **Location of the manufacturing site:** CONFIDENTIAL information - data provided separately - Volume 4
1.4.3. Trade name or proposed trade name and producer's development code number of the plant protection product	Trade name: Timorex Gold Company code number: BM 608

<table>
<tr><td colspan="2">1.4.4. Detailed quantitative and qualitative information on the composition of the plant protection product</td></tr>
<tr><td>1.4.4.1. Composition of the plant protection product</td><td>Pure active substance
<table>
<tr><td>content of pure active substance:</td><td>222.5 g/L</td><td>23.8% w/w</td></tr>
<tr><td>Limits (± 6% as given by FAO 2016):</td><td>209.2 g/L</td><td>235.9 g/L</td></tr>
</table>
Technical active substance (Purity)
The purity of the technical active substance Tea Tree Oil is not applicable, as the active substance is UVCB substance and have a complex composition with specification according to ISO 4730:2004</td></tr>
<tr><td>1.4.4.2. Information on the active substances</td><td>
<table>
<tr><td>Type</td><td>Name/Code Number</td></tr>
<tr><td>ISO common name</td><td>Tea tree oil[4]</td></tr>
<tr><td>CAS No</td><td>68647-73-4</td></tr>
<tr><td>EC No</td><td>-</td></tr>
<tr><td>CIPAC No</td><td>Not assigned</td></tr>
<tr><td>Salt, ester anion or cation present</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>1.4.4.3. Information on safeners, synergists and co-formulants</td><td>CONFIDENTIAL information - data provided separately in the Confidential Part (see Volume 4).</td></tr>
<tr><td>1.4.5. Type and code of the plant protection product</td><td>Emulsifiable Concentrate [Code: EC]</td></tr>
<tr><td>1.4.6. Function</td><td>Fungicide</td></tr>
<tr><td>1.4.7. Field of use envisaged</td><td>BM 608 (Timorex Gold) is a fungicide product for control of powdery mildew and grey mold in tomato (indoor and outdoor) and in vineyards. The product is used for foliar application along the whole crop cycle.</td></tr>
<tr><td>1.4.8. Effects on harmful organisms</td><td>BM 608 (Timorex Gold) leads to an inhibition of spore germination and fungal growth of powdery mildew and grey mold in vineyard and tomato</td></tr>
</table>

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

[4] TTO does not have an ISO common name

1.5.1 Details of representative uses – Timorex Gold (BM 608)

Intended uses supported in the EU for which data have been provided

GAP - updated date: 2017-12-06

PPP (product name/code)	**Timorex Gold/BM 608**	**Formulation type:**	**EC (Emulsifiable Concentrate)**
active substance 1	**Tea Tree Oil**	**Conc. of as 1:**	**222.5 g/L**
safener	**no**	**Conc. of safener:**	**--**
synergist	**no**	**Conc. of synergist:**	**--**
Applicant:	**Stockton Europe Ltd.**	**professional use**	☒
Zone(s):	**EU countries / EU-S**	**non professional use**	☐
Verified by MS:	**No**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate***			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	EU Countries	Tomato	G	Powdery mildew	Overall foliar spraying	Along crop cycle	a) 4 b) 4 (7)	a) 1.5 b) 6.0	a) 0.334 b) 1.335	500-1000	*	0.15 – 0.3 L/hL
2	EU Countries	Tomato	G	Grey Mold	Overall foliar spraying	Along crop cycle	a) 4 b) 4 (7)	a) 2.0 b) 8.0	a) 0.445 b) 1.780	500-1000	*	0.2 – 0.4 L/hL
3	EU-S	Vineyard	F	Powdery mildew	Overall foliar spraying	Along crop cycle from Spring till Autumn	a) 4 b) 4 (7)	a) 1.5 b) 6.0	a) 0.334 b) 1.335	500-1000	*	0.15 – 0.31 L/hL
4	EU-S	Vineyard	F	Grey Mold	mainly on the bunches	Along crop cycle from Spring till Autumn	a) 4 b) 4 (10)	a) 2.0 b) 8.0	a) 0.445 b) 1.780	500-1000	*	0.2 – 0.4 L/hL

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate***				
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
5	EU-S	Tomato	F	Powdery mildew	Overall foliar spraying	Along crop cycle from Spring till Autumn	a) 3 b) 3 (7)	a) 1.5 b) 6.0	a) 0.334 b) 1.335	500-1000	*	0.15 – 0.31 L/hL
6	EU-S	Tomato	F	Grey Mold	Overall foliar spraying	Along crop cycle from Spring till Autumn	a) 3 b) 3 (7)	a) 2.0 b) 8.0	a) 0.445 b) 1.780	500-1000	*	0.2 – 0.4 L/hL

(a) For crops, the EU and Codex classification (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)
(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
(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
(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 synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage at 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
(k) Indicate the minimum and maximum number of application 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

1.5.2 Further information on representative uses

The details of the formulated product and its proposed use are as follows:

Active substance:	Tea tree oil
Chemical group:	plant extract of *Melaleuca alternifolia*
Formulation	Emulsifiable concentrate (EC)
Type:	Fungicide
Target organism:	Powdery mildew
Crop:	Vineyard
Single application rate:	1.5 L/ha
Application timing:	along crop cycle from spring till autumn
Maximum number of applications:	4
Minimum interval between applications:	7 days

Active substance:	Tea tree oil
Chemical group:	plant extract of *Melaleuca alternifolia*
Formulation	Emulsifiable concentrate (EC)
Type:	Fungicide
Target organism:	grey mold
Crop:	Vineyard
Single application rate:	2.0 L/ha
Application timing:	along crop cycle from spring till autumn
Maximum number of applications:	4
Minimum interval between applications:	10 days

Active substance:	Tea tree oil (plant extract of *Melaleuca alternifolia*)
Chemical group:	plant extract of *Melaleuca alternifolia*
Formulation	Emulsifiable concentrate (EC)
Type:	Fungicide
Target organism:	grey mold
Crop:	Tomato (indoor and outdoor)
Single application rate:	2.0 L/ha
Application timing:	along crop cycle from Spring till Autumn
Maximum number of applications:	4
Minimum interval between applications:	7 days

Active substance:	Tea tree oil (plant extract of *Melaleuca alternifolia*)
Chemical group:	plant extract of *Melaleuca alternifolia*
Formulation	Emulsifiable concentrate (EC)
Type:	Fungicide
Target organism:	Powdery mildew
Crop:	Tomato (indoor and outdoor)
Single application rate:	1.5 L/ha
Application timing:	along crop cycle from Spring till Autumn
Maximum number of applications:	4
Minimum interval between applications:	7 days

Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops - Not relevant.
Proposed Instructions for Use - Timorex Gold (BM608)
The following label text is proposed for Tea Tree Oil:

PLANT PROTECTION PRODUCTS FOR PROFESSIONAL USERS

Instruction

Timorex Gold

BIOFUNGICIDE
EMULSIFABLE CONCENTRATE

For the control or suppression of listed diseases on table and wine grape, greenhouse and field tomato.

COMMERCIAL

READ THE LABEL BEFORE USING
WARNING - EYE AND SKIN IRRITANT
POTENTIAL SKIN SENSITIZER

GUARANTEE:
Tea Tree Oil …….. 23.80% (222.5 g/L)
Ethanol ……....4% (37.4 g/L)

Manufactured by:
Stockton (Israel) Ltd.
17 Ha'Mefalsim St.
Petach Tikva P.O. Box 3517
Israel 4951447
www.stockton-ag.com

Manufacturing site:
CONFIDENTIAL information - data provided separately in the Confidential Part (see Volume 4).

Net Contents: 1, 5, 20 litres

MODE OF ACTION
Timorex Gold contains tea tree oil belonging to the Cell Membrane Disruption Group 46, Target Site Group F7.

CLASSIFICATION, HAZARD AND PRECAUTIONARY STATEMENTS
Timorex Gold classification is proposed according to Regulation (EU) no. 1272/2008:

Signal words	Classification	Pictograms
Warning	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1B Asp. Tox. 1 Repr. 2 Aquatic Acute 1 Aquatic Chronic 3	 GHS02  GHS07  GHS08

CMR category	Repr. 2
Specific Target Organ Toxicity	None

Hazard Statements

H226 - Flammable liquid and vapour
H302 - Harmful if swallowed
H332 - Harmful if inhaled
H315- Causes skin irritation
H317 - May cause an allergic skin reaction

H304 - May be fatal if swallowed and enters airways
H361f - Suspected of damaging fertility
H400 - Very toxic to aquatic life
H412 - harmful to aquatic life with long lasting effects

Precautionary statements:

General:
P101: If medical advice is needed, have product container or label at hand.
P102: Keep out of reach of children
P202: Do not handle until all safety precautions have been read and understood.

Prevention:
P210: Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P233: Keep container tightly closed
P240: Ground/bond container and receiving equipment.
P242: Use only non-sparking tools
P243: Take precautionary measures against static discharge
P264: Wash hands thoroughly after handling.
P273: Avoid release to the environment
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P331: Do NOT induce vomiting

RESPONSE:
P301 + P310: IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313: If eye irritation persists: Get medical advice/attention
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention
P362 + P364: Take off contaminated clothing and wash it before reuse.
P308 + P313: IF exposed or concerned: Get medical advice/attention.

STORAGE:
P403 + 235: Store in a well-ventilated place. Keep cool.
P405: Store locked up

DISPOSAL:
P501: Dispose of content/container to in accordance with local and national regulations.

Others:
EUH401: To avoid risks to human health and the environment, comply with the instructions for use

HAZARDOUS COMPONENTS
Contains Tea Tree Oil and ethanol. May produce allergic reaction.

FIRST AID

General notes	Remove victim from area of exposure. Wash off remaining material with plenty of water. Seek medical assistance. Never leave the victim alone.
Eye contact	Wash out with water with the eyelid held wide open for at least 15 minutes. Get medical attention.
Skin contact	Remove contaminated clothing. Wash away remainder with water and soap
Inhalation	Remove victim to fresh air. If breathing is difficult: artificial respiration. Get medical attention.
Ingestion	Do NOT induce vomiting Wash out mouth with plenty of water. Get medical attention. Never give anything by mouth to an unconscious person.
Take container, label or product name and registration number with you when seeking medical attention. There is no antidote. Treat symptomatically.	

Emergency phone no.: +972-72-2570000 (office hours)

STORAGE
Keep only in the original container. Keep container tightly closed in a cool, dry, well ventilated place away from direct sunlight and ignition/heat sources.
Keep from overheating or freezing.
To prevent contamination, store this product away from food or feed.

HANDLING
Avoid contact with skin and eyes. Ventilation required. When handling, wear suitable protective clothing. Keep away from ignition sources -Do not smoke. Protect against electrostatic charges.

WASTE DISPOSAL INSTRUCTIONS

- Triple- or pressure-rinse the empty container. Add the rinsings to the spray mixture in the tank.
- Follow provincial instruction for any required additional cleaning of the container prior to its disposal.
- Make the empty container unsuitable for further use.
- Dispose of the container in accordance with provincial requirements.
- For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of a spill, and for clean-up of spills.

DIRECTIONS FOR USE

GENERAL USE INFORMATION:
Timorex Gold is a broad spectrum, preventative and curative biofungicide for the control or suppression of listed plant diseases in greenhouse and field crop. It is applied as a foliar spray as a solo application or in an alternating spray program with other registered crop protection products. For maximum effectiveness, use Timorex Gold at the first sign of disease development. Under heavy disease pressure, use Timorex Gold in a rotational program with other registered fungicides.

Timorex Gold has to be applied by ground application equipment. Timorex Gold is recommended for control of powdery mildew and gray mold in greenhouse and field tomato and grape.

USE DIRECTIONS:
SHAKE WELL BEFORE USE.

Half fill the spray tank, turn on agitation and add the required amount of Timorex Gold. With agitation on, add the remaining water to the spray tank. Maintain agitation during mixing, standing and spraying. DO NOT allow the diluted spray to remain in the spray vat for more than 24 hours.

APPLICATION DIRECTIONS:
DO NOT enter or allow worker entry into treated areas until full dry out.
Apply in slow drying conditions for best uptake of spray solution and product effectiveness.

DO NOT spray during the warm hours of the day and in hot seasons with temperatures above 35ºC (95ºF)

DO NOT apply this product through any type of irrigation system.

Apply Timorex Gold at recommended rates of spray volume (see table of Application Rates).

Make applications in the early stages of disease infestation for best control. Early treatment prevents continued disease development. Apply Timorex Gold in the greenhouse or field to listed crops using conventional spray equipment. Good coverage and wetting of the foliage is required. Use enough spray solution to completely penetrate the leaf canopy and cover both the top and underside of all leaves until runoff. The amount of spray solution varies with crop and amount of plant growth. Prepare enough solution based on foliage and consider plant density to insure sufficient coverage, while maintaining the concentration spray volume. Reapply Timorex Gold throughout the growing season at recommended intervals (detailed below).

Use higher application rates and shorter spray intervals when disease pressure is high.

APPLICATION RATES and level of disease control for Timorex Gold

Crop	Disease	Level of control	Max. Product application rate L/ha	Spray volume L/ha
Grape (tables and wine	Powdery mildew (*Erysiphe necator*)	Control	1.5	500-1000
	Gray Mold (*Botrytis cinerea*)	Control	2.0	500-1000
Greenhouse tomato	Powdery mildew (*Oidium neolycopersici, O. lycopersici* or *Leveillula taurica*)	Control	2.0	500-1000
	Gray Mold (*Botrytis cinerea)*	Control	1.5	500-1000
Tomato open field	Powdery mildew *(Oidium neolycopersici, O. lycopersici or Leveillula taurica)*	Control	1.5	500-1000

FREQUENCY OF TREATMENTS
Recommended number of applications per season – max. 4 applications, for field tomatoes up to 3 only.

For preventative treatments, apply at 7-10 day intervals, depending on disease level. Use the shorter application interval under conditions that promote rapid disease development.

For curative control apply Timorex™ Gold at the first signs of the disease,

Timorex Gold has been evaluated for phytotoxicity on a variety of crops under various normal growing conditions. However, testing all crop varieties, in all mixtures and combinations is not feasible. Test a small portion of the crop for sensitivity prior to treating entire crop.

DO NOT use to control aquatic pests
DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.

DO NOT allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters.

Use of the following spray methods or equipment DO NOT require a buffer zone: hand-held or backpack sprayer and spot treatment.

For tank mixes, consult the labels of the tank mix partners and observe the largest (most restrictive) buffer zone of the products involved in the tank mixture

CAUTION:
Causes eye, skin and mucous membrane irritation. DO NOT get in eyes, on skin or clothing, or inhale sprays, mists or vapors. Potential skin sensitizer.
Wash thoroughly with soap and water after handling. Remove and wash contaminated clothing before reuse.

Apply only when the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools, and recreational areas is minimal. Take into consideration wind speed, wind direction, temperature inversion, application equipment, and sprayer settings.

PERSONAL PROTECTIVE EQUIPMENT (PPE):
Workers potentially exposed to the product through mixing, loading, applying, clean-up and repair activities must wear chemical-resistant goggles or a face shield, long-sleeved shirt and long pants, chemical-resistant gloves and shoes plus socks. Follow manufacturer's instructions for cleaning / maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS:
Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing. Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

ENVIRONMENTAL HAZARDS:
TOXIC to aquatic organisms.

To reduce runoff from treated areas into aquatic habitats avoid application to areas with a moderate to steep slope, compacted soil, or clay.

Avoid application when heavy rain is forecast.

Contamination of aquatic areas as a result of runoff may be reduced by including a vegetative strip between the treated area and the edge of the water body.

SPRAYER CLEAN-UP
Rinse the tank and other sprayer parts twice with water and once with water and detergent.
Collected rinses should be disposed of according to local regulations.

COMPATIBILITY
Carefully clean the spray tank, if you have used these products recently. When adding another fungicide or an insecticide, observe the cautions and pre-entry and pre-harvest intervals on edible crops printed on the other pesticide label. Always use caution when trying a new spray mixture, by applying to a few test plants.

RESISTANCE MANAGEMENT
Timorex Gold can be considered an effective tool for resistance management programs. Timorex Gold has a unique mode of action against fungal plant pathogens. In addition, it contains multiple compounds that support multi-site functional activity. This supports a very low or no probability for the development of resistance in plant pathogens. Therefore, Timorex Gold can be included in a spray programme to avoid cross resistance during the season. It can be rotated or tank mixed in applications with chemical products to which fungal plant pathogen populations have shown reduced sensitivity. To minimize risk, use strictly in accordance with label instructions

BENEFICIAL ARTHROPODS
Timorex Gold is of low toxicity to honeybees and bumble bees. In addition, it has low toxicity to beneficial arthropods and soil macro- and microorganisms.

NOTICE TO USER
This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *Pest Control Products local laws* to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any such use of this product.

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

No other uses applied for to support the setting of MRLs beyond the representative uses.

1.5.4 Overview on authorisations in EU Member States

Authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications - growth stages and where appropriate, season)		**Actual uses, if current practice is known to deviate from the authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications - growth stages and where appropriate, season)**
1	Austria	
2	Belgium	
3	Bulgaria	
4	Czech Republic	
5	Cyprus	

Authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications - growth stages and where appropriate, season)		**Actual uses, if current practice is known to deviate from the authorised uses (crops, harmful organisms, rates of application, number of applications, timings of applications - growth stages and where appropriate, season)**
6	Denmark	
7	Estonia	
8	Finland	
9	France	
10	Germany	
11	Greece	
12	Hungary	
13	Ireland	
14	Italy	
15	Latvia	
16	Lithuania	
17	Luxemburg	
18	Malta	
19	Netherlands	
20	Poland	
21	Portugal	
22	Romania	
23	Slovenia	
24	Slovak Republic	
25	Spain	**Crops**: zucchini, cucumber, **harmful organism**: powdery mildew, botrytis; **rates of application:** 0.4-0.6%; **number of applications:** 4 (7 days interval between applications); **spray volume (L/ha)**: 300-700 (zucchini); 500-1000 (cucumber); **timings of applications: growth stages:** Along crop cycle (BBCH 10 – 89) – cucumber. Along crop cycle from spring till autumn (BBCH 10 – 89) – zucchini.
26	Sweden	
27	United Kingdom	

.

Level 2

Tea Tree Oil (TTO)

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

Tea Tree Oil, Oil of *Melaleuca alternifolia* is naturally occurring substance having complex composition (UVCB substance). UVCB stands for unknown or variable composition, complex reaction products or for materials of biological origin as defined in ECHA 'Guidance for identification and naming of substances under REACH and CLP' Version 2.1 - May 2017.
TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The specified components according to ISO 4730:2004.

FAO: No FAO specification available (EFSA Journal 2012;10(2):2542, p. 6)

The purity of the technical active substance Tea Tree Oil is not applicable, as the active substance is UVCB substance and have a complex composition with specification according to ISO 4730:2004 (in table below):

Name	CAS No.	EC No.	Min. %	Max. %
Terpinen-4-ol	562-74-3	209-235-5	30	48
γ-Terpinene	99-85-4	202-794-6	10	28
α-Terpinene	99-86-5	202-795-1	5	13
α-Terpineol	98-55-5	202-680-6	1.5	8
α-Terpinolene	586-62-9	209-578-0	1.5	5
α-Pinene	80-56-8	201-291-9	1	6
p-Cymene	99-87-6	202-796-7	0.5	8
1,8-Cineole (Eucalyptol)	470-82-6	207-431-5	trace	15
Limonene	138-86-3	205-341-0	0.5	1.5
Aromadendrene	489-39-4	207-694-6	0.5	3
δ-Cadinene	483-76-1	--	trace	3
Sabinene	3387-41-5	222-212-4	trace	3.5
Globulol	489-41-8	207-696-7	trace	1
Viridiflorol	552-02-3	209-003-3	trace	1
Ledene	21747-46-6	244-565-3	trace	3

2.1.1 Summary of identity

Acceptable information has been provided by the applicant on the sources and identity of Tea Tree Oil and the representative plant protection product. The reference specification remains appropriate.
The five batch study of technical Tea Tree Oil is provided and indicates that no new components are present and the variability of all components are within the specification according to ISO 4730:2004 (please see confidential section for full information).

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

Tea Tree Oil is a colourless to pale yellow liquid which has a boiling point between 177 – 191°C. The water solubility and the vapour pressure of its main component Terpinen-4-ol were measured to be 3.28 g/L and 14.9 Pa at ambient temperature, respectively. The partition coefficient n-octanol/water of its main component Terpinen-4-ol, log P_{ow}, is 2.643 at pH 5.85 and at 23.5°C, which indicates that the partitioning of the substance to organic matter in soil and water, and to biota, is slightly higher than to the aqueous phase. However, the n-octanol/water partition coefficient (log Pow) for the most of the remaining components of TTO (α-Pinene, Limonene, γ-Terpinene, Terpinolene, p-Cymene, α-Terpinene, Aromadendrene, δ-Cadinene, Sabinene, Globulol, Viridiflorol, Ledene) is above 4. The measured surface tensions of neat (28.4 mN/m) and 1% w/v solution (45.6 mN/m) TTO are lower than 60 mN/m, thus the active substance has to be regarded as a surface-active material. Tea Tree Oil is not oxidizing and not explosive. Its auto-ignition temperature and flash point is 269°C and 55°C at approx. 1007 hPa, respectively. Therefore, TTO should be classified as flammable liquid category 3 (Flam. Liq. 3; H226: Flammable liquid and vapour) according to classification criteria of Regulation (EC) No 1272/2008.

Reliability statement: The data in the table below have been compiled from laboratory studies as well as open scientific literature. All laboratory studies have been evaluated for deviations from the respective test guidelines, accordance to quality criteria and overall scientific reliability. All the studies fulfilled their respective validity criteria. Only minor deviations have been identified in some of the studies which were not at all relevant for their scientific reliability and regulatory suitability. Hence, the laboratory studies are considered reliable (reliability score: 1).

The literature studies have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for regulatory relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

According to CLP regulation, all available relevant data shall be included in the CLH proposal. There is a REACH registration dossier for *Melaleuca alternifolia*, ext. (EC no 285-377-1) available. According to Annex VI, part 2 of the CLP regulation the information from registration dossiers shall be considered when preparing dossiers for harmonised classification and labelling. Therefore all available relevant data were included in the CLH proposal.

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	Colourless to pale yellow liquid	Anonymous 2016a	Observed
Melting/freezing point	No melting point down to - 100°C at atmospheric pressure	Anonymous 2016a	Measured
	-22°C	ECHA dissemination site[5]	Measured (EU A.1)
Boiling point	177 – 191°C	Anonymous 2016a	Measured

[5] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/3

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>97-220°C</td><td>ECHA dissemination site[6]</td><td>Measured (EU A.2)</td></tr>
<tr><td rowspan="2">Relative density</td><td>d_{20}^{20} = 0.902</td><td>Anonymous 2017</td><td>Measured</td></tr>
<tr><td>$D_{20/4}$ = 0.89</td><td>ECHA dissemination site[7]</td><td>Measured (EU A.3)</td></tr>
<tr><td rowspan="3">Vapour pressure</td><td>1,8-Cineole: 385 Pa at 20°C, 501 Pa at 25°C, 2048 Pa at 50°C
Terpinen-4-ol: 14.9 Pa at 20°C</td><td>Anonymous 2016b
Parsons, A.(2007)</td><td>Measured</td></tr>
<tr><td>
<table>
<tr><th>Components</th><th>Vapor pressure</th></tr>
<tr><td>γ-Terpinene</td><td>103 Pa at 23.5°C[1]; 145 Pa at 25°C*</td></tr>
<tr><td>α-Terpinene</td><td>145 Pa at 25°C*</td></tr>
<tr><td>α-Terpineol</td><td>5.69 Pa at 23.5°C[1]; 5.64 Pa at 25°C*</td></tr>
<tr><td>α-Terpinolene</td><td>99 Pa at 23.5°C[1]; 222 Pa at 25°C**</td></tr>
<tr><td>α-Pinene</td><td>544 Pa at 23.5°C[1]; 633 Pa at 25°C*</td></tr>
<tr><td>p-Cymene</td><td>219 Pa at 25°C*</td></tr>
<tr><td>Limonene</td><td>222 Pa at 23.5°C[1]; 192 Pa at 25°C*</td></tr>
<tr><td>Aromadendrene</td><td>5.27 Pa at 25°C**</td></tr>
<tr><td>δ-Cadinene</td><td>2.51 Pa at 25°C**</td></tr>
<tr><td>Sabinene</td><td>981 Pa at 25°C**</td></tr>
<tr><td>Globulol</td><td>0.00495 Pa at 25°C**</td></tr>
<tr><td>Viridiflorol</td><td>0.00495 Pa at 25°C**</td></tr>
<tr><td>Ledene</td><td>2.72 Pa at 25°C**</td></tr>
</table>
[1] Experimental value from a literature study
*From Episuite v4.11, experimental
** From Episuite v4.11, calculated
</td><td>Li, J., Perdure, E.M. et al., (1998)

Anonymous 2018</td><td>Measured, estimated</td></tr>
<tr><td>The maximum vapour pressure of tea tree oil:
2100 Pa at 25°C
(vapour pressure readings of the samples with time show a decrease, thus the maximum reading from samples as the maximum vapour pressure of the test substance was reported)</td><td>ECHA dissemination site[8]</td><td>Measured (EU A.4)</td></tr>
<tr><td rowspan="2">Surface tension</td><td>45.6 mN/m for 1% w/v aqueous solution at 20°C
28.4 mN/m for neat Tee Tree Oil at 20 °C</td><td>Parsons, A. (2007)</td><td>Measured</td></tr>
<tr><td>51.0 mN/m of 90% saturated aqueous solution of tea tree oil at 20°C
55.0 mN/m of 1g/L aqueous solution of tea tree oil at 20°C</td><td>ECHA dissemination site[9]</td><td>Measured (EU A.5)</td></tr>
<tr><td rowspan="3">Water solubility</td><td>1,8-Cineole Solubility in water at 20°C:
2.76 g/L (pH = 5.2)</td><td>Anonymous 2016c</td><td>Measured</td></tr>
<tr><td>Terpinen-4-ol Solubility in water at 20°C:
3.28 g/L (pH = 5.85)</td><td>Parsons, A., (2007)</td><td>Measured</td></tr>
<tr><td>
<table>
<tr><th>Components</th><th>Solubility in water [mg/L]</th></tr>
<tr><td>α-Pinene</td><td>2.53 at 23.5 °C [1]</td></tr>
<tr><td>Limonene</td><td>6.32 at 23.5 °C [1]</td></tr>
<tr><td>γ-Terpinene</td><td>8.68 at 23.5 °C [1]</td></tr>
<tr><td>Terpinolene</td><td>9.48 at 23.5 °C [1]</td></tr>
<tr><td rowspan="2">α-Terpineol</td><td>626.7 at 23.5 °C [1]</td></tr>
<tr><td>1980 *</td></tr>
<tr><td>p-Cymene</td><td>23.4 at 25 °C [2]</td></tr>
</table>
</td><td>Li, J. Perdure, E.M. et al., (1998)
Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C., (1980)
Anonymous 2018</td><td>Measured, estimated</td></tr>
</table>

[6] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/4
[7] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/5
[8] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/7
[9] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/11

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>
<table>
<tr><td>α-Terpinene</td><td>8.68 *</td></tr>
<tr><td>1,8-Cineole</td><td>3500*</td></tr>
<tr><td>Aromadendrene</td><td>0.07057**</td></tr>
<tr><td>δ-Cadinene</td><td>0.04863**</td></tr>
<tr><td>Sabinene</td><td>2.494**</td></tr>
<tr><td>Globulol</td><td>11.98**</td></tr>
<tr><td>Viridiflorol</td><td>11.98**</td></tr>
<tr><td>Ledene</td><td>0.07057**</td></tr>
</table>
[1] Experimental value from the literature study Li, J. Perdure, E.M. et al., 1998
[2] Experimental value from the literature study Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C., 1980
*From Episuite v4.11, experimental database match
** From Episuite v4.11, estimated at 25°C</td><td></td><td></td></tr>
<tr><td></td><td>tea tree oil: 1420mg/L
Alpha-terpinene: 1.7 mg/l
p-cymene: 3.0 mg/l
Cineole: 73 mg/l
Gamma-terpinene: 3.0 mg/l
Terpinen-4-ol: 1140 mg/l
Alpha-terpineol: 87 mg/l at 20°C, pH=6.7</td><td>ECHA dissemination site[10]</td><td>Measured (EU A.6)</td></tr>
<tr><td>Partition coefficient n-octanol/water</td><td>Terpinen-4-ol
Log P_{OW} = 2.643 at 23.5°C and pH of 5.85.</td><td>Parsons, A. (2007)</td><td>Measured</td></tr>
<tr><td></td><td>
<table>
<tr><th>Components</th><th>Log Pow</th></tr>
<tr><td>Terpinen-4-ol</td><td>2.80[3]</td></tr>
<tr><td>α-Pinene</td><td>4.83[1];4.48[3]</td></tr>
<tr><td>Limonene</td><td>4.57[1];4.38[3]</td></tr>
<tr><td>γ-Terpinene</td><td>4.50[1];4.36[3]</td></tr>
<tr><td>Terpinolene</td><td>4.47[1];4.24[3]</td></tr>
<tr><td>α-Terpineol</td><td>2.98[1];3.28[3]</td></tr>
<tr><td>p-Cymene</td><td>6.34[2];;;4.10*</td></tr>
<tr><td>α-Terpinene</td><td>4.25[3]</td></tr>
<tr><td>1,8-Cineole</td><td>2.74[3]</td></tr>
<tr><td>Aromadendrene</td><td>6.13**</td></tr>
<tr><td>δ-Cadinene</td><td>6.32**</td></tr>
<tr><td>Sabinene</td><td>4.69**</td></tr>
<tr><td>Globulol</td><td>4.63**</td></tr>
<tr><td>Viridiflorol</td><td>4.63**</td></tr>
<tr><td>Ledene</td><td>6.18**</td></tr>
</table>
[1] Experimental value from the literature study Li, J. Perdure, E.M. et al., 1998
[2] Experimental value from the literature study Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. 1980
[3] Experimental value from the literature study Griffin, S.; Wyllie, S.G. & Markham, J., 1999
*From Episuite v4.11, experimental database match
** From Episuite v4.11, estimated</td><td>Li, J. Perdure, E.M. et al., 1(998)

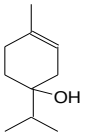
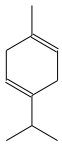
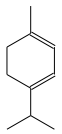
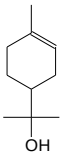
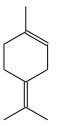
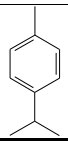
Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. (1980)

Griffin, S.; Wyllie, S.G. & Markham, J., (1999)</td><td>Measured, estimated</td></tr>
</table>

[10] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/9

Property	Value	Reference	Comment (e.g. measured or estimated)
	The range of partition coefficient values for Tea Tree Oil was found to be 2510 to 314000 (logPow= 3.4 to 5.5) at 30°C. Alpha-terpineol: logPow = 3.4 Terpinen-4-ol: logPow = 3.5 Alpha-terpinene: logPow = 5.2 Gamma-terpinene: logPow = 5.3 The effect of pH on the partition coefficient was not assessed since the test substance does not possess a dissociation constant in environmental pH ranges.	ECHA dissemination site[11]	Measured (EU A.8)
Flash point	55°C at 1007 hPa	Parsons, A. (2007)	Measured
	54°C at 1022 hPa	ECHA dissemination site[12]	Measured (EU A.9) closed cup method
	55°C at 1021 hPa	ECHA dissemination site[13]	Measured (EU A.9) Equilibrium method
Flammability	Auto-ignition temperature is 269°C at 1008 hPa	Parsons, A. (2007)	Measured
Explosive properties	No likely or realistic possibility of this substance being an explosive hazard.	Parsons, A. (2007)	Reasoned case
Self-ignition temperature	Auto-ignition temperature is 269°C at 1008 hPa.	Parsons, A. (2007)	Measured
	The auto-ignition temperature of Tea Tree Oil: 252°C at 1020 hPa	ECHA dissemination site[14]	Measured (EU A.15) auto-ignition temperature-liquids
Oxidising properties	No likely or realistic possibility of this substance being an oxidation hazard.	Parsons, A. (2007)	Reasoned case
Granulometry	not relevant		
Stability in organic solvents and identity of relevant degradation products	Tea Tree Oil: Solubility at 20°C: n-Heptane: > 25 – 29 g/L Acetone: ≥ 500 g/L Dichloroethane: ≥ 500 g/L Ethyl acetate: ≥ 500 g/L Methanol: ≥ 500 g/L p-Xylene: 50 – 57 g/L	Anonymous2016d	Measured

[11] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/8
[12] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12
[13] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12/?documentUUID=4e36aeff-be3a-44cc-a78d-934d00ed43c2
[14] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/13

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>Terpinen-4-ol:
Solubility at 20°C:
n-Heptane: > 250 g/L
Acetone: > 250 g/L
Dichloroethane: > 250 g/L
Ethyl acetate: > 250 g/L
Methanol: > 250 g/L
p-Xylene: > 250 g/L</td><td>Parsons, A. (2007)</td><td>Measured</td></tr>
<tr><td>Dissociation constant</td><td>
<table>
<tr><th>Name</th><th>Structural formula</th><th>Findings, Guideline, Method, Comments</th></tr>
<tr><td>Terpinen-4-ol</td><td>  </td><td>No dissociation constant available in data bases. No acidic hydrogen present in the molecule and dissociation not considered relevant at acid to slightly alkaline pH values.</td></tr>
<tr><td>γ-Terpinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Terpinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Terpineol</td><td>  </td><td>No dissociation constant available in data bases. No acidic hydrogen present in the molecule and dissociation not considered relevant at acid to slightly alkaline pH values.</td></tr>
<tr><td>α-Terpinolene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Pinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>p-Cymene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
</table>
</td><td>--</td><td>Statement based on the structure</td></tr>
</table>

Property	Value	Reference	Comment (e.g. measured or estimated)
Viscosity	Kinematic viscosity of tea tree oil: 2.86 mm2/s at 20°C 1.71 mm2/s at 40°C Dynamic viscosity: 2.54 mPa/s at 20°C 1.52 mPa/s at 40°C	ECHA dissemination site[15]	Measured (OECD TG 114) using a reverse flow viscometer
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	see Volune 3 B.2 (AS) for full information		

2.2.1.1 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
Reasoned case	No likely or realistic possibility of this substance being an Explosive Hazard	-	Parsons, A. (2007)

[15] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/23

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

As all the ingredients are, as natural products, clearly substances of long standing which have for a long time been used on a large scale, with no recorded problems in respect of explosivity, it would suggest that it is extremely unlikely to pose any threat from this perspective.
Reference to the Safety Literature (e.g. Sax[16], Bretherick[17] etc.) shows no suggestions of any explosive hazards associated with any of the materials present in the oil and the chemical structures of the compounds concerned do not contain any of the groups more likely to lead to explosivity problems (i.e. groups such as Nitrate, Perchlorate, Pierate etc.).
A sample of the product was subjected to Differential Scanning Calorimetry on a Perkin Elmer, Pyris 6 DSC. The sample was examined over the range 30°C to 400°C, programmed at a rate of 10°C/min and two replicate runs were carried out.
It was found that there were no significant exothermic events that occurred during this test, which would indicate that it is very unlikely that a thermally induced explosive reaction is likely to occur with this material.

It is therefore no likely or realistic possibility of this material being an explosive hazard.

2.2.1.1.1.2 Comparison with the CLP criteria

The substance does not meet the CLP criteria for classification for this hazard class.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

The substance does not have explosive properties. Data is conclusive but not sufficient for classification.

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

Hazard class not applicable: The substance is a liquid.

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

Hazard class not applicable: The substance is a liquid.

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

Hazard class not applicable: The substance is a liquid.

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

Table 3: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A9- Flash point	55°C at 1007 hPa	-	Parsons, A. (2007)
EU A.9 – flash point Equilibrium method	55°C at 1021 hPa	-	ECHA dissemination site[18]
EU A.9 – flash point Closed cup method	54°C at 1022 hPa	-	ECHA dissemination site[19]

[16] Sax's Dangerous Properties of Industrial Materials, John Wiley & Sons, 2004, ISBN: 9780471476627
[17] Bretherick's Handbook of Reactive Chemical Hazards, Academic Press, 2006, ISBN: 9780123725639
[18] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12/?documentUUID=4e36aeff-be3a-44cc-a78d-934d00ed43c2
[19] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

The Flash Point of one of the submitted specimens of Tea Tree Oil was determined, in duplicate according to EC Method A.9 and using a Pensky-Martens, Closed Cup Flashpoint Apparatus equipped with an IP15C, -5 to 110°C Thermometer.
The results obtained were as follows :
a) 56°C
b) 55°C
The Atmospheric Pressure at the time of the determinations was 1007 hPa. Hence, the Flash Point of the Tea Tree Oil, being the lowest temperature at which the vapour from the material ignites, is 55°C at 1007 hPa.
Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the flash point of Tea Tree Oil. The study followed the requirements of EEC method A9 without significant deviation. The flash point of Tea Tree Oil was found to be 55.0°C and 54.0°C in duplicate tests using the equilibrium method and a Mensky-Martens closed tester, respectively.

2.2.1.1.5.2 Comparison with the CLP criteria
The substance has to be classified as flammable liquid in the category 3 according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments) since its flash point is ≥ 23°C and ≤ 60°C.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids
The substance has to be assigned to the category 3 for flammable liquids. Labelling proposed is **H226- Flammable Liquid.**

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

Hazard class not applicable: The substance is a liquid.

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

Table 4: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Differential Scanning Calorimetry	No significant exothermic events	-	Parsons, A. (2007)
EC A15- Autoflammability	269°C	-	Parsons, A. (2007)
EC A15- Autoflammability	252°C at 1020 hPa	-	ECHA dissemination site[20]

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

A sample of the product was subjected to Differential Scanning Calorimetry on a Perkin Elmer, Pyris 6 DSC. The sample was examined over the range 30°C to 400°C, programmed at a rate of 10°C/min and two replicate runs were carried out.
It was found that there were no significant exothermic events that occurred during this test, which would indicate that it is very unlikely that a thermally induced, explosive reaction is likely to occur with this material.
The testing was carried out with a modified, temperature programmed, Gas Chromatograph Oven. A Portec Type K, PI 8013, Electronic Thermometer and an RS Ltd., Digital Timer.
The following results were obtained :
The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 hPa.Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the auto-ignition temperature of Tea Tree Oil. The study followed the requirements of EEC method A15 without significant deviation. The auto-ignition temperature of Tea Tree Oil was found to be 252°C.

2.2.1.1.7.2 Comparison with the CLP criteria

The substance does not meet the CLP criteria for classification for this hazard class.

[20] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/13

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Not a self-reactive substance. Data is conclusive but not sufficient for classification.

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

Table 5: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
EC A.15- Autoflammability	269°C	-	Parsons, A. (2007)

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

The testing was carried out with a modified, temperature programmed, Gas Chromatograph Oven containing the flask etc. A Portec Type K, PI 8013, Electronic Thermometer and an RS Ltd., Digital Timer.
The following results were obtained :
The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 hPa.

2.2.1.1.8.2 Comparison with the CLP criteria

The substance does not ignite within 5 min when added to an inert carrier and exposed to air, nor does it ignite or char a filter paper on contact with air within 5 min.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

The substance is no pyrophoric liquid. Data is conclusive but not sufficient for classification.

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

Hazard class not applicable: The substance is a liquid.

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

Table 6: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A15- Autoflammability	269°C	-	Parsons, A. (2007)
EC A15- Autoflammability	252°C at 1020 hPa	-	ECHA dissemination site

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 hPa.
Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the auto-ignition temperature of Tea Tree Oil. The study followed the requirements of EEC method A15 without significant deviation. The auto-ignition temperature of Tea Tree Oil was found to be 252°C.

2.2.1.1.10.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not a self-heating substance. Data is conclusive but not sufficient for classification.

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

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

Based on the chemical structure of the substance and the experience in manufacture and handling, the substance does not react with water. Thus, a study does not need to be conducted according to Regulation (EC) No 1272/2008, Annex I, part 2 (2.12.4.1).

2.2.1.1.11.2 Comparison with the CLP criteria

Not required.

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

Hazard class not applicable.

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

Table 7: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Reasoned case	No likely or realistic possibility of this substance being an Oxidation Hazard.	-	Parsons, A. (2007)

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

From a consideration of each of the individual ingredients of the substance there is no indication to suggest that either alone or in combination, they are likely to be an oxidation hazard.
As also all the ingredients are products of long standing which have been used on a large scale, with no recorded problems in respect of an oxidation hazard, it would suggest that it is extremely unlikely to pose any threat from this perspective.
The following points also apply when considering the possibility of this formulation constituting an Oxidation hazard:
a) In general, Oxidizing Properties are not expected if an organic molecule does not contain oxygen, chlorine or fluorine at all, or if, as in this case, the molecules of the major constituents of the Tea Tree Oil, either do not contain any oxygen or, if they do, that it is chemically bonded to carbon or hydrogen only.
b) None of the major ingredients of the Tea Tree Oil contains a chemical group that might indicate the potential presence of oxidizing properties (e.g. peroxide, chlorate, perchlorate, nitrate, bromate, chromate etc.)
c) For an organic chemical, a very important factor in deciding whether that system might be an oxidation hazard, is the so-called, 'Oxygen Balance'. This is the difference between the oxygen content of the actual compound(s) and that required to fully oxidise the Carbon, Hydrogen and other oxidizable elements present in it, to carbon dioxide, water, etc. If there is a large deficiency of oxygen present, as there is with all the major compounds present (i.e. Terpinen-4-ol, Cineole, alpha-Terpineol, gamma-Terpinene, p-Cymene, alpha-Terpinene), then the balance is said to be negative meaning it is less likely that this compound/mixture will be an oxidising agent.
d) In the Differential Scanning Calorimetry experiments carried out earlier in connection with the explosive properties, there were no indications of any significant reactions when the material was heated up to 400°C, which would again tend to indicate that the material was stable and unlikely to react readily with other materials and that as such was unlikely to form an oxidising hazard.
It is therefore our considered opinion from all the above, that there is no likely or realistic possibility of this material being an oxidation hazard and that therefore, the actual oxidation properties testing should not be required.

2.2.1.1.12.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not an oxidising substance. Data is conclusive but not sufficient for classification.

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

Hazard class not applicable: The substance is a liquid.

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

Hazard class not applicable: The substance is not an organic peroxide.

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

No test data are available. However, based on the experience in manufacture and handling the substance does not materially damage metallic containers.

2.2.1.1.16 Desensitized explosives *[equivalent to section 8.16 of the CLH report template]*

The formulation does not contain substances to suppress or reduce their explosive properties.

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

The appearance of the product Timorex Gold (BM 608) is that of a light yellow-brown liquid, with a characteristic odour. It is not explosive, has no oxidising properties. The self-ignition temperature and flash point are 313°C and 39°C, respectively. Therefore, Timorex Gold fulfils the criteria of category 3 for flammability (Flam. Liq. 3, H226: Flammable liquid and vapour) according to Regulation (EC) 1272/2008. In aqueous solution (1%w/v), it has a pH value of 9.4. The surface tension, persistent foam, emulsifiability, re-emulsifiability, emulsion stability all meet the acceptable criteria. The formulation BM 608 is considered as a surface-active material. The stability data indicate a shelf life of at least 2 years at ambient temperature and an accelerated storage at 54°C for 2 weeks in the commercial packaging (HDPE), with no significant loss of active substance content. Its technical characteristics are acceptable for an Emulsifiable Concentrate. However, low temperature (at 0°C) stability study was not performed for Timorex Gold. According to Commission Regulation (EU) No 284/2013 the effect of low temperatures on stability shall be determined and reported for liquid plant protection products.

2.3 DATA ON APPLICATION AND EFFICACY

The results of 15 trials conducted with BM 608 Timorex Gold throughout Europe during the period 2015 to 2017 representing relevant locations for the Mediterranean EPPO zone according to EPPO Standard PP1/241 (1). On the basis of submitted data it can be concluded that Timorex Gold can be used for the control of powdery mildew in vineyard and tomato with a maximum dose rate of 1.5 L/ha and for the control of grey mold in vineyard and tomato with a maximum dose rate of 2.0 L/ha; both with a maximum number of 4 applications. The field treatments can be applied along crop cycle from spring till autumn, and also the greenhouse uses can be performed along crop cycle.

2.3.1 Summary of effectiveness

The active substance of BM- 608 (Timorex Gold) is a Tea tree plant extract. According to the Fungicide Resistance Action Committee (FRAC) this active substance is an organic oil and cannot be classified by its mode of action (MoA); therefore, it belongs the Cell Membrane Disruption Group 46, Target Site Group F7. Resistance is not known for the members of this group. Compared to the chemical fungicides, which show already a strong development of resistance to powdery mildew and grey mold, Tea tree oil (plant extract of Melaleuca alternifolia) is an effective tool for the control of these target diseases as part of an integrated pest management program. The actual resistance risk of Tea tree oil is regarded low.

2.3.2 Summary of information on the development of resistance

To be filled

2.3.3 Summary of adverse effects on treated crops

There is no evidence that BM- 608 (Timorex Gold) has any effect on the quality of plants or plant products or transformation processes. It is generally considered that there are no negative effects on the yield of tomato and grapes when the product is applied at the recommended rates. In all trials assessed for yield effects, no significant reductions in yield were observed. Assessments for phytotoxic effects of BM 608 were made directly in the efficacy trials. In the efficacy trials in tomato only one phytotoxic effect could be observed in one trial (field), but this was of no of statistical significance.

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

Impact on succeeding crops
Based on the absence of any adverse effects in typical cropping situations, it was concluded that the fungicide BM 608 (Timorex Gold) poses no risk to succeeding crops. There is no waiting period or other precautions between last application and sowing or planting succeeding crops; there is no limitation on choice of succeeding crops

Impact on adjacent crops
It is considered that BM 608 (Timorex Gold) has no herbicidal activity. Furthermore, the crop safety following application is well proven for many years and established through commercial use of Tea tree oil (plant extract of *Melaleuca alternifolia)* on several crops. Therefore, it is concluded that BM 608 (Timorex Gold) is not expected to have any harmful impact on other crops.

Impact on seed viability
Not relevant, no use for propagation is intended.

Impact on beneficial and other non-target organisms
No effects on beneficial or other non-target species were reported in any of the field trials carried out to assess the effectiveness of BM 608 (Timorex Gold). For summary of results of studies performed with non-target species and the risk assessment, please refer to point 2.9 of this document.

2.4 FURTHER INFORMATION

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

Handling: Avoid contact with skin and eyes. Ventilation required. When handling, wear suitable protective clothing.
Keep away from ignition sources - Do not smoke. Protect against electrostatic charges.

Storage: Keep only in the original container. Keep container tightly closed in a cool, dry, well-ventilated place away from direct sunlight and ignition/heat sources

Transport

UN number:	1993
ADR Class:	3
OMI/IMDG Class:	3
Packaging group:	III
Marine Pollutant	Yes
UN proper shipping name:	Flammable Liquid. N.O.S , (Tea Tree Oil, Ethanol)

Fire:

Extinguishing media:
Foam, carbon dioxide (CO_2), dry chemical. Avoid using water jet

Special hazards arising from the substance:
Fire and explosive hazards: Flash point: 39°C; Flash back may occur a long vapor trail. Vapours may form explosive mixture with air.
Hazardous thermal (de)composition products: Carbon oxides.

Advice for fire-fighters:
Use breathing apparatus with independent air supply. Cool containers at risk with water spray jet.

2.4.2 Summary of procedures for destruction or decontamination

Package the waste and contact the local authorities to make sure that the waste will be led to controlled incineration or safe waste disposal according to the official regulations.
The pyrolytic behaviour of the active substance does not need to be reported as the content of halogens of the active substance in the preparation is <60%.
In addition, the effectiveness of cleaning procedure was conducted and resulted to a removal of 97.7 % w/w Tea Tree Oil after 'triple rinse procedure without tank-cleaner'
No other methods of safe disposal than controlled incineration are proposed.

2.4.3 Summary of emergency measures in case of an accident

First aid measures:
General notes:
Remove victim from area of exposure. Wash off remaining material with plenty of water.
Inhalation:
Remove victim to fresh air. If breathing is difficult: artificial respiration. Get medical attention.
Skin contact:
Remove contaminated clothing. Wash away remainder with water and soap.
Eye contact:
Wash out with water with the eyelid held wide open for at least 15 minutes. Get medical attention.

Ingestion:
Wash out mouth with plenty of water. Get medical attention. Never give anything by mouth to an unconscious person.

Accidental release measures:

Personnel precautions:
Wear suitable protective clothing, protective gloves and tightly sealed goggles
Environmental precautions:
Prevent spills to reach any water course, surface and ground water. In case of leakage to water course inform the respective authorities.
Methods and material for containment and cleaning up:
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Dispose contaminated material as waste according to the local waste regulation authority. Ensure adequate ventilation. Do not flush with water or aqueous cleansing agents.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1 Analysis of the active substance as manufactured

The content of the active ingredient in the Tea Tree Oil Technical was determined by means of GC-FID analytical method using external standardization. The samples were diluted in ethyl acetate. The quantification of the 15 components were performed by a relevant calibration function generated over a suitable concentration range.
The analytical method for quantitation of the 15 components of technical Tea Tree Oil using GC-FID is considered fully validated in accordance with EU Guidance SANCO/3030/99 rev. 4. with respect to linearity, accuracy, precision and specificity.

2.5.1.2 Formulation analysis

The content of the active ingredient in the product BM 608 (Timorex Gold) was determined by means of GC-FID method. The quantitation of Tea Tree Oil is performed by a combination of external standard and internal standard based on the selected markers components of Tea Tree Oil.
The analytical method for determination of the content of Tea Tree Oil (sum of the following components of TTO: α-Terpinene, γ Terpinene, p-Cymene, 1,8-Cineole and Terpinen-4-ol) in Timorex Gold (BM 608) using GC-FID is considered fully validated in accordance with EU Guidance SANCO/3030/99 rev. 4. with respect to linearity, accuracy, precision and specificity.

2.5.1.3 Methods for Risk Assessment

Plants and plant products
For the determination of residues during field trial studies in grape and tomato, a GC/MS and GC/MS/MS methods were successfully developed and validated to measure the content of the representative components of Tea Tree Oil in samples of tomato and grape, which were extracted with ethyl acetate. The lowest limit of quantification achieved was 0.01 mg/kg.
The analytical methods for determination residues of components of Tea Tree Oil in tomato and grape using GC/MS and GC-MS/MS are considered validated in accordance with requirements of Guidance SANCO/825/00 rev 8.1 and SANCO/3030/99 rev. 4 with respect to calibration, recovery and repeatability and selectivity.

Food of animal origin
No new analytical method for risk assessment was necessary or was conducted or validated.

Soil
Within the aerobic soil degradation studies of the Tea Tree Oil components in soil, a GC-MS analytical method was developed and validated for the quantification of the components of Tea Tree Oil (γ-Terpinene, p-Cymene, 1,8-Cineole, (+)-Aromadendrene and (-)-Globulol) in soil. After extraction with Acetonitrile/water (80/20, v/v) and further with acetone from the soil samples, the combined extracts were analysed for the test item residues. The limit of quantification (LOQ) was determined to be 0.044 mg/kg. The method is considered fully validated in accordance with requirements of Guidance SANCO/3030/99 rev. 4 with respect to linearity, accuracy (mean recovery between 70% and 110%), precision (5 determinations at each fortification level, RSD≤20%), specificity and LOQ.

Water and sediment
During the water/sediment study, a GC-MS analytical method was developed and validated for the quantification of the components of Tea Tree Oil (γ-Terpinene, p-Cymene, 1,8-Cineole, (+)-Aromadendrene and (-)-Globulol) in water and sediment. After extraction with Acetonitrile/water (80/20, v/v) and further with acetone from the samples, the combined extracts were analysed for the test item residues.
For γ-Terpinene, p-Cymene and 1,8-Cineole, the limit of quantification (LOQ) was determined to be 3.7 µg in sediment and 7.4 µg/L in water /. The LOD was set to 20% of the LOQ (LOD = 0.7 µg in sediment and 1.4 µg/L in water).
For (-)-Globulol, the limit of quantification (LOQ) was determined to be 25 µg in sediment and 50 µg/L in water /. The LOD was set to 20% of the LOQ (LOD = 4.72 µg in sediment and 9.44 µg/L in water).
For (+)-Aromadendrene, the limit of quantification (LOQ) was was determined to be 50 µg in sediment and 100µg/L in water./. The LOD was set to 20% of the LOQ (LOD = 9.46 µg in sediment and 18.9 µg/L in water).

The method is considered fully validated in accordance with requirements of Guidance SANCO/3030/99 rev. 4 with respect to linearity, accuracy (mean recovery between 70% and 110%), precision (5 determinations at each fortification level, RSD≤20%), specificity and LOQ.

Air
In the context of risk assessment, an analytical method for the determination of the actual concentration of Tea Tree Oil (based on its representative components) in the test chamber atmosphere applied for the acute inhalation toxicity study of the formulation BM 608 was validated. The method was based on GC-FID with a LOQ of 3.1993 µg/mL, 4.7827 and 12.2449 µg/mL for α-Terpinene, γ-Terpinene and Terpinen-4-ol, respectively.
In the context of risk assessment, an analytical method for the determination of Tea Tree Oil in air was validated to monitor the operator and worker inhalation exposure. The method is based on GC-MS/MS. A LOQ of 1 µg/specimen i.e. 1 µg/tube is achieved.
The methods are considered fully validated in accordance with requirements of Guidance SANCO/3030/99 rev. 4 with respect to linearity, accuracy, precision and specificity.

Body fluids and tissues
No analytical method for risk assessment was necessary or was conducted or validated.

2.5.2 Methods for post control and monitoring purposes

Plants and plant products
No analytical method for monitoring purposes was necessary or was conducted or validated.

Food of animal origin
No analytical method for monitoring purposes was necessary or was conducted or validated.

Soil
No analytical method for monitoring purposes was necessary or was conducted or validated.

Water
No analytical method for monitoring purposes was necessary or was conducted or validated.

Air
For the determination of Tea Tree Oil in air a GC/MS/MS analytical method was successfully developed and validated according to the guidance documents SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The limit of quantification was 1 µg/specimen, i.e. 1 µg/tube.

Body fluids and tissues
No analytical method for monitoring purposes was necessary or was conducted or validated

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]*

No ADME studies were available for Tea Tree Oil. However, since the major part of the terpene components of TTO are commonly occurring compounds found in many plants, there is extensive data and publicly available literature for these components available. Thus, for the AIR-4 renewal process of TTO, no specific ADME study was provided for TTO in order to avoid further animal testing.
An extensive literature search for TTO and its single components was performed according to EFSA Journal 2011; 9(2):2092.
Several publications were found for the single terpene components of TTO, most for 1,8-Cineole, p-Cymene, d-Limonene, and α-Pinene. Few for α-terpineol, γ-Terpinene, Terpinen-4-ol and δ-Cadinene. During literature search, no study was found for Sabinene, Aromadendrene, Ledene, Globulol and Viridiflorol.

Besides being naturally present in plants, most of the terpene components of TTO were globally used as fragrances and flavouring additives in consumer products. The health effects and risk for consumers has already been assessed by the International Agency for Research and Cancer (IARC/WHO) and the European Food Safety Authority (EFSA) (for reference details please refer to DRAR, Volume 3, B.6 (AS))).

Thereby, terpenes were allocated to different chemical groups according to their chemical structure and risks were assessed for group members based on available data for supporting substances of similar chemical structure. For consumer risk assessment, information on ADME is crucial and was therefore also evaluated within these official documents (DRAR, Volume 3, B.6 (AS))
For the purpose of characterization of ADME of TTO, these official evaluations serve as a base for the following assessment. Those terpenes which were not part of the evaluation by EFSA/WHO itself were allocated by the notifier to the respective chemical groups according to their chemical structure.
Due to the structural similarity to the already evaluated substances (same or no additional functional groups) no different metabolic pathways as described for the evaluated substances are expected. The following grouping was performed:

	Chemical group			
	Aliphatic and alicyclic hydrocarbons	**Aromatic hydrocarbons**	**Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances**	**Alicyclic ethers**
Terpenes evaluated under WHO/EFSA	<u>monocyclic</u> • d-Limonene • α-Terpinene • γ-Terpinene • α-Terpinolene <u>bicyclic</u> • α-Pinene • δ-cadinene	• p-Cymene	• α-terpineol • Terpinen-4-ol	• 1,8-Cineole
Terpenes grouped according to their chemical structure	• Sabinene • Aromadendrene • Ledene	--	• Globulol • Viridiflorol	--
Reference	EFSA J. 4053 EFSA J. 4067 EFSA J. 931 EFSA J. 918 WHO TRS 928	EFSA J. 931	WHO TRS 891	WHO TRS 922 EFSA J. 639, EFSA J. 3092

Additionally, to those information provided by EFSA/WHO, further peer reviewed literature studies, which were not used within the EFSA/WHO evaluations, were assessed to broaden information on ADME of the TTO terpene components. A large number of open literature studies is available. Main focus was set on absorption, distribution, elimination and identification of metabolites in animals and humans. Furthermore, special focus was also set on inhalative absorption since TTO and the vast majority of its components were highly volatile

In the following, an overview table is given for ADME of the different terpenes belonging to the above mentioned different chemical groups.

Table 8: Toxicokinetic properties and metabolism of TTO components

Chemical class of terpenes	Terpenes evaluated within the chemical group	Absorption, Distribution and Excretion	Metabolism
Aliphatic and alicyclic hydrocarbons	Monocyclic: • d-Limonene • α-Terpinene • γ-Terpinene • α-Terpinolene Bicyclic: • α-Pinene • δ-Cadinene • Sabinene Tricyclic: • Aromadendrene • Ledene	Being lipophilic, the aliphatic and alicyclic hydrocarbons in this group are likely to cross biological membranes by passive diffusion. After oral, dermal and inhalation exposure, they are rapidly absorbed, distributed to lipophilic body tissues and extensively metabolized. Elimination from blood follows a triphasic pattern, with a slow terminal phase. Elimination occurs mainly via urinary excretion in the form of conjugated polar metabolites; only small amounts are excreted via faeces or by exhalation.	On the basis of the available data, it is anticipated that all the aliphatic and alicyclic hydrocarbons in this group will participate in similar pathways of metabolic detoxification in mammals, including humans. After absorption, these hydrocarbons are oxidized to polar oxygenated metabolites via cytochrome P450 (CYP) enzymes and alcohol and aldehyde dehydrogenases. The aliphatic and alicyclic substances are oxidized either by side-chain oxidation or by epoxidation of an exocyclic or endocyclic double bond. Alkyl oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine. If a double bond is present, epoxide metabolites may form and these metabolites are detoxified either by hydrolysis to yield diols, or by conjugation with glutathione.
Aromatic hydrocarbons	• p-Cymene	p-Cymene is rapidly and well absorbed following oral and dermal administration, or exposure by inhalation. After absorption, p-Cymene was immediately distributed in body tissues. Excretion was rapidly and nearly complete within 48 h.	*p*-Cymene undergoes extensive oxidation of the methyl substituent and isopropyl side-chain to yield polar oxygenated metabolites. Oxidation on the carbons of the benzene ring was seldom. A large percentage of the urinary metabolites was conjugated both to glucuronic acid and glycine.
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally	• α-Terpineol • Terpinen-4-ol • Globulol • Viridiflorol	Alicyclic tertiary terpenoid alcohols are rapidly absorbed after oral administration and inhalation exposure. In humans and animals, terpenoid tertiary alcohols are conjugated with glucuronic	Unsaturated terpenoid alcohols may undergo allylic oxidation to form polar diol metabolites, which may be excreted either free or conjugated. If the diol contains a primary alcohol function, it may under-go further oxidation to the

Chemical class of terpenes	Terpenes evaluated within the chemical group	Absorption, Distribution and Excretion	Metabolism
related substances		acid and are excreted in the urine and faeces	corresponding carboxylic acid. Metabolic oxidation is mediated by CYP450 enzymes.
Alicyclic ethers	• 1,8-Cineole	In humans and animals, 1,8-Cineole is rapidly absorbed after oral and inhalation exposure, extensively metabolized and eliminated by conjugation to polar metabolites. Elimination occurs in a biphasic pattern within a few hours. There are no indications for tissue accumulation. 1,8-Cineol is further excreted by exhalation. 1,8-Cineole and its metabolites are able to cross the blood-milk-barrier.	In humans and animals, 1,8-Cineole, has been shown to be oxidised via P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated and excreted or further oxidised and excreted. 1,8-Cineole principally undergoes ring-hydroxylation to form amongst others 2-or 3-hydroxy-1,8-Cineole. Cleavage of the ether is, at most, a very minor metabolic pathway.

***ADME Summary*:**

- Based on ADME data of TTO constituents, TTO is metabolized and excreted from experimental animals within 2-3 days, mainly via urine (d-limonene in Wistar rats cleared within 48 hours).
- There is no evidence of bioaccumulation due to major biotransformation reactions taking place in the liver and to a lesser extent in other organs.
- Due to the structural similarity to the already evaluated substances (same or no additional functional groups) no different metabolic pathways as described for the evaluated substances are expected; metabolism of the components is comparable. Therefore it can be concluded that no dangerous/non-toxic metabolites are synthetized.

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]

The acute oral toxicity of Tea Tree Oil has been assessed in rats.

Table 9: 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}	Reliability score	Reference
Acute oral toxicity study in rats OECD 425 (2008) (Acute Oral Toxicity Up-and-Down Procedure) GLP	Rat, Wistar Hsd Han: females, 3/group	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO specification)	550 mg TTO/kg (Group 1) and 2000 mg TTO/kg (Group 2) 14 days	1049 mg/kg bw <u>Clinical signs:</u> 550 mg TTO/kg: no clinical signs or mortality 2000 mg TTO/kg: Hypoactivity, slight tremors, recumbency, death on day 1 – 2 after dosing.	1	Anonymous 2015a

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reliability score	Reference
Acute oral toxicity of Tea Tree Oil in the rat OECD 401 GLP not stated	Rat, Sprague Dawley SPF rats - Specific Pathogen-free and non-SPF-rats males, females, 5/group	Tea Tree Oil (no information on composition available)	3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) 14 days	2.6 mL/kg bw in SPF rats 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non-SPF rats Clinical signs: Surviving SPF and non-SPF rats: lack of tonus in the forelimbs, weeping eyes, bloodied noses.	2	Anonymous 1989a and ECHA disseminatio n site[21]
Acute oral toxicity in oral: gavage OECD Guideline 423; EU Method B.1; EPA OPPTS 870.1100 GLP	mouse (CRL:(NMRI) BR Mouse) female 3/group	Melaleuca alternifolia, ext., purity: 100% (complies with ISO 4730:2017specifitaion)	2000mg TTO/kg bw (Group 1) and 2000 mg TTO/kg bw (Group 2) Observation period: 14d	LD_{50}: >2000 mg/kg bw (female) based on: (test mat.)	1	ECHA disseminatio n site[22]

Literature studies

Open scientific literature search has been performed and some data on the acute oral toxicity of the Tea Tree Oil components have been found. The toxicity of tested monoterpenes is found to be comparable.
The following table presents all the data found for the individual components.

Table 10: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Observations	Reliability score	Reference
Acute oral / Rat	1,8-Cineole	LD_{50} = 1280 mg/kg > 100 mg/kg: Tremor, convulsion, abnormal gait and ataxia, increased respiration, decreased activity, unresponsiveness to writhing test, flaccid paralysis (leading to recumbency). Mortality latency: 1000 mg/kg: >6, <15 h 1600 mg/kg: >2, <10 h 2900 mg/kg: >2, <3 h Intestinal transit: 20-120 mg/kg: Slight and non-significant decrease in traversing in the small intestine.	2	Jalilzadeh-Amin *et al*. (2015)
Acute oral / Mice	1,8-Cineole	LD_{50} = 3849 mg/kg Lethal dose: Rapid cyanosis, stupor, irregular breathing, extreme sensitivity to noise and convulsions.	2	Xu *et al*. (2014)

[21] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2
[22]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2/?documentUUID=77b26afa-3b23-4d8a-a1a7-7c769040fa3c

Type of study/data	Test substance	Observations	Reliability score	Reference
		21.38 and 64.15 mg/kg: Central venous congestion of liver lobule, granular degeneration of hepatocytes. 192.45 mg/kg: Central venous congestion, granular degeneration, vacuolar degeneration and hepatic necrosis; distorted and fractured endoplasmic reticulum, ribosomes scattered into cytoplasm, swollen mitochondria with disorganized cristae. (liver, kidney) 64.15 and 192.45 mg/kg: Capillary of glomerulus and interstitial angiectasis, hyperemia, renal tubular epithelial cells swelling, granular degeneration and partially separating from basement membrane, amount of eosinophilic protein exudation existing in tubular lumen		
Acute oral / Rat	1,8-Cineole	1500 mg/kg < LD_{50} < 1750 mg/kg <u>Single dosing:</u> 1500 mg/kg: sedation, tremor, significant increase in consumption of food, water and body weight. 1750 and 2000 mg/kg: sedation, tremor, diarrhea, difficulty breathing and seizures, death within less than 24 h. <u>Repeated dosing:</u> 500 and 1000 mg/kg: diarrhoea and reduced body weight during the first week, followed by an increase until the end of treatment. <u>Hematology and biochemistry:</u> 500 and 1000 mg/kg: Significant increase of MCV and decrease in MCHC and MPV (males); increase in the level of urea (females). 100 mg/kg: Decrease in the level of alkaline phosphatase (males). <u>Morphology:</u> 500 and 1000 mg/kg: Decrease in absolute weigth of the lungs and spleen (males), lymphocytic infiltrate in the liver (females). 1000 mg/kg: increase in absolute and relative weight of liver (females), increase of glomerular space in kidneys. All doses: Eosinic and lymphocytic infiltrate in the lungs (males and females), in the liver (males) and into the uterus (females). <u>Reproductive toxicity:</u> All doses: Significant decrease in maternal weight gain during pre-implantation and organogenesis. 1000 mg/kg: Significant decrease in maternal weight gain during pregnancy, dead fetuses and reduction of the mass of fetuses. 250 mg/kg: Reduction in the number of corpora lutea.	2	Caldas *et al*. (2016)
Acute oral / Rat, Mice	γ-Terpinene	LD_{50} > 2000 mg/kg No sign of evident toxicity, no behavioural and clinical alterations. 12.5 and 25 mg/kg: Significant reduction of the licking time of pain-stimulated paw. ≤ 6.25 mg/kg: Significant inhibition of glutamate-induced nociception.	2	de Brito Passos *et al*. (2015)
Acute oral / Rat	Terpinen-4-ol (4-Carvomenthenol)	LD_{50} = 1300 mg/kg	4	RIFM Report

Type of study/data	Test substance	Observations	Reliability score	Reference
				number 1695 (1977)**
Acute oral / Rat	γ-Terpinene	LD_{50} = 3650 mg/kg	4	Moreno (1973b)*
Acute oral / Rat	α-Terpinene	LD_{50} = 1680 mg/kg	4	Moreno (1973a)*
Acute oral / Rat	α-Pinene	LD_{50} = 3700 mg/kg	4	Moreno (1972e*)
Acute oral / Rat	p-Cymene	LD_{50} = 4750 mg/kg	4	Jenner (1964)*
Acute oral / Rat	α-Terpinolene	LD_{50} = 3784 mg/kg	4	Brownleer (1940)*

*Cited in T.B. Adams et al./Food and Chemical Toxicology 49 (2011)2471-2494. The studies are published and were not available for reliability assessment.
** Cited in S. P. Bhatia *et al*. (2008), Food and Chemical Toxicology 46 (2008) 91-94.

Reliability statement: The literature studies from which the data listed in the above table have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information). Studies with reliability score 3 (not reliable) are only presented in section 11 (aquatic ecotoxicity). Studies attributed with a reliability score of 4 (not assignable) were not available for reliability assessment and are thus not relied upon.

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

Study 1, Anonymous 2015a, Tea Tree Oil: Acute oral toxicity study (Up-and-Down Procedure) in Wistar rats, OECD 425, GLP.

Reliability statement: The study is conducted in accordance with OECD 425. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the 2015 study, Tea Tree Oil, was tested for its potential acute hazard after a single oral administration at a dosage volume of 0.13 mL resulting in a target dose of 550 mg/kg for the group 1 and approx. 0.47 mL resulting in a target dose of 2000 mg/kg for the group 2. Six female Wistar rats were dosed sequentially by gavage, each group at 3 steps at a minimum of 48 hours as follow:
Each of the six female Wistar rats were sequentially dosed by gavage with Tea Tree Oil at a target dose as follow:
No mortality and clinical signs were observed in all the animals dosed with 550 mg TTO/kg bw (Group 1) throughout the entire 14-day observation period.
The rats dosed with 2000 mg TTO/kg bw (Group 2) exhibited clinical signs such as hypoactivity and slight tremors and died on day 1 or day 2.
All the survived rats gained weight during the 14-day observation period. The pre-terminal dead rats lost weight when compared to their initial body weight.
Based on the results obtained, the estimated acute oral LD_{50} of Tea Tree Oil is 1049 mg/kg bw. with 95% confidence interval of 550 to 2000 mg/kg body weight in female rats.
In accordance with the EC Directives on dangerous preparations 1272/2008, Tea Tree Oil is classified as acute oral Category 4 ($300 < ATE \leq 2000$).

Study 2, Anonymous 1989a, Acute oral toxicity of Tea Tree Oil in the rat, OECD 401.

Reliability statement: The study was accepted during previous evaluation and is conducted in accordance with OECD 401. However, it shows some deviations in terms of methodology and reporting, which were not deemed to have an influence on its overall scientific reliability. More deviations would become obvious when comparing

the study to the current test guidelines for oral toxicity studies, which show a significantly altered design. Overall, the study is therefore considered reliable with restrictions (reliability score: 2).
The acute oral toxicity of Tea Tree Oil was assessed according to the OECD Guideline no. 401.
Groups of 5 male and 5 female Sprague Dawley rats weighing between 146 and 219 grams received a single oral dose of 3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) as a suspension in peanut oil. The samples were diluted with peanut oil w/w at 3 different concentrations: 1/3, 1/4 and 1/5. The animals were fed on a diet of rat and mouse cubes and tap water *ad libitum*, and fasted for 24 hrs prior to treatment. The animals were observed during the experimental period of 14 days after treatment for mortality and signs of toxicity.
The LD_{50} for mortality was found to be 2.6 mL/kg bw in SPF rats and 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non- SPF rats, respectively.
Surviving animals showed lack of tonus in the forelimbs, weeping eyes and bloodied noses.
Based on data on ECHA dissemination site a GLP-compliant study was conducted in accordance with OECD Guideline 423 (acute toxic class method) to determine the acute oral toxicity of Tea Tree Oil to female CRL:(NMRI)BA mice. In this two-step study, three animals were dosed in the initial step with Tea Tree Oil (formulated in PEG 400) at a dose level of 2000 mg/kg bw, followed by an observation period of 14 days. In the absence of any mortalities, a confirmatory group of three animals was then tested at the same dose level. There were no mortalities or macroscopic findings related to treatment and no clear indications of effects on bodyweight. Clinical signs included decreased activity, hunched back position, incoordination, piloerection, decreased grip reflex, decreased respiratory rate and/or dyspnoea, none of which persisted beyond day 8 of treatment. In conclusion, under the conditions of this study, the acute oral LD_{50} of Tea Tree Oil was > 2000 mg/kg bw, when administered to female mice.
The literature studies show that the individual components of Tea Tree Oil do not lead to lower LD_{50} values and therefore support the allocation of Tea Tree Oil into the category 4 of the acute toxicity hazard.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

In accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments, Tea Tree Oil is classified as acute oral Category 4 ($300 < ATE \leq 2000$).

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

TTO is proposed to classify as Acute oral Category 4with hazard statement **H302- Harmful if swallowed**. Proposed $ATE_{oral\ acute}$ = 1049 mg/kg bw/d.

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

The acute dermal toxicity of Tea Tree Oil was tested in male and female rats and rabbits. The new study from 2015 was conducted with rats.

Table 11: 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}	Reliability score	Reference
Tea Tree Oil: Acute dermal toxicity study in Wistar rats OECD 402 (1987) GLP	Rats, Wistar male, female, 2 groups, 5/sex	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO-specification)	Undiluted test item, 2000 mg/kg bw (2.24 ml/kg bw) 24 hours	> 2000 mg/kg bw No clinical signs occurred.	1	Anonymous 2015b
Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit OECD 402 GLP not stated	Rabbit, New Zealand White 5 males and 5 females	Tea Tree Oil (no information on composition available)	Undiluted test item, 2000 mg/kg bw 24 hours	> 2000 mg/kg bw Clinical signs: Slight diarrhoea in 1/10 animals.	2	Anonymous 1989b and ECHA dissemination site[23]

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

Study 1, Anonymous 2015b, Tea Tree Oil: Acute dermal toxicity study in Wistar rats, OECD 402 (1987), GLP.

Reliability statement: The study was conducted according to the former version of OECD 402. The design and methodological structure of the updated OECD guideline 402 (2017) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. Therefore, the study is considered reliable without restrictions (reliability score: 1)

In the new study from 2015, the acute dermal toxicity of Tea Tree Oil was tested in male and female Wistar rats. Based on the individual body weights, the undiluted test item at dose of 2000 mg/kg bw. (2.24 mL/kg bw.) was applied directly to the clipped skin of the animal to cover about 10% of the body surface of the animal. The applied area was covered with cotton gauze. The test item contact period with the skin was for 24 hours.

After the 24 hour contact period, the dressing was removed and the applied area was washed with water.

All the rats were observed for clinical signs of toxicity and mortality for 14 days post application. At the end of the observation period, all animals were euthanized and subjected to necropsy.

There were no clinical signs and mortality observed. All rats gained weight during the experimental period. No abnormalities detected at the necropsy.

Study 2, Anonymous 1989b, Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit, OECD 402.

Reliability statement: The study was largely conducted according to the former version of OECD 402 and accepted during previous evaluation. The design and methodological structure of the updated OECD guideline 402 (2017) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. However, the study reveals some considerable deviations also from the former

[23] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/4

guideline in terms of methodological and reporting deficiencies. Yet, these were not deemed to have an influence on its overall scientific reliability. Therefore, the study is overall considered reliable with restrictions (reliability score: 2).
In support, in the 1989 study (pre-GLP), the undiluted test sample was applied dermally at a dose of 2000 mg/kg bw and held in contact with the skin for 24 hours over an approximate skin area of 175 cm^2.
The animals were then observed during the 24 hour exposure period and daily for 14 days thereafter. Observations were made for any signs of toxicity and abnormal behaviour. The body weight was determined on days 0, 7 and 14. No mortality was observed and there were no other signs of toxicity or abnormal behaviour. No significant loss of weight was observed during the observations period.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Based on the results obtained, the estimated acute dermal LD_{50} of Tea Tree Oil is above 2000 mg/kg bw for male and female rats as well as male and female rabbits. The application on the guidance of the CLP criteria (Regulation (EC) 1272/2008) gives a cut off LD_{50} value of 2000 mg/kg bw for acute dermal toxicity classification.
Therefore, Tea Tree Oil does not require to be classified for the acute dermal toxicity.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified. Data conclusive but not sufficient for classification.

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

The acute toxicity via the inhalation route with Tea Tree Oil was assessed with Wistar rats.

Table 12: 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}	Reliability score	Reference
Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats OECD 403 (2009) GLP	Rats, Wistar HsdCpb: WU males, females, 5 per group	Tea Tree Oil, 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol aerosol (complies with ISO-specification)	0.77, 3.69 and 5.06 mg TTO/L of chamber air Continuous exposure for 4 hours	3.64 mg/L air (4 h, male & female rats) Clinical signs: Please refer to Table 20	1	Anonymous. 2010a
Acute Inhalation Toxicity Study in Wistar Rats Inhalation: aerosol (nose only) OECD 403 GLP	Rats, Wistar CRL:(WI) BR males, females, 5 per group	Melaleuca alternifolia, ext., purity: 100% (complies with ISO 4730:2017specifitaion) MMAD: 2.31 - 3.51µm GSD: 2.05 – 2.42	1.94, 3.70, 5.04 mg/L Duration of exposure: 4 h	LC_{50}: 5.23 mg/L air (male) LC50: 4.29 mg/L air (female) LC50: 4.78 mg/L air (male/female)	1	ECHA dissemination site[24]

[24] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/3

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

Study 1, Anonymous 2010a, Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats, OECD 403 (1987), GLP.

Reliability statement: The study is conducted in accordance with OECD 403. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

The aim of this study was to assess the possible inhalation toxicity potential of Tea Tree Oil. The acute inhalation toxicity study with Tea Tree Oil was conducted in male and female Wistar rats by nose only exposure using 30%, 50% and 70% w/v aerosol of the test item diluted in Dimethyl sulphoxide to 3 groups of rats (G2, G3 and G4). The aerosol was generated by a glass atomizer with an injection rate of 0.4 mL/min. Similarly rats in the vehicle control group (G1) were exposed to Dimethyl sulphoxide aerosol only. The rats were continuously exposed to the test item aerosol for 4 hours in an inhalation exposure chamber. The post-treatment observation period was 14 days.

Mortality and clinical signs were observed immediately after exposure and thereafter once daily during days 2 to 15. Body weights were determined during acclimatization, on Day 1, 3, 8, 15 and at death. Macroscopic examination was performed after terminal sacrifice (Day 15).

Table 13: Clinical signs and mortality after exposure of TTO in an Acute Inhalation Study in Wistar Rats (a total of 10 animals per dosing group)

Test Item Concentration	**Dose level** mgTTO/L air		**Toxic Signs [No. of incidences]**										**Mortality [%]**	**Necropsy findings [%]**	**Body weight**
			Ataxia	Dispnoea	Dullness	Lethargy	Nasal discharge	Perineum wet with urine	Recumbency	Slight salivation	Tremor	Death			
Vehicle control	**0**		-	-	-	-	-	-	-	-	-	-	0	0	+
30 % w/v in DMSO	0.77	Day 1				10	10				1		10	0	+**
		Day 2			3	9									
		Day 3			1	1						1			
		Day 4*													
50 % w/v in DMSO	3.69	Day 1	1	1		10	8	2	1	4	8		40	0	+**
		Day 2	1		8	9		1			1	1			
		Day 3			1	2						3			
		Day 4*													
70 % w/v in DMSO	5.06	Day 1	6	8		2	9	1		5	3	1	70	0	+**
		Day 2		4	7	7						2			
		Day 3			1	4						3			
		Day 4*										1			

*: All surviving animals were normal from day 4 onwards;

**: Pre-terminally dead animals lost body weight when compared to its initial body weight

The test item concentration in the air inhalation sample columns were analysed using a validated analytical method. The analytical determined mean test item concentrations in the air inhalation sample columns were 0, 0.77, 3.69 and 5.06 mg TTO/L of chamber air.
No mortality occurred in the control group G1. Mortality of 10, 40 and 70% occurred in G2, G3 and G4 groups, respectively without sex preference.
The acute inhalation LC_{50} (4 h) of Tea Tree Oil in Wistar rats was established to be 3.64 mg/L of air for both male and female rats.
Based on data on ECHA dissemination site a GLP-compliant study was carried out to determine the acute inhalation toxicity of Tea Tree Oil to rats. The study followed the requirements of OECD guideline 403, without significant deviation. Three groups of ten Wistar rats (five males and five females) were exposed to an aerosol atmosphere. The animals were exposed for a single four-hour period using a nose-only exposure system, followed by a fourteen day observation period. Seven mortalities (2/5 males, 5/5 females) occurred at the highest test concentration; a specific cause of death was not clearly determined. No mortalities occurred at the two lower test concentrations. The surviving males from Group 1 and the majority of surviving males from Group 3 showed bodyweight loss during the first week of the observation period. Necropsy of the surviving animals on completion of the fourteen day observation period did not reveal any test item-related gross findings up to a concentration of 5.04 mg/L. The acute inhalation median lethal concentrations (4-hr LC_{50}) and 95% confidence limits of Tea Tree Oil in rats were calculated to be:

- Male & Female : 4-hr LC_{50}: 4.78 (3.94 - 5.32) mg/L
- Male only : 4-hr LC_{50} : 5.23 mg/L
- Female only (4-hr LC_{50}: 4.29 (3.41 - 6.41) mg/L.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

The acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats was established to be 3.64 mg/L of air for both male and female rats.
Based on these results Tea Tree Oil (Anonymous 2010a, ECHA dissemination site) has to be classified as category 4 ($1.0<LC_{50}\leq5.0$) with regard to the acute inhalation toxicity according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments). Proposed $ATE_{inhalation\ acute}$ = 3.64 mg/L.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Harmonised classification proposed. Tea Tree Oil has to be classified as acute toxicity hazard category 4 when regarding inhalation exposure. Labelling proposed is **H332- Harmful if inhaled**. Proposed $ATE_{inhalation\ acute}$ = 3.64 mg/L.

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

The potential of TTO to induce skin corrosion/irritation was tested in two rabbit studies.

Table 14: 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	Reliability score	Reference
Tea Tree Oil: Acute dermal irritation / corrosion	New Zealand White rabbits males one rabbit	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene,	0.5 ml of Tea Tree Oil 4 hours contact time	Mean score reactions, indicating that the test item is a "moderate-irritant" according to Draize´s evaluation method: Mean scores for individual animals at 7 observation times	1	Anonymous 2015c

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td>study in New Zealand White rabbits OECD 404 (2002) GLP</td><td>for the initial test and two rabbits for the confirmatory test</td><td>2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO specification)</td><td></td><td>(values reported as sum of erythema/oedema per number of observation dates):
<table><tr><td></td><td>Mean24-72h score for erythema</td><td>Mean 24-72h score for edema</td></tr><tr><td>Rabbit 1</td><td>2.67</td><td>1.00</td></tr><tr><td>Rabbit 2</td><td>2.00</td><td>1.00</td></tr><tr><td>Rabbit 3</td><td>2.00</td><td>1.00</td></tr></table>Clinical signs such as scale formation and peeling / desquamation were observed in all the rabbits.
The reaction was reversible after 7 day.</td><td></td><td></td></tr>
<tr><td>Acute dermal irritation in the rabbit of Tea Tree Oil batch 88/375. No OECD Guideline GLP not stated</td><td>New Zealand White albino rabbits 6 young and mature animals</td><td>Tea Tree Oil</td><td>Undiluted test item</td><td>Irritation reactions were observed on both intact and abraded skin after the treatment with the test item. The primary irritation index was found to be 5.0. Evaluation of the skin reactions according to the EU criteria revealed that the test material produced mean irritation scores of 3.08 and 1.83 for erythema and oedema for intact skin and mean irritation scores of 3.25 and 2.0 for erythema and oedema for abraded skin, respectively.
Mean Scores for individual animals at 2 observation times (24 and 72
<table><tr><td rowspan="2">Animal No.</td><td colspan="2">Intact Skin</td><td colspan="2">Abraded Skin</td></tr><tr><td>Erythema</td><td>Edema</td><td>Erythema</td><td>Edema</td></tr><tr><td>1</td><td>2.5</td><td>1.5</td><td>2.5</td><td>1.5</td></tr><tr><td>2</td><td>3.0</td><td>2.0</td><td>3.0</td><td>2.0</td></tr><tr><td>3</td><td>3.5</td><td>2.0</td><td>3.5</td><td>2.0</td></tr><tr><td>4</td><td>3.5</td><td>2.0</td><td>3.5</td><td>2.5</td></tr><tr><td>5</td><td>3.0</td><td>2.0</td><td>3.5</td><td>2.0</td></tr><tr><td>6</td><td>3.0</td><td>1.5</td><td>3.5</td><td>2.0</td></tr></table></td><td>2</td><td>Anonymous 1989c</td></tr>
</table>

Literature studies
Open scientific literature search has been performed and a study on skin irritation of the Tea Tree Oil components have been found.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by

identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 15: Summary table of other studies relevant for skin corrosion/irritation

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reliability score</th><th>Reference</th></tr>
<tr>
<td>Literature study
Correlations of the components of Tea Tree Oil with its antibacterial effects and skin irritation

Reliable with restrictions
Supportive information
•No OECD guideline or GLP defined</td>
<td>TTOs were isolated from the leaves (TTO-L), twigs, and branches of M. alternifolia by steam distillation, and the components analysed by gas chromatography–mass spectrometry. Results showed that components of TTO-L satisfied the International Organization for Standardization (ISO) 4730 guidelines.

Yields:TTO_{Leaves} = 2.2%
TTO_{Twigs} = 0.59 %
$TTO_{Branches}$ = 0.01 %
Major components in TTO_{Leaves}:Terpinen-4-ol: 47.3%□-terpinene: 20.59%□-terpinene: 9.58%1,8-cineole: 1.71%</td>
<td>Draize skin irritation assay (female 8-12 week old Wistar rats, 5 animals per test concentration)
Chronic liver toxicity : 3 animals
TTO test concentrations: 0.625 %, 1.25 %, 2.5 %, 5 %.
Vehicle not specified for sensitization test. In other parts of the study: dilution in jojoba oil.</td>
<td>In the Draize skin irritation assay TTO-L, did not cause significant skin irritation at 2.5 % per site.
At concentrations of 5% and 10% TTO irritating effects were seen:
<table>
<tr><th></th><th colspan="2">Draize score</th></tr>
<tr><th></th><th>5 % TTO</th><th>10 % TTO</th></tr>
<tr><td>After 0 h</td><td></td><td></td></tr>
<tr><td>Edema</td><td>0</td><td>0</td></tr>
<tr><td>Erythema</td><td>0</td><td>0</td></tr>
<tr><td>After 24 h</td><td></td><td></td></tr>
<tr><td>Edema</td><td>1</td><td>1</td></tr>
<tr><td>Erythema</td><td>1</td><td>2</td></tr>
<tr><td>After 48 h</td><td></td><td></td></tr>
<tr><td>Edema</td><td>1</td><td>1</td></tr>
<tr><td>Erythema</td><td>1</td><td>2</td></tr>
</table>
Terpinen-4-ol did not cause skin irritation at up to 1.5%, whereas 1,8-cineole induced skin irritation in female Wistar Rats (8-10 weeks old) at dose rates of 0.75% and 1.5% but not at 0.375% and lower concentrations.</td>
<td>2</td>
<td>Lee, C.-J., Chen, L.-W., Chen, L.-G., Chang, T.-L., Huang, C.-W., Huang, M.-C., Wang, C.-C.; 2013</td>
</tr>
</table>

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

Study 1, Anonymous, 2015c, Tea Tree Oil: Acute dermal irritation / corrosion study in New Zealand White rabbits, OECD 404 (2002), GLP.

Reliability statement: The study is conducted in accordance with OECD 404. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

In the 2015 study, the acute dermal irritation with New Zealand White rabbits was performed to evaluate the skin irritation potential of Tea Tree Oil.

A volume of 0.5 mL of undiluted test item was applied in between the prepared area of the skin and the cotton gauze of approx. size of 6 cm^2. A control patch was applied 3 – 4 cm anterior to the 4 hour test patch. All the patches were secured to the body of the animals by an adhesive tape, and a crepe bandage (except for 3 min patch) was wrapped around the torso of the animal. After a contact period of 4 hours, the treated are was washed with de-

ionised water. The study was conducted in a stepwise manner (i.e., one rabbit for the initial test and two rabbits for the confirmatory test).
The degree of irritation was evaluated and scored by Draize´s evaluation method (1959) at 1, 24, 48 and 72 hours and day 7 and 14 post removal of the test patch.
The mean score reactions from gradings at 24, 48 and 72 hours after patch removal calculated for each individual animal were 2.67, 2.00, 2.00 for erythema and 1.00, 1.00, 1.00 for oedema,. Clinical signs such as scale formation and peeling / desquamation were observed in all the rabbits were reversible after 7 day observation. There were no pre-terminal deaths observed and no abnormality was detected at necropsy.

Study 2, Anonymous., 1989c, Acute dermal irritation in the rabbit of Tea Tree Oil batch 88/375.
Reliability statement: Although no GLP-study, it was largely conducted according to the former version of OECD 404 and accepted during previous evaluation. The design and methodological structure of the updated OECD guideline 404 (2015) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. However, the study reveals some considerable deviations also from the former guideline in terms of methodological and reporting deficiencies. Yet, these were not deemed to have an influence on its overall scientific reliability. Therefore, the study is overall considered reliable with restrictions (reliability score: 2) and being a vertebrate test it is relied upon also for reasons of animal welfare.
In the 1989 study, the skin irritating potential TTO was determined in six young and mature New Zealand White albino rabbits. The test substance was administered undiluted to the intact and abraded skin of mature New Zealand rabbits. All animals were observed for signs of toxicity and abnormal behaviour during the experimental period of 72 h. The skin reactions were assessed according to the scoring scheme of Draize.
Overall mean irritation scores of 3.08 and 1.83 for erythema and oedema for intact skin and mean irritation scores of 3.25 and 2.0 for erythema and oedema for abraded skin, respectively were assessed.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Based on results of study 2 (Anonymous 1989c):means scores for erythema in all tested animals from gradings at 24 - 72 hours after patch removal) were of ≥ 2,3 and ≤ 4,0, therefore Tea Tree Oil has to be classified as skin irritant Category 2 in accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments).

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Harmonised classification proposed. Tea Tree Oil has to be classified as Skin irritation category 2 with hazard statement **H315- causes skin irritation**.

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

The potential of TTO to induce serious eye damage/eye irritation was investigated in rabbits.

Table 16: Summary table of animal studies on serious eye damage/eye 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	Relia-bility score	Reference
Tea Tree Oil: Acute eye irritation/ corrosion study in New Zealand white rabbit OECD 405	New Zealand White rabbits Three males	Tea Tree Oil 9.7 % α-Terpinene, 2.6 % 1,8-Cineole, 17.8 % γ-Terpinene, 1.5 % p-Cymene and 41.5 %	1 ml of undiluted test item One administration, observation duration 7 days	Mean total scores for each individual animal 24, 48 and 72 h are presented in y post-instillation and scored. Reversibility: Conjunctivitis completely regressed on day 7. There were no clinical signs of toxicity. No mortality and	1	Anonymous 2015d

(2012) GLP		Terpinen-4-ol (complies with ISO-specification)		abnormal behaviour were observed at necropsy in any of the tested animals.		
OECD Guideline 405 GLP	Rabbit (New Zealand White)	Melaleuca alternifolia, ext., Purity 100% (no vehicle)	0.1 mL Tea Tree Oil, purity: 100%	cornea opacity score (animal #1 - mean) 0 of max. 0 (Time point: 24/48/72 h) iris score (animal #1 - mean) 0 of max. 0 (Time point: 24/48/72 h) conjunctivae score – redness (animal #1 - mean) 1 of max. 2 (Time point: 24/48/72 h) fully reversible within: 72 h chemosis score (animal #1 - mean) 0.33 of max. 1 (Time point: 24/48/72 h) fully reversible within: 48 h cornea opacity score (animal #2 - mean) 0 of max. 0 (Time point: 24/48/72 h) iris score (animal #2 - mean) 0 of max. 0 (Time point: 24/48/72 h) conjunctivae score – redness (animal #2 - mean) 0.67 of max. 1 (Time point: 24/48/72 h) fully reversible within: 72 h chemosis score (animal #2 - mean) 0.33 of max. 1 (Time point: 24/48/72 h) fully reversible within: 48 h	1	ECHA dissemination site[25]
In vitro/ex vivo study According to OECD Guideline 437 (Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants) [before 26 July 2013] GLP	Cattle (strain not specified) 3 corneas for the test material and each control.	Melaleuca alternifolia, ext., Purity 100% (no vehicle)	0.75 mL of Tea Tree Oil or control item TTO was applied to isolated bovine corneas for 10 minutes. Duration of post- treatment incubation (in vitro): 120 minutes positive control negative control	in vitro irritation score Tea Tree Oil; value 2.2 Negative controls Irritancy score = 2.3 Positive controls Irritancy score = 44.5	1	ECHA dissemination site[26]

[25] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/4/3

[26] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/4/3/?documentUUID=e5d53c32-8d2a-4375-9a5c-a878b16d59c4

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

Study 1, Anonymous 2015d, Tea Tree Oil: Acute eye irritation/corrosion study in New Zealand white rabbit; OECD 405 (2012); GLP

Reliability statement: The study is conducted in accordance with OECD 405. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

0.1 ml of the undiluted TTO was installed into the conjunctival sac of the left eye of the three New Zealand White rabbits after gently pulling the lower lid away from the eyeball. The right eye remained untreated and served as a control.

The eye of rabbit were examined at 1, 24, 48, 72 hours and on the 7th day post-instillation and scored.

Table 17: Mean eye irritation scores

Rabbit No.	Time after appl.	CONJUNCTIVA			IRIS	CORNEA	
		Redness A	Chemosis B	Discharge C	Pupil D	Opacity E	Area of opacity F
RBa 858	1 hour	0	0	1	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	0	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.0**	**0.0**	**0.0**	**--**
Overall mean		1.0			0.0	0.0	
RBa 859	1 hour	1	1	1	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	0	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.0**	**0.0**	**0.0**	**--**
Overall mean		**1.0**			**0.0**	**0.0**	
RBa 860	1 hour	1	2	2	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	1	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.3**	**0.0**	**0.0**	**--**
Overall mean		1.1			0.0	0.0	

NA – not applicable Total score: sum of conjunctive, iris and cornea

No mortality was observed during the study. The individual mean scores of eye reactions are reported in y post-instillation and scored.

.

A study was performed to assess the irritancy potential of Tea Tree Oil to the eye of the New Zealand White rabbit (ECHA dissemination site). A single application of Tea Tree Oil to the non-irrigated eye of two rabbits produced mean conjunctival redness and chemosis scores of < 2 following grading at 24, 48 and 72 hours. The treated eyes of both animals appeared normal at the 72-hour observation.

A study to assess the ocular irritancy potential of Tea Tree Oil to isolated bovine cornea (ECHA dissemination site) concluded that tea tree oil was not an ocular corrosive or severe irritant.

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

Based on these results of two *in vivo* studies the mean scores following grading at 24, 48 and 72 hours after installation of the test material did not meet in any tested animals any of the following criteria for category 2 for reversible effects on the eye according to Regulation (EC) No 1272/2008: corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2., Tea Tree Oil does not have to be

classified and has no obligatory labelling requirement for eye irritation according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments).

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

Tea Tree Oil does not need to be classified for serious eye damage or eye irritation. Data is conclusive but not sufficient for classification.

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

No data on respiratory sensitisation available. Tea Tree Oil was negative in two skin sensitisation studies (see below); therefore, it is unlikely that it would induce respiratory sensitisation.

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

Not relevant

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

Not relevant

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed

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

The skin sensitising potential of Tea Tree Oil was investigated in two guinea-pig maximisation tests according to OECD guideline 406 in 2015 and 1989.

Table 18: 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	Reliability score	Reference
Tea Tree Oil: Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs OECD 406 (1992) GLP	Guinea-Pig Albino, NIH (Duncan Hartley) males and females 10 per control, 20 in the test item group	Tea Tree Oil 9.7 % α-Terpinene, 2.6 % 1,8-Cineole, 17.8 % γ-Terpinene, 1.5 % p-Cymene and 41.5 % Terpinen-4-ol	Induction: 25% (w/w) in propylene glycol Boosting: 50% (w/w) in acetone Challenge: 100% TTO (undiluted) Test duration was 48 h	In the control and treatment group, there were no skin reactions at 24 and 48 hours post removal of the test patch. In the positive control group, 6/10 guinea pigs had score of 1 (discrete or patchy erythema) at 24 and 48 hours post removal of the test patch.There were no clinical signs of toxicity. No mortality was observed during the study.	1	Anonymous 2015e
Skin	Guinea-Pig	Tea Tree Oil	Two weeks	No dermal	2	Anonymous

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reliability score	Reference
sensitization potential in the guinea-pig of Tea Tree Oil batch 88/375 OECD 406 (Magnusson & Kligmann) GLP not stated	HA-strain 20 animals		after induction application, the test group animals were challenged by application of the maximum sub irritant concentration of the test compound (30% (w/w) dilution of TTO in petroleum jelly) on one flank under occlusive conditions for a period of 24 hours.	responses at challenge. No mortality and abnormal behaviour was observed in all the tested animals during the test period.		1989d
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay); GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions.	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde in acetone/ olive oil (4/1 v/v)	Stimulation index (SI) (Mean): 2.4 at 2% (SD=1.4) SI (Mean): 6.9 at 20% (SD=2.0) SI (Mean): 16 at 100% (SD=6.3) EC3=4.4%(w/v) Positive control results provided in study 2006a, below	1	ECHA dissemination site (study report 2006)[27]
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions.	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive	SI (Mean): 1.6 at 2% (SD=0.4) SI (Mean): 2.8 at 20% (SD=0.7) SI (Mean): 5.7 at 100% (SD=1.6) EC3=25.5% (w/v)	1	ECHA dissemination site (study report 2006a)[28]

[27] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=d97775d8-17e8-47b6-82c5-bad02fd3e225

[28] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=476b7293-ced5-48a6-b1d3-c51085f726b8

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reliability score	Reference
Node Assay); GLP			control: alpha-hexylcinnam-aldehyde in acetone/ olive oil (4/1 v/v)	Positive control results: SI (Mean): 1.8 at 5% SI (Mean): 2.9 at 10% SI (Mean): 6.2 at 25% EC3=10.5% (w/v)		
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay); GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde in acetone/ olive oil (4/1 v/v)	SI (Mean): 1.8 at 2% (SD=0.4) SI (Mean): 2.8 at 20% (SD=1.2) SI (Mean): 6.5 at 100% (SD=2.3) EC3=24.3% (w/v) Positive control results provided in study 2006a, above	1	ECHA dissemination site (study report 2006b)[29]
Skin sensitisation: *in vivo* (LLNA) No guideline followed, method similar to OECD Guideline 429 GLP	Mouse (CBA/J) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions, according to Test Article Characteriza-tion	5%, 25% and 50% in PEG 400 a negative control group was treated with PEG 400 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde 25% in PEG 400	SI (Mean): 2.1 at 5% (SD=0.7) SI (Mean): 7.7 at 25% (SD=4.0) SI (Mean): 7.9 at 50% (SD=3.2) EC3=8.3% (w/v) Positive control results: SI (Mean): 21.2 at 25% (SD=7.7)	2	ECHA dissemination site (study report 2007)[30]

Literature studies
Open scientific literature search has been performed and some studies on skin sensitisation of the Tea Tree Oil components have been found.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the

[29] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=113e4b90-df3c-407d-8c4d-d213eeac7dcb
[30] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=2ea26c41-7e89-421f-9540-4bdf0ab30d7b

publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 19: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
Contact allergy to essential oils cannot always be predicted from allergy to fragrance markers in the baseline series. Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	*Melaleuca alternifolia* oil (composition not specified)	Test system: patch test on human skin Concentrations tested: 5% in petrolatum Number of test individuals: 2104 patients Readings mostly on day 2 and 4, but in some patients on day3 and 5.	Reactions after: 11 (0.5%) positive, 2 (0.1%) doubtful, 3 (0.1%) irritant	2	Sabroe, R.A.. Holden C.R., Gawkrodger, D.J. (2016) Contact Dermatitis, 74, 236-2111
Is tea trea oil an important contact allergen? Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	*Melaleuca alternifolia* oil (meeting Australian standard for min. + max concentrations of e.g. 1.8-Cineol, d-Limonene, Aromadendrene, α-Terpinene, Terpinolene and α-Pinene)	Test system: Patch test on human skin Concentrations: 10 % in pet., 5 % commercial lotion Number of test individuals: 217 patients (140 ♀, 77 ♂) Placing of patch with test substance on upper back, removed after 2 days, assessment after 3 days	50 of 140 women and 15 of 77 men had 1 or more positive patch test. 1♀: ++ to 10% TTO in pet. and lotion (5% TTO) 3x (1.4%) non-relevant weakly positive reaction to the lotion containing 5% TTO 44 patients (20.3%) had weak, irritant reaction to the lotion	2	Veien, N.K., Rosner, K., Skovgaard, G.L. (2004) Contact Dermatitis 50(6):378-9
		Test system: Patch test on human skin Concentrations:4commercial lotions containing 5 % TTO Number of test individuals: 160 patients (117 ♀, 43 ♂) Placing of patch with test substance on upper back, removed after 2 days, assessment after 3 days	No allergic reactions 5 patients (3.1%) irritant reactions		
Comparison of human skin irritation patch	α-terpineol (purity 95%)	Test system: 4-hr HPT (human patch test) Concentrations: 0.2 mL,	4 hour HPT: Non irritating	2	Jírová, Basketter, D., Liebsch,

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
test data with in vitro skin irritation assays and animal data. Irritation measurement: 4-hr HPT (human patch test) Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system		undiluted Number of test individuals: 30 human volunteers Placing of patch with test substance on upper outer arm. 4 hours exposure time, Observations after 24. 48, 72 hours after patch removal	Positive reactions: 0/29 Positive reactions to SLS: 23/29 (pos. control)		M., Bendová, H., Kejlova, K., Marriott, M., Kándarová, H.; 2010 Contact Dermatitis 2010 (62): 109-116
α-Terpinene, an antioxidant in Tea Tree Oil, autoxidizes rapidly to skin allergens on air exposure. Sensitization measurement: Following OECD 429 Reliable with restrictions Supportive information No GLP	α-Terpinene, p-Cymene	Test system: Female CBA/Ca mice Concentrations tested: 0.1, 1, 5, 10 and 30% w/v No. of animals per treatment group: 3 The sensitizing potential of p-Cymene, a degradation product of α-Terpinene, was investigated with the murine local lymph node assay. 8 week old female CBA/Ca mice were used, with test concentration of 0.1, 1, 5, 10 and 30%. 5 h after exposure, the draining auricular lymph nodes were excised and the relative [^{3}H]thymidine incorporation was measured by β-scintillation. Results were expressed as mean dpm/lymph node for each experimental group and as stimulation index (SI) i.e., test group/control group. Test material that caused an SI greater than 3 were considered to be positive in the LLNA. EC3 values were calculated by linear interpolations. Sensitization potency of the test compound was classified according the following: <0.1 extreme; ≥ 0.1 - <1 strong; ≥ 1 - < 10 moderate; ≥ 10 - <100 weak.	At any dose tested, p-Cymene did not reach SI values above 3. The EC_3 value was determined as >30% and therefore considered as weak sensitizer in the LLNA assay. For α-Terpinene, EC3 values of 0.9 and 1.0 were determined. It is therefore considered as strong sensitizer.	2	Rudbäck, J., Bergström, M.A., Börje, A., Nilsson, U. (2012) Chemical Research in Toxicology 25: 713 - 721
Assessment of sensitization	(-)-menthol 1,8-cineole	Test system: Female Wistar rats	Primary assay: PLNA was positive	2	Friedrich, K., Delgado, I.,

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
potential of monoterpenes using the rat popliteal lymph node assay. Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	(+/-) citronellal (+)-limonene (+/-) camphor terpineol.	Test concentrations: Primary assay: 0.5, 2.5 or 5 mg; secondary assay: 0.5 mg The rat popliteal lymph node assay (PLNA) has been used to evaluate the immuno-sensitizing potential of 10 monoterpenes. The primary or direct PLNA was performed with the monoterpenes, and chlorpromazine (CPZ) and barbital were used as positive and negative controls, respectively. Female, 7-8 week-old Wistar rats were injected subcutaneously (50 µL) with the test substance (0.5, 2.5 or 5 mg) into the right hind footpad while the contralateral footpad was injected with the vehicle (DMSO) alone. Weight (WI) and cellularity (CI) indices for draining PLNs were determined 7 days after treatment. A secondary PLNA, a T-cell priming test, was carried out with the four substances that had been positive in the primary assay. Six weeks after being locally primed with 5 mg/paw, rats were sc injected into the same footpad with a dose (0.5 mg/paw) of the substance that had been previously found to be insufficient to cause a positive response. WI and CI were then calculated 4 and 7 days after the second injection. No. of animals per treatment group: (-)-menthol 9 1,8-cineole 10 (±)-citronellal 10 (+)-limonene 10 (±)-camphor 10 Terpineol 10 Barbital 8 DMSO 47 Saline 50 Citral 7, 8,	(WI >or= 2 and CI >or= 5) for CPZ, citral, alpha-terpinene, beta-myrcene and (-)-alpha-pinene, and negative for barbital, DMSO, (-)-menthol, 1,8-cineole, (+/-) citronellal, (+)-limonene, (+/-) camphor and terpineol. Secondary assay: CPZ was also positive in the secondary assay thereby confirming that it is a sensitizing agent. Citral, alpha-terpinene, beta-myrcene and (-)-alpha-pinene, however, were negative in the secondary assay. In summary, no monoterpene proved to be a sensitizing agent in the PLNA.		Santos, L., Paumgartten, F. (2007) Food and Chemical Toxicology

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
		□-Terpinene 10* □-Myrcene 7, 7, 10* (-)-□-pinene 7, 8 ,11* Chlorpromazine 8, 8, 10* 4, 6, 11* *: Dose 0.5, 2.5, 5.0 mg/paw			
Limonene hydroperoxide analogues differ in allergenic activity. Reliable with restrictions Supportive information • No OECD guideline or GLP defined	R-limonene Oxidized limonene	Test system: Mice Test concentrations: R-limonene: 25, 50 and 100% w/v , oxidized limonene: 1, 5 and 25% w/v No. of animals per treatment group: 4 The sensitizing potential of R-Limonene was investigated with the murine local lymph node assay. 4 mice per treatment group were used, with test concentration of 25, 50 and 100%. The draining auricular lymph nodes were excised and the relative [³H]thymidine incorporation was measured by β-scintillation. Results were expressed as mean dpm/lymph node for each experimental group and as stimulation index (SI) i.e., test group/control group. Test material that caused an SI greater than 3 were considered to be positive in the LLNA. EC3 values were calculated by linear interpolations. Sensitization potency of the test compound was classified according the following: <0.1 extreme; ≥ 0.1 - <1 strong; ≥ 1 - < 10 moderate; ≥ 10 - <100 weak.	At 50 and 100 %, R-Limonene showed SI values above 3, thus had to be considered as positive in the LLNA The EC3 value was determined to be 30% and therefore considered as weak sensitizer in the LLNA assay.	2	Christensson, J.B., Johansson, S., Hagvall, L., Jonsson, C., Börje, A., Karlberg, A.T. (2008) Contact Dermatitits 59(6): 344-352

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

The skin sensitising potential of Tea Tree Oil was investigated in two guinea-pig maximisation tests according to OECD guideline 406 in 2015 and 1989 and four LLNA studies provided in REACH registration dossier (available on ECHA dissemination site).
Furthermore, six literature studies with different components of Tea Tree Oil are summarized above, three of these studies reported effects on human skin. A further publication reporting effects on human skin is presented below.

Study 1, Anonymous 2015e, Tea Tree Oil: Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs; OECD 406 (2012); GLP

Reliability statement: The study is conducted in accordance with OECD 425. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

In the new OECD study, in the control group, at 24 and 48 hours (post administration) observation period erythema score of 1 and oedema score of 1 was observed in 10/10, 0/10 and 10/10 at sites 1, 2 and 3, respectively.

In the treatment group, at 24 hours (post administration) observation period erythema score of 1 was observed in 20/20, 20/20 and 20/20 at sites 1, 2 and 3, respectively. Oedema score of 1 was observed in 20/20, 16/20 and 17/20 at sites 1, 2 and 3, respectively.

At 48 hours (post administration) observation period erythema score of 1 was observed in 20/20, 20/20 and 20/20 at sites 1, 2 and 3, respectively. Oedema score of 1 was observed in 20/20, 13/20 and 14/20 at sites 1, 2 and 3, respectively. There were no clinical signs of toxicity and pre-terminal deaths were observed.

Study 2, Anonymous 1989d, Skin sensitization potential in the guinea-pig of Tea Tree Oil batch 88/375 OECD 406.

Reliability statement: The study was accepted during previous evaluation and appears to be conducted in overall accordance with OECD 406. Apart from that, it shows some deviations in terms of methodology and shortcomings in reporting, which were, however, not deemed to have an influence on its overall scientific reliability. The study is therefore considered reliable with restrictions (reliability score: 2).

In the old OECD study, following administration of the topical challenge dose, animals were free of irritation responses at challenge. On the basis of the results obtained in the GPMT test performed according to the method of Magnusson and Kligmann, the tested TTO did not demonstrated a skin sensitizing potential.

A GLP-compliant LLNA study (ECHA dissemination site, study report 2006) was carried out to determine the possible contact allergenic potential of Tea Tree Oil. The study followed the requirements of EU method B.42, OECD method 429, without significant deviation. Three groups each of five female mice were treated with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted) by topical application to the dorsum of each ear lobe (left and right) on three consecutive days. A negative control group of five mice was treated with an equivalent volume of the vehicle polyethylene glycol 300 (PEG 300) only. Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine, ^{3}HTdR). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per animal. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were washed subsequently and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}HTdR measured in a β-scintillation counter. All treated animals survived the scheduled study period.

Neither clinical/local signs nor other findings were observed in any animals of the control group. One day after the first or the second topical application, a slight ear erythema was observed at both dosing sites in all mice of Group 2 (2%), Group 3 (20%) and Group 4 (100%, undiluted), persisting for the remainder of the in-life phase of the study (Groups 3-4), or persisting for a total of two days (Group 2). In addition, two days after the third topical application (Day 5) and prior to sacrifice (Day 6), scales were found on both ears in all mice of Group 4 (100%, undiluted). No significant difference of dpm/ LN was determined at the test item concentration of 2% (w/v) in PEG 300 compared with the vehicle control group at $p \leq 0.05$ (two sides). A significant difference of dpm/LN was determined at the test item concentrations of 20% in PEG 300 and 100% (undiluted) compared with the vehicle control group at $p \leq 0.05$ (two sides).

A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentrations resulted in 3-fold or greater increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the S.I. In this study S.I. of 2.4, 6.9 and 16.0 were determined with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 4.4% (w/v) was derived.

The second LLNA study (ECHA dissemination site, study report 2006a) was conducted in the same way as above study however no erythema or scales were found on ears of all mice after topical application. In this study S.I. of 1.6, 2.8 and 5.7 were determined with the test item at concentrations of 2 %, 20 % (w/v) in PEG 300 and 100 % (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 25.5 % (w/v) was derived.

The third LLNA study (ECHA dissemination site) was conducted in the same way as above two studies. One day after the second topical application (Day 3) and on Day 4, a slight ear erythema and hypersensitivity to touch on both ears were observed at both dosing sites in all mice of Group 2 (2%). One day after the first topical application (Day 2), a slight ear erythema was observed at both dosing sites in all mice of Group 3 (20%), persisting for the remainder of the in-life phase of the study. On Days 3-4, the ears of all mice in this group were hypersensitive to

touch (both ears). On Day 6 (prior to necropsy), scales were found on both ears in all mice of Group 3 (20%). One day after the first or the second topical application, a slight to moderate ear erythema and/or slight ear swelling were observed at both dosing sites in all mice of Group 4 (100%, undiluted), persisting for the remainder of the in-life phase of the study. On Days 3-4, the ears of all mice in this group were hypersensitive to touch. On Day 4, scales were found on both ears in all mice of this group, persisting for the remainder of the in-life phase of the study. In this study S.I. of 1.8, 2.8 and 6.5 were determined with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 24.3% (w/v) was derived.

The GLP-compliant study (ECHA dissemination site, study report 2007) followed MB Research Protocol 5650A-06 and determined the sensitizing potential of topically applied Melaleuca alternifolia. A preliminary screening test was conducted with three groups of healthy female CBA/J mice (2 per group) to determine the concentrations of the test article to be used in the main study. Because the chosen vehicle, PEG 400, had not been validated, two additional groups of 2 mice each were added, one treated with the PEG 400 vehicle and one treated with 25% HCA in PEG 400. The initial screening study showed no irritation at any of the test article concentrations tested, including the maximum soluble concentration of 50%. No irritation was detected following treatment of the PEG 400 vehicle, whereas 25% HCA in PEG 400 elicited irritation. Concentrations of 5%, 25% and 50% of the test article were chosen for the definitive Local Lymph Node study by the sponsor.

For the definitive study, three separate groups of five healthy female CBA/J mice were treated with increasing concentrations of Tea Tree Oil by topical application to the dorsum of each ear, once daily for three consecutive days. A vehicle control group of five mice was treated with PEG 400 and another group of five mice were treated with the positive control, 25% HCA (in PEG 400), in exactly the same manner.

Five days following the initial dose, and five hours prior to sacrifice, the mice were given an intraperitoneal injection of the thymidine analog 5-bromo-2'deoxy-uridine (BrdU), and at sacrifice the auricular lymph nodes were isolated and single-cell suspensions of lymph node cells (LNC) were generated. For each animal, the LNC suspension was analysed for BrdU incorporation and total number of LNC by flow cytometry. The amount of proliferating (BrdU+) LNC was determined as a measure of the proliferative response of the local lymph node. The stimulation index (SI) was calculated by dividing the proliferative response (BrdU incorporation) of each test article group by the proliferative response of the vehicle control group. Test articles that yielded a SI $\geq$ 3 were characterized as sensitizing substances.

All animals survived the in-life phase of the study and appeared normal. Body weight changes were normal. Ear swelling measurements and individual animal observations indicated that none of the treatments resulted in dermal irritation.

The SI of the positive control, 25% HCA, was 21.2, similar to 25% HCA in more common vehicles. The SI values for the test article at 5%, 25% and 50% were 2.1, 7.7 and 7.9, respectively. Since topical application of the test article at 25% and 50% in PEG 400 resulted in a stimulation index greater than 3, this test article is considered to be a dermal sensitizer in the Local Lymph Node Assay. The EC3 for this test article was calculated to be 8.3%, which would classify it as extremely weak for sensitizing potency.

> *Publication (Review): Larsen, J.R. and Borling, P. (2000), Tea Tree Oil. Safety aspects. Danish Toxicology center (DTC), Denmark. Publication evaluated under Reg. 91/414 and presented in the Draft Assessment Report of TTO (2007), Vol. 3, Annex B.6.*

This review of the toxicity of TTO contains references to 35 peer reviewed scientific publications. Those addressing effects on skin after topical application are reported in the following.

28 volunteers were treated with 25% TTO, containing 1.5-28.8 of 1,8-Cineole. Irritancy was not detected, however, 3 of the 28 had severe allergic response.

Seven patients with pre-existing skin conditions were patch-tested with 11 constituents of TTO.

All seven patients were reactive to 1% TTO. In addition, six reacted to Limonene, five to α-terpinene and aromadendrene, two to terpinen-4-ol and one each to p-cymene and α-phellandrene.

In Denmark since 1991 more than 30 cases of patients sensitized by using TTO topically have been documented. About 5 new cases of allergy to TTO out of 1000 patients are seen per year.

Oxidised TTO caused three times stronger reaction than freshly distilled solutions, monoterpene fraction was stronger sensitizer than the sesquiterpene fraction and the sensitizing constituents were p-cymene, aromadendrene, ascaridol, terpinolene and α-terpinene. TTO undergoes photooxidation within a few days to several months, leading to creation of sensitizing degradation products (*e.g*. ascaridol).

A 45-year-old man with a long history of dermatitis was dermally treated with undiluted TTO and experienced worsening of his dermatitis after an initial improvement. He was then advised to ingest the oil mixed with honey (dose unknown), which resulted in obvious exacerbation of the dermatitis. A patch test with the main ingredients of Tea Tree Oil revealed 1,8-cineole to be the actual allergen.

A 33-year-old woman, who had been treating her acne for several years with Tea Tree Oil, was presented with a 1-week history of dermatitis. Patch test showed a reaction at the site of Tea Tree Oil and at the site of colophony.

Cross reaction between colophony and oil of turpentine has previously been reported. The actual allergen in Tea Tree Oil proved to be 1,8-cineole.
A 74-year-old man developed contact dermatitis from Tea Tree Oil in wart paint within 24 hours of application. Patch test showed a reaction towards Tea Tree Oil (1 %) and also to fragrance mix, but no reactions were seen to the individual constituents of Tea Tree Oil that were tested.
The RMS concluded that this publication can be relied upon and that the effect of TTO is related to skin irritation and sensitization in humans after dermal contact. The symptoms initially observed were reversible and recovery was demonstrated. On the basis of effects observed in humans classification Xi, R43 (May cause sensitisation by skin contact) was proposed. Fresh TTO seems to be better tolerated so a date of minimum durability should be considered.
Further literature studies of single TTO components were provided as supportive data. It was described that in LLNA (or PLNA) the tested terpenes can be considered as weak or non-sensitizing.

In the following table, the test results are compared to the results in the ECHA C&L database.

Compound	**Test results**	**ECHA C&L database**
α-terpineol (purity 95%)	not irritating not sensitizing (PLNA)	Skin irrit. 2
α-Terpinene CAS no.: 99-86-5 (content in TTO 5-13%)	pure: moderate sensitizer, strong sensitizer after bioactivation and autoxidation	RAC Opinion no CLH-O-0000001412-86-274/F: Skin Sens. 1
p-Cymene	weak sensitizer	Skin irrit. 2
1,8-cineole	not sensitizing (PLNA)	Skin-sens. 1B
Limonene CAS no.: 138-86-3 (content in TTO 0.5-1.5%)		Harmonised classification - Annex VI of CLP Regulation: Skin Sens. 1

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

In accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments), a sensitising potential of a substance is identified, if response in a guinea-pig maximisation tests at least 30% of the animals is considered as positive (redness score ≥ 1).

Within the available GLP compliant GPM test, no erythema was observed in any of the test animals after intradermal induction with a test item concentration of 25 % (w/v). In a second GPMT which was not reported in such detail, but which was also performed according to the method by Magnusson & Kligmann, there were again no animals with signs of erythema.

Hence, since no positive reactions were observed in GPMT for >30% of treated animals the criteria for classification with regard to skin sensitization are not met for Tea Tree Oil.

It is noted that four LLNA are available for TTO in REACH registration dossier. TTO concentrations tested in LLNAs were between 2-100%. The stimulation index obtained in the LLNAs at concentration of 2% was between 1.6 – 2.4. The EC3 values for TTO tested in LLNAs at concentrations above 2% and up to 100% ranged between 4.4% and 25.5% thus TTO meets classification criterion as a skin sensitiser. Noting that subcategory 1A can be excluded since at concentrations of 2% TTO did not induced stimulation index above 3, the substance warrants classification Skin Sens.1B because the EC3 values found in several LLNA were above 2%.

It is also noted that α-Terpinene present in TTO at concentrations 5-13% has been classified as Skin Sens.1 in the RAC Opinion No CLH-O-0000001412-86-274/F and Limonene present in TTO at concentrations of 0.5-1.5% has harmonised classification as Skin Sens.1 (Annex VI of CLP Regulation)

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Based on all provided data, classification as Skin Sens. 1B, with hazard statement H317: May cause an allergic skin reaction is proposed for Tea Tree Oil

2.6.2.8 Phototoxicity

According to Regulation (EC) No 1107/2009, a phototoxicity study with the active substance Tea Tree Oil is required if the UV/VIS molar extinction coefficient (ε) of the active substance is > 10 L x mol^{-1} x cm^{-1} at 290-700 nm.
The UV spectra of the marker components of Tea Tree Oil have been measured.
None of the Tea Tree Oil components noteworthily absorb at > 290 nm in neutral aqueous media (at pH 6), as shown in the representative UV spectra.
Accordingly, a phototoxicity study with Tea Tree Oil is not required.

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

Table 20: Summary table of evidence for aspiration hazard

	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
experimental study OECD TG 114 GLP	Melaleuca alternifolia, ext., purity 100%	The viscosity of Tea Tree Oil was determined in triplicate at 20°C and 40°C using a reverse flow viscometer.	Kinematic viscosity of tea tree oil: 2.86 mm2/s at 20°C 1.71 mm2/s at 40°C Dynamic viscosity: 2.54 mPa/s at 20°C 1.52 mPa/s at 40°C	Comb (2007) (REACH registration dossier, 2020)[31]

Tea Tree Oil is a substance having complex composition of several components (please refer to point 1.1), that can be attributed to different chemical subgroups.
According to information provided on ECHA website several components of TTO have been notified with classification and labelling for aspiration hazard according to Regulation (EC) No. 1272/2008 by different notifiers. The content of these components in Tea Tree Oil may vary between 19 and 68% (see Table 64).

Table 21: Tea Tree Oil components: occurrence in the extract (acc. to ISO 4730:2004) and allocation to chemical groups (hydrocarbons as defined above are underlined) with data on theirs classification under Regulation (EC) No. 1272/2008 (acc. to substance information on ECHA website: https://echa.europa.eu)

	Name	Min. %	Max. %	C&L as Asp. Tox. 1, H304
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	**γ-Terpinene**	**10**	**28**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >90% notifications)
	α-Terpinene	**5**	**13**	Classified as Asp. Tox. 1 according to RAC opinion proposing harmonised classification and labelling of alpha-terpinene (CAS No: 99-86-5) No: CLH-O-0000001412-86-274/F (Adopted 15 March 2019)
	α-Terpinolene	**1.5**	**5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 100% notifications)
	Limonene	**0.5**	**1.5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 50% notifications)
	p-Cymene	**0.5**	**8**	Classified as Asp. Tox. 1 according to RAC opinion proposing harmonised classification and labelling of p-cymene

[31] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20921/4/23

	Name	Min. %	Max. %	C&L as Asp. Tox. 1, H304
				EC Number: 202-796-7 (CAS No: 99-87-6) No: CLH-O-0000001412-86-273/F (Adopted 15 March 2019)
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	30	48	Not classified
	α-Terpineol	1.5	8	Not classified
Bicyclic monoterpenes	1,8-Cineole (Eucalyptol)	trace	15	Not classified
	α-Pinene	**1**	**6**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >75% notifications)
	Sabinene	**trace**	**3.5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >60% notifications)
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	trace	3	Not classified
Polycyclic sesquiterpenes Aromadendrene group	**Aromadendrene**	**0.5**	**3**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 100% notifications)
	Ledene (Viridiflorene)	trace	3	Not classified
Polycyclic sesquiterpenes, Aromadendrene group, Alcohols	Globulol	trace	1	Not classified
	Viridiflorol	trace	1	Not classified

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

Based on information on aspiration hazard of Tea Tree Oil and its constituents TTO should be classified in Category 1 for aspiration toxicity in accordance with the Regulation (EC) No 1272/2008.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Under Regulation (EC) No. 1272/2008: "where the mixture itself has not been tested to determine its aspiration toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazard of the mixture, these data shall be used in accordance with the bridging principles set out in section 1.1.3. of Annex I to CLP regulation. However, in the case of application of the dilution bridging principle, the concentration of aspiration toxicant(s) shall be 10% or more". Taking into account that concentration of aspiration toxicants in Tea Tree Oil is higher than 10% (19 - 68%) the TTO should be classified for aspiration toxicity, too.

In the TTO REACH registration dossier 2020 (https://echa.europa.eu/) kinematic viscosity of tea tree oil (containing >10% hydrocarbons) is 1.71 mm^2/s at 40°C (Comb (2007). According to Regulation (EC) 1272/2008 mixture which contains a total of 10 % or more of a substance or substances classified in Category 1, and has a kinematic viscosity of 20,5 mm 2 /s or less, measured at 40 o C, shall be classified in Category 1.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

The classification for Aspiration Hazard Cat. 1 (Asp. Tox. 1, H304: May be fatal if swallowed and enters airways) for Tea Tree Oil is warranted.

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

The acute studies that are relevant for the assessment of the specific target organ toxicity of Tea Tree Oil after single exposure are reported in sections dealing with assessment of acute toxicity

Table 22: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reliability score	Reference
Acute oral toxicity study in rats OECD 425 (2008) Acute Oral Toxicity Up-and-Down Procedure GLP Rat, Wistar, Hsd Han: females, 3/group	Tea Tree Oil Purity: 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (in compliance with ISO specification) Administration: oral, single dose Dose levels: 550 mg TTO/kg (Group 1) and 2000 mg TTO/kg (Group 2) Observation period 14 days	LD_{50} 1049 mg/kg bw Clinical signs: 550 mg TTO/kg: no clinical signs or mortality 2000 mg TTO/kg: Hypoactivity, slight tremors recumbeny, death on day 1 – 2 after dosing.	1	Anonymous (2015)
Acute oral toxicity of Tea Tree Oil in the rat OECD 401 Rat, Sprague Dawley SPF rats - Specific Pathogen-free and non-SPF-rats males, females, 5/group GLP not stated	Tea Tree Oil Purity: not stated Administration: oral, single dose Dose levels: 3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) Observation period 14 days	LD_{50} 2.6 mL/kg bw in SPF rats 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non-SPF rats Clinical signs: Surviving SPF and non-SPF rats: lack of tonus in the forelimbs, weeping eyes, bloodied noses.	2	Anonymous 1989a and ECHA dissemination site[32]
Tea Tree Oil: Acute dermal toxicity study in Wistar rats OECD 402 (1987) GLP Rats, Wistar male, female, 2 groups, 5/sex	Tea Tree Oil Purity: 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (in compliance with ISO specification) Administration: dermal, Undiluted test item, Dose level: 2000 mg/kg bw (2.24 ml/kg bw) Exposure duration: 24 hours	LD_{50} > 2000 mg/kg bw	1	Anonymous (2015b)
Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit OECD 402 Rabbit, New Zealand White 5 males and 5 females	Tea Tree Oil Undiluted test item, 2000 mg/kg bw 24 hours	LD_{50} > 2000 mg/kg bw Clinical signs: slight diarrhoea in 1/10 animals on day 3	2	Anonymous 1989b and ECHA dissemination site[33]
Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 %	The acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats	1	Anonymous (2010a)

[32] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2
[33] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/4

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reliability score	Reference
OECD 403 (2009) GLP Rats, Wistar HsdCpb: WU males, females, 5 per group	1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Administration: aerosol Dose levels: 30%, 50% and 70% w/v aerosol of the test item (0.77 ± 0.10 mg/L, 3.69 ± 0.41 mg/L and 5.06 ± 1.13 mg/L air) Exposure duration: Continuous exposition for 4 hours	was established to be 3.64 mg/L of air for both male and female rats. Clinical signs: Wet fur was recorded both during and for several hours after exposure, whilst fur staining on the head was recorded on removal from restraint and persisted for several days. In addition, fur staining by the test item was detected in all groups during exposure		

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

Two acute oral studies were available to assess the specific target organ toxicity of Tea Tree Oil upon single exposure.

Study 1, Anonymous (2015a), Tea Tree Oil: Acute oral toxicity study (Up-and-Down Procedure) in Wistar rats, OECD 425, GLP.

In an acute oral toxicity study from 2015 (see section 10.1), no mortality and clinical signs were observed in all the animals dosed with 550 mg TTO/kg bw (Group 1) throughout the entire 14-day observation period.
The rats dosed with 2000 mg TTO/kg bw (Group 2) exhibited clinical signs such as hypoactivity and slight tremors and died on day 1 or day 2.
All the surviving rats gained weight during the 14-day observation period. The pre-terminal dead rats lost weight when compared to their initial body weight. No abnormalities were observed at necropsy.

Study 2, Anonymous (ECHA dissemination site), Acute oral toxicity of Tea Tree Oil in the rat, OECD 401.

In the second oral toxicity study according to OECD guideline 401, the LD_{50} of TTO was determined in two environmentally derived types of Sprague-Dawley rat. There was a difference in toxicity observed between the two sources of rat. The LD_{50} was found to be 2.6 mL/kg bw in SPF rats and 1.9 mL/kg bw (≈ 1682 – 1721 mg/kg bw) in non-SPF rats, respectively. In rats with different environmental status (i.e. SPF and non-SPF, respectively), no sex-specific difference in the sensitivity towards the test material was observable. The major reaction caused by TTO was complete lack of muscular tone in forelimbs. The surviving animals, however, recovered after few days.

Study 1, Anonymous, (2015b) , Tea Tree Oil: Acute dermal toxicity study in Wistar rats, OECD 402 (1987), GLP.

In an acute dermal toxicity study no clinical signs and mortality were observed after application of the undiluted test item at dose of 2000 mg/kg bw (2.24 mL/Kg bw). All rats gained weight during the experimental period. No abnormalities were detected at the necropsy.

Study 2, Anonymous, (ECHA dissemination site), Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit, OECD 402.

In the second dermal toxicity test in rabbits from 1989, no mortality was observed and there were no other signs of toxicity or abnormal behaviour. No significant loss of weight was observed during the observations period.

Study 3, Anonymous, (2010a), Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats, OECD 403 (1987), GLP.

In an acute inhalation toxicity study the acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats was determined to be 3.64 mg/L of air for both male and female rats.
In the control group G1, no toxic signs were recorded throughout the observation period. Toxic signs such as nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnea, perineum wet with urine, dullness and recumbency were observed in the treated rats (G2, G3 and G4).
The body weights of all surviving animals in the control and the test item groups were increased throughout the observation period. Body weight loss was noted for all dead animals in the test item groups (G2, G3 and G4).
Lung congestion was observed at necropsy in one pre-terminally dead rat of the treated group G4. No abnormalities were found at macroscopic *post mortem* examination of the other animals.

No human data are available

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

According to the Regulation (EC) No 1272/2008 (CLP), specific target organ toxicity (single exposure) categories 1 and 2 is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture, which are not covered by the other hazard classes. Category 3 covers transient effects, occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE).
Categories 1 and 2
In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. There were no significant non-lethal toxic effects observable that would warrant a classification for specific target organ, single exposure.
After inhalation exposure, clinical signs such as nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnea, perineum wet with urine, dullness and recumbency were observed in the treated rats at doses of 3.69 mg/L (close to the LC_{50} value).
According to the CLP Guidance, care should be taken not to give a "double classification" for the same effect and as these effects occurred close to the lethal doses they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE.

Category 3
This evaluation is usually based primarily on human data. No human data is available for Tea Tree Oil. However, appropriate animal data, e.g. clinical signs or histopathology data from acute inhalation studies can also be used if available.
According to the CLP Guidance section 3.8.2.2, clinical signs (e.g. dyspnoea, rhinitis etc) and histopathology (hyperaemia, oedema, minimal inflammation, thickened mucous layer) observed in inhalation toxicity studies may justify classification for RTI and lethargy, lack of coordination, loss of righting reflex and ataxia observed in animal studies may justify classification for NE.
Dyspnoea was observed in the tested animals but was closely related to the acute toxicity caused by inhalation and is therefore covered by the classification of Tea Tree Oil as hazard category 4 when regarding inhalation exposure.

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

Tea Tree Oil does not need to be classified for STOT SE. Data conclusive but not sufficient for classification.

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]

The specific target-organ toxicity of Tea Tree Oil upon short-term repeated exposure has been investigated in 28-day and two 90-day studies in rats, and a 90-day study in dogs. A 28-day dermal toxicity study in rabbits is also available. Chronic/carcinogenicity studies in rats and mice are not available. Reproductive and developmental toxicity studies were also considered for STOT RE evaluation.

Table 23: Summary table of animal studies on STOT RE

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
28 day oral gavage rat (Sprague-Dawley) 5 m and 5 f/group (40 in total). OECD 407 EPA OPPTS 870.3050 GLP	Melaleuca alternifolia, ext., Purity: 100% Compliant with ISO 4730:2017	5 mg/kg bw/day (actual dose received) 15 mg/kg bw/day (actual dose received) 45 mg/kg bw/day (actual dose received) Vehicle: corn oil Exposure: At least 28 days (7 days/week).	No effects observed. NOAEL: 45 mg/kg bw/day (actual dose received) (male/female) based on: (test mat.)	1	ECHA dissemination site (2017b)[34]
28-days, feeding, rats (Wistar rats) Non-GLP	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in clompliance with ISO specification) Vehicle: Groundnut oil Administration: gavage Dose levels: 0, 62.5, 125, 250 mg/kg bw/day	NOEL = 62.5 mg/kg body weight/day	<u>At 125 mg/kg bw/day</u> • Degenerative changes in testes • Oligospermia • Epididymal cell debris • Pale liver • Hepatocyte vacuolation • ↑Liver weight <u>At 250 mg/kg bw/day</u> ↓ absolute and relative weights of testes and epididymides • Small sized epididymides and testes • Degenerative changes in testes • Aspermia • Pale liver • Hepatocyte vacuolation • Zona fasciculata hypertrophy (adrenals) • ↑Liver weight • ↑Adrenal weight *More detailed information in presented in Table 56.*	2	Anonymous (2010b) N896

[34] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/6/2

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
90-days, feeding, rats (Wistar rats – HsdCpb) OECD 408 GLP	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage Dose levels: 0, 30, 60, 120 mg/kg bw/day	Males: NOAEL (90 days) = 30 mg/kg bw/day Females: NOAEL (91 days) = 60 mg/kg bw/day	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms <u>At 120 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms ↓ absolute and relative weights of testes and epididymides -degenerative changes in seminiferous tubules -cell debris in tubular lumen of testes and atrophic appearance -sertoli cell vacuolation -sperm granuloma -cell debris in epidydimal duct lumen • Spleen vacuolation (minimal degree) • Tubular dilatation in kidneys (minimal degree) *More detailed information in presented in Table 57*	1	Anonymous (2011b) G7153
90-days, feeding, rats (Wistar rat - Hsd Han) OECD 408 GLP	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	Not estimated.	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms - Sperm granuloma - Oligospermia, - Single cell necrosis, - Luminal cell debris - Degeneration/atrophy of seminiferous tubules *More detailed information is presented in Table 58*	1	Anonymous (2016a) G11089

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
	Dose levels: 0, 60 mg/kg bw/day				
90-days oral, dogs (Beagle) OECD 409 GLP	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Sesame oil Administration: gavage Dose rates: 0, 30, 75/60, 180/20 mg/kg bw/day (dose reduction from test day 27 on due to signs of intoxication)	NOAEL (90 days) = 30 mg/kg bw/day	<u>At 75/60 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids <u>At 180/120 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids • Clinical signs (starting within 5 minutes after administration and lasting up to 20 minutes) • ↓Body weight/gain (180mg) • ↓Food consumption (180mg) *More detailed information is presented in Table 59-* *Table 61*	1	Anonymous (2018a) 34433

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
Two generation study in the rat OECD 416 Oral (gavage) GLP	Generation-P: 0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) ↓Body weight/gain (M) ↓Food consumption (M+F) *More detailed results are presented in* *Table 33- Table 37 (Section 10.10.1)*	1	Anonymous (2017b)
Prenatal Developmental Toxicity Study in the rat Oral (gavage) OECD 414 GLP Wistar rats – HsdHan Females	Tea Tree Oil Purity: 8.18 % α-Terpinene, 1.80 % 1,8-Cineole, 14.23 % γ-Terpinene, 3.86 % p-cymene and 41.73 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 75, 150 and 300 mg/kg/day and	NOAEL, maternal toxicity: 30 mg/kg/day NOAEL, fetal toxicity: 60 mg/kg/day	<u>60 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake <u>120 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake ↓Fetal weight <u>150 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake <u>300 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight	1	Anonymous (2012a)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
	0, 30, 60 and 120 mg/kg/day.		↓Maternal food intake ↑Resorptions *More detailed results are presented in* *49 - 50 (Section 10.10.5)*		
Prenatal Developmental Toxicity Study in the rabbit Oral (gavage) OECD 414 GLP New Zealand white rabbits 24/group	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 15, 30, and 75 mg/kg/day	NOAEL, maternal toxicity: 75 mg/kg/day NOAEL, fetal toxicity: 30 mg/kg/day NOAEL teratogenicity: 75 mg/kg/day	75 mg/kg day: ↑Post implantation loss *More detailed results are presented in Table 51-* *Table 52 (Section 10.10.5)*	1	Anonymous (2018b)

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

Study 1, Anonymous (2010b), Tea Tree Oil: 28-Day Repeated Dose Toxicity Study in Wistar Rats, Non-GLP.

Reliability statement: The study is conducted in accordance with OECD 407. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. However, not all required organs have been fixed for histopathological examination and the study reports no information as to GLP. Hence, it is considered reliable with restrictions (reliability score: 2).

28 days after oral application at doses of 62.5, 125, and 250 mg/kg bw/day, at the highest dose, decreased weights of testes and epididymides, degenerative changes in both organs and aspermia were observed. At 125 mg/kg bw/day, again degenerative changes in tested and epididymides and oligospermia were observed. Liver and adrenals were affected at the highest dose.

90 days after oral application at doses of 30, 60 and 120 mg/kg bw/day to rats, in males testes and epididymides were affected. At 120 mg/kg bw /day degenerative changes in testes and in epididymides became apparent and remain present after a recovery period of 28 days. At 60 and 120 mg/kg bw/day sperm number, motility and morphology were affected with a trend of recovery after 28 days. Spleen and kidneys were minimally affected.

Table 24: Clinical observations in Wistar rats during a 28-day repeated dose toxicity study with TTO

Parameter	**Concentration [mg/kg Bwt/day]**							
	Males				**Females**			
	0	**62.5**	**125**	**250**	**0**	**62.5**	**125**	**250**
No. of Animals per Concentration	6	6	6	6	6	6	6	6
Gross pathology								
Epididymides – small sized	0	0	0	2	NA	NA	NA	NA
Testes – small sized	0	0	0	2	NA	NA	NA	NA
Liver – pale, diffuse	0	0	4	1	0	0	0	2
Histopathology								
Adrenals- hypertrophy-zona fasciculata	0	0	0	0	0	0	0	0
minimal	---	---	---	3	---	---	---	2
Testes								
- degenerative changes-bilateral	0	0	4	6	NA	NA	NA	NA
minimal	---	---	4	3	NA	NA	NA	NA
mild	---	---	---	3	NA	NA	NA	NA
Epididymides - aspermia	0	0	0	5	NA	NA	NA	NA
- oligospermia	0	0	2	1	NA	NA	NA	NA
- cell debris in lumen	0	0	4	0	NA	NA	NA	NA
Liver -hepatocyte vacuolation	0	1	2	6	1	0	1	5
minimal	---	1	2	4	1	---	1	5
mild	---	---	---	2	---	---	---	---
Organ weights								
Epididymides - absolute	-	-	-	↓(34)	NA	NA	NA	NA
- relative	-	-	-	↓(28)	NA	NA	NA	NA
Testes - absolute	-	-	-	↓(43)	NA	NA	NA	NA
- relative	-	-	-	↓(39)	NA	NA	NA	NA
Liver - absolute	-	-	-	-	-	-	↑(19)	↑(23)
- relative	-	-	-	↑(14)	-	-	↑(18)	↑(32)
Adrenals - relative	-	-	-	↑(18	-	-	-	↑(18)

↑: Statistically significant increase; ↓: Statistically significant decrease; -: no statistical significance

Study 2, Anonymous, (ECHA dissemination site), Tea Tree Oil: 90-day repeated dose toxicity study in Wistar Rats, OECD 408, GLP.

Reliability statement: The study is conducted in accordance with the former version of OECD 408. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. OECD 408 has been updated to place additional emphasize on measurement of endocrine endpoints, such as T3, T4, TSH, LDL, HDL. Since these requirements were not in place at the time of study conduction, the endpoints have not been measured. However, this does not impair the overall reliability and regulatory suitability of the study. Hence, the study is nevertheless considered reliable without restrictions (reliability score: 1).

Table 25: Clinical observations in Wistar rats during a 90-day repeated dose toxicity study with TTO

Inlife phase	**Treatment Period**				**Recovery Period**			
Concentration [mg/kg Bwt/day]	0	**30**	**60**	**120**	**0**	**120**	**0**	**120**
No. of Animals per Concentration	10	10	10	10	10	10	10	10
Sacrified on day	91				15		29	
Sperm evaluation (Males)								
Progressive motile sperms %	60.1	---	43.1 ↓* (28)	3.8 ↓ (94)	60.8	7.6 ↓ (88)	63.0	10.2 ↓ (84)
Motile sperms %	84.2	---	64.6 ↓* (23)	16.5 ↓ (80)	82.6	30.8 ↓ (63)	83.0	41.0 ↓ (51)
Sperm morphology (Males)								
Normal sperms	98.9	---	78.5 ↓ (21)	3.8 ↓ (96)	99.4	15.8 ↓ (84)	97.8	22.3 ↓ (77)
Abnormal sperms	106	---	21.5 ↑*	86.18 ↑	0.60	84.2 ↑	2.20	77.7 ↑
Sperm counts (Males)								
Cauda epididymis wt	0.247	---	---	---	0.255	---	0.258	0.199 ↓ (23)

Inlife phase	Treatment Period				Recovery Period			
Concentration [mg/kg Bwt/day]	0	**30**	**60**	**120**	**0**	**120**	**0**	**120**
No. of sperms per cauda epididymis	190.03	---	---	---	205.40	---	187.15	63.40 ↓ (66)
No. of sperms per gram of cauda epididymis	770.88	---	604.59 ↓ (22)	457.31 ↓ (41)	803.51	353.59 ↓ (56)	727.65	310.61 ↓ (57)
Gross pathology (Males)								
Epididymides – Abscess	0	0	0	6	0	4	0	1
Testes – small and flacid	0	0	0	1	0	0	0	4
- flaccid	0	0	0	3	0	0	0	0
- small	0	0	0	0	0	2	0	4
Histopathology (Males)								
Testes No. examined	10	10	10	10	5	10	5	10
Degenerative changes –								
seminiferous tubules	0	0	0	8	0	9	0	8
minimal	0	0	0	4	0	3	0	2
mild	0	0	0	2	0	4	0	4
moderate	0	0	0	1	0	2	0	2
marked	0	0	0	1	0	0	0	0
Sertoli cell vacuolation	0	0	0	9	0	10	0	9
minimal	0	0	0	7	0	6	0	5
mild	0	0	0	2	0	4	0	4
Sperm stasis	0	0	0	0	0	4	0	0
minimal	0	0	0	0	0	4	0	0
Epididymides No. examined	10	10	10	10	5	10	5	10
Sperm granuloma	0	0	0	4	0	6	0	1
Cell debris in lumen	0	0	1	7	0	9	0	9
minimal	0	0	1	6	0	1	0	2
mild	0	0	0	0	0	6	0	5
moderate	0	0	0	0	0	1	0	2
marked	0	0	0	0	0	1	0	0
Oligospermia	0	0	0	3	0	5	0	6
Aspermia	0	0	0	1	0	0	0	0
Kidneys No. examined	10	10	10	10	5	10	5	10
Dilatation of tubules	0	0	0	3	0	0	0	0
minimal	0	0	0	3	0	0	0	0
Spleen No. examined	10	10	10	10	5	10	5	10
Vacuolation	0	0	0	5	0	0	0	0
minimal	0	0	0	5	0	0	0	0
Organ weights (Males and females)								
Epididymides (males)								
- absolute	1.497	---	---	---	1.561	---	1.521	1.154 ↓ (24)
- ratios to Bwt	0.382	---	---	---	0.400	---	0.390	0.290 ↓ (26)
Testes (males)								
- absolute	3.787	---	---	---	3.808	---	3.760	2.855 ↓ (24)
- ratios to Bwt	0.970	---	---	---	0.974	---	0.967	0.717 ↓ (26)
Liver (females)								
- ratios to Bwt	2.766	---	3.077 ↑ (11)	3.025 ↑ (9)	---	---	---	---

↑: Statistically significant increase; ↓: Statistically significant decrease; *: Apparent decrease -: No statistical significance; Values in parenthesis indicate % change

Study 3: Anonymous (2016a) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats, OECD 408 (1998)

A second 90-day study especially designed with a prolonged recovery period (Anonymous (23), 2016) was performed in rats only at 60 mg/kg bw/day. Testicular and epididymal findings were comparable to those of the initial 90-day study in rats. All effects recovered after 8 weeks without dosing.

Table 26: Clinical observations in Wistar rats during a 90-day repeated dose toxicity study with TTO

Inlife Period		**Treatment Period**		**Recovery Period**					
Day of Sacrifice		**91**		**147 (8 weeks)**		**175 (12 weeks)**		**203 (16 weeks)**	
Concentration (mg/kg bw/day)		**0**	**60**	**0**	**60**	**0**	**60**	**0**	**60**
No. of Animals per Concentration		10	10	10	10	10	10	10	10
Sperm evaluation									
Motility	Progressive motile sperms %	68.20	19.40* ↓(72)	65.30	68.60	54.70	48.50	61.30	59.10
	Motile sperms %	94.10	83.00* ↓(12)	87.30	89.30	76.00	70.60	82.60	81.20
Morphology	Normal sperms	98.00	25.35* ↓(74)	97.20	96.25	86.90	85.39	96.60	95.30
	Abnormal sperms	2.00	74.65* ↑(38folds)	2.80	3.75	13.10	14.61	3.40	4.70
Cauda epididymal sperm counts	Cauda epididymis weight (g)	0.22	0.26	0.24	0.24	0.22	0.25	0.25	0.24
	No. of sperms per cauda epididymis (x 10^6)	198.53	127.88	189.70	181.38	158.03	214.35	214.40	201.05
	No. of sperms per gram of cauda epididymis	902.83	474.35* ↓(850)	799.00	767.03	672.36	785.78	858.89	835.89
Gross Pathology									
EPIDIDYMIDES – Abscess(es); bilateral; tail		0	1	0	0	0	1	0	0
– small; bilateral		0	0	0	0	1	0	0	0
TESTES – Enlarged; unilateral		0	1	0	0	0	0	0	0
– Small/flaccid; bilateral		0	0	0	0	1	2	0	0
– Focus (i); white; bilateral; multiple		0	0	0	0	0	1	0	0
Histopathology									
LEFT EPIDIDYMIS No. examined		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Sperm granuloma; cauda; focal		0	2	0	0	0	1	0	0
mild		—	1	—	—	—	—	—	—
moderate		—	1	—	—	—	1	—	—
Oligospermia		0	5	0	0	1	2	0	0
minimal		—	5	—	—	—	—	—	—
moderate		—	—	—	—	1	2	—	—
Cellular debris in duct lumen		0	1	0	0	1	2	0	0
minimal		—	1	—	—	—	1	—	—
mild		—	—	—	—	1	1	—	—
Single cell necrosis; caput		0	1	0	0	0	0	0	0
minimal		—	1	—	—	—	—	—	—
Chronic active inflammation; cauda		0	1	0	0	0	0	0	0
minimal		—	1	—	—	—	—	—	—
TESTES No.examined		(10)	(10)	(-)	(-)	(1)	(2)	(-)	(-)
Degeneration/atrophy; seminiferous tubule; unilateral		0	1	0	0	0	0	0	0
mild		—	1	—	—	—	—	—	—
Degeneration/atrophy; seminiferous tubule; bilateral		0	0	0	0	1	2	0	0
mild		—	—	—	—	—	1	—	—
moderate		—	—	—	—	1	—	—	—
severe		—	—	—	—	—	1	—	—

Inlife Period	**Treatment Period**		**Recovery Period**					
Day of Sacrifice	**91**		**147 (8 weeks)**		**175 (12 weeks)**		**203 (16 weeks)**	
Concentration (mg/kg bw/day)	**0**	**60**	**0**	**60**	**0**	**60**	**0**	**60**
No. of Animals per Concentration	10	10	10	10	10	10	10	10
Hypertrophy/hyperplasia; interstitial cell; bilateral	0	0	0	0	1	2	0	0
minimal	—	—	—	—	1	2	—	—
Sperm granuloma; bilateral	0	0	0	0	0	2	0	0
minimal	—	—	—	—	—	1	—	—
moderate	—	—	—	—	—	1	—	—

↑: Statistically significant increase; ↓: Statistically significant decrease; *: Statistically significant; Values in parenthesis indicate % change

Study 4, Anonymous , (2018a), Repeated dose 90-Day oral toxicity study of Tea Tree Oil in Beagle dogs, OECD 409, GLP.

Reliability statement: The study is conducted in accordance with OECD 409. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

In a 90-day studies performed in dogs at 0, 30, 75/60, 180/20 mg/kg bw/day, viability and motility of the canine spermatids were decreased at medium and high concentrations. No further signs of toxicity were observed.

Table 27: Changes in sperm viability and motility of Beagle dogs treated with Tea Tree Oil compared to the control group at the end of the treatment period (test week 13)

Parameter / Concentration		**Control**	**75/60 mg/kg**	**180/120 mg/kg**
Sperm Viability %	Alive / Dead	83.4 / 16.6	54.8 / 45.2*	63.1 / 36.9*
Sperm Motility %	Progressive motile sperms	54.5	9.7*	29.4*
	estimated motility	68.8	20.0*	43.0*
	Immotility	29.3	76.0*	55.6*

*: statistically significant at $p \leq 0.01$ (chi²-test)

Table 28: Statistically significant differences results of the sperm analysis in Beagle dogs treated with Tea Tree Oil compared to the control

Parameter	**Increase ↑ Decrease ↓**	**Concentration / Sex**	**Test day(s)**	**Statistical significance**	**Reason**
Weight of Ejaculate	↑	75/60 mg/kg / m	13	$p \leq 0.05$	A
Estimated Motility	↑	30 mg/kg / m	13	$p \leq 0.01$	A
Immotility	↓	30 mg/kg / m	13	$p \leq 0.01$	A

m: male; A: the slight alteration in comparison to control animals is without any biological relevance

Table 29: Food and drinking water consumption during a repeated dose 90-day oral toxicity study of Tea Tree Oil in Beagle dogs

Increase ↑ Decrease ↓	Concentration / Sex	Test week(s)	Statistical significance	Reason
Summary of the food and drinking water consumption during the treatment period				
↑	180/120 mg/kg / m	6 12	$p \leq 0.05$ $p \leq 0.01$	A A
↑	30 mg/kg / f	6 7	$p \leq 0.01$ $p \leq 0.05$	A A
↑	180/120 mg/kg / f	6, 13	$p \leq 0.01$	A
Summary of the food and drinking water consumption during the recovery period				
↑	180/120 mg/kg / f	15 16	$p \leq 0.05$ $p \leq 0.01$	A A

m: male; f: female; A: the slight alteration in comparison to control animals is without any biological relevance

Besides the adverse effects on reproduction described under Point 10.10, within the reproductive and developmental toxicity studies only (in part reversible) reduction in body weight, body weight gain and food consumption were described as further treatment related effects. The post-implantation loss observed in the developmental toxicity study in rabbits (Anonymous (33), 2018b) can be considered as a consequence of maternal toxicity adversely supported by extreme overexposure of TTO caused by gavage administration.

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

Regarding all available repeated dose toxicity studies, it becomes clear that Tea Tree Oil has a detrimental effect on spermatogenesis. However, as extensively discussed under Point 10.10., it is most likely that these effects were due to the administration type (gavage vs. dietary). Effects were seen in studies where Tea Tree Oil was administered by gavage. For other terpenes (which were also content of TTO) it was shown that sperm damage does not occur after dietary administration. Gavage administration can be regarded as a non-relevant route of exposure to humans. Furthermore, no exposure of TTO as a plant protection product to humans is expected since there is a no-residue situation of the treated crops. Therefore, no classification is warranted for STOT RE with respect to sperm impairment.
The repeated-dose toxicity of TTO by the oral route has been investigated in a 28-day and two 90-day studies in rats, a 90-day study in dogs, a 3-week dermal toxicity study in rats and by a two-generation toxicity study in rats. There were no long-term/chronic studies available for TTO. Although the developmental studies are also repeated dose toxicity studies, they were not considered for an assessment of STOT RE here. The studies lack histopathological examination of the dams and organ weights and necropsy were also limited. Taken together that extrapolation of effective doses using Haber's law can lead to large uncertainties, especially in studies with short exposure durations, and the toxicological gain is limited by the study design, it appeared reasonable not to include this study type for STOT RE evaluation. No human data is available.

In the following, effects on the liver after repeated dose administration of TTO are discussed:

In the non-GLP 28-day oral study in rats, liver weights were increased in the highest dose group (250 mg/kg bw/day) in males and at the mid (125 mg/kg bw/day) and high dose group in females. At the highest dose, minimal/mild liver vacuolation was observed histopathologically in both sexes (6 males and 5 females affected) at the highest dose and in males at the mid dose (2 animals affected).

In the 90-day oral study in rats relative liver weights were increased in females at the mid (60 mg/kg bw/day) and the highest (120 mg/kg bw/day) tested dose. There were no corresponding histopathological alterations found. After a recovery period of 8 weeks, liver weights were not different from control animals, indicating an adaptive response during test item application. In males, the liver remained totally unaffected.

A similar picture was observed within the second 90-day study in rats. Minimal liver weight increase at 60 mg/kg bw/day in males was then absent after a recovery period up to 16 weeks.

Within the 90-day study in beagle dogs, there were no liver effects observed in any treatment group which were different from the control animals.

In the parental animals (P and F1) within the two-generation toxicity study, there were minimal weight decreases observed in males and females without dose-dependency and without corresponding alterations in histopathology. The study authors did not consider these weight changes as toxicologically significant.

There is no information of liver effects after dermal application of TTO for 30 days.

Hepatic vacuolation as exclusively observed in the non-GLP 28-day study in rats is often described to be involved in adaptive processes to resist further insults by foreign substances. Also increases in liver weight, which is described in the 90-day and 28-day studies in rats, can be observed during adaptive processes after exposure to xenobiotics. The fact that after a short recovery period no increase in liver weight was observable any longer supports the assumption that both, vacuolation and weight increase are part of an adaptive process rather than an effect of toxicological significance.

As described in the guidance on the application of the CLP criteria[35], more weight should be usually given to studies of a longer duration (28 days or more) because animals may not have fully adapted to the exposure in studies of shorter durations and also because longer duration studies tend to include more thorough and extensive investigations (e.g. in terms of detailed pathology and haematological effects etc) which can generally give more substantial information compared to shorter duration studies. Since the available 28-day study in rats was not performed under GLP conditions, in the present case, the 90-day studies should clearly be given preference.

Taken together, within all available animal studies of sufficient reliability, no life-threatening changes (e.g. necrosis) have been observed in the liver. There were no further indications of functional impairment (e.g. increased serum levels of liver enzymes) after TTO administration. Therefore, the available data does not support a classification for specific target organ toxicity following repeated exposure.

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

No classification is warranted for STOT RE. Data conclusive but not sufficient for classification.

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

The genotoxic properties of Tea Tree Oil were investigated with *in vitro* tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and with an *in vivo* test for DNA damage (mouse micronucleus test).

Table 30: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
Bacterial reverse mutation test OECD 471 (1997) GLP	Tea Tree Oil 9.13% α-Terpinene, 1.55 % 1,8-Cineole, 18.45% γ-Terpinene, 1.66 % p-cymene and 40.50% Terpinen-4-ol	Test system: TA 98, TA 100, TA 1535 and TA 1537 strains of *Salmonella typhimurium* and WP2 uvrA (pKM 101) strain of *Escherichia coli*. Concentrations tested: Initial test: 50, 158, 500, 1581 and 5000 µg/plate (± S9) Confirmation assay: 100, 266, 707, 1880 and 5000 µg/plate (± S9) (Concentration selection based on a cytotoxicity pre-tests)	Tea Tree Oil was not mutagenic in this bacterial reverse mutation assay up to the highest tested concentration of 5000 µg/plate.	1	Anonymous (2010b)

[35] ECHA-17-G-21-EN; 10.2823/124801

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
	(analyzed)				
In vitro mammalian cell gene mutation test OECD 476 (1997) GLP	Melaleuca alternifolia, ext.	mouse lymphoma L5178Y cells Assay 1: - With S9-mix (3 h treatment): Cells evaluated at 100, 75, 50, 25, 10 and 5 µg/mL. - Without S9 mix (3 h treatment): Cells evaluated at 70, 60, 40, 20, 10 and 5 µg/mL Positive control substance(s): 4-nitroquinoline-N-oxide; cyclophosphamide Assay 2: - With S9-mix (3 h treatment): Cells evaluated at 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL - Without S9 mix (24 h treatment): Cells evaluated at 40, 30, 20, 10 and 5 µg/mL. Positive control substance(s): 4-nitroquinoline-N-oxide; cyclophosphamide	No mutagenic effect of tea tree oil nor any formed metabolites was observed either in the presence or absence of a metabolic activation system under the conditions of this Mouse Lymphoma Assay.	1	ECHA dissemination site(study report 2010)[36]
In Vitro Mammalian Chromosome Aberration Test OECD 473 (2016) GLP	Melaleuca alternifolia, ext.	Chinese hamster lung fibroblasts (V79) [mammalian cell line] (Met. act.: with and without) Experiment A with 3/20h treatment/ sampling time: - Without S9 mix: 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (Ethyl methanesulphonate): 1.0 µl/mL. - With S9 mix (50 µl/mL): 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (N-Nitrosodimethylamine): 1.0 µl/mL. Experiment B with 20/28h treatment/ sampling time: - Without S9 mix: 2.34 µl DMSO/mL (solvent control); 4.88, 9.76, 19.53 and 39.06 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). Metaphase	Tea Tree Oil tested up to cytotoxic concentrations, both with and without metabolic activation, did not induce structural chromosome aberrations in this test in V79 Chinese Hamster lung cells. Therefore, Tea Tree Oil and its metabolite(s) are not considered to be clastogenic in this test system.	1	ECHA dissemination site (study report 2009)[37]

[36]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2/?documentUUID=78a01fb0-6711-46b3-8e26-748d62eba1dc

[37] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
		analysis was not conducted in a treatment at 58.59 µg Tea Tree Oil/mL. Positive control (Ethyl methanesulphonate): 0.4 µl/mL. Experiment B with 3/28h treatment/ sampling time: - With S9 mix (50 µl/mL): 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (N-Nitrosodimethylamine): 1.0 µl/mL. Positive control substance(s): ethylmethanesulphonate			
Bacterial Reverse Mutation Test comparable to guideline study with acceptable restrictions GLP no	Tea Tree Oil	Test system: TA98, TA100 and TA102 strains of *Salmonella typhimurium*. Concentrations tested: 10, 25, 50, 100, 150 µl µg/plate (± S9) Confirmation assay: 100, 266, 707, 1880 and 5000 µg/plate (± S9) (Concentration selection based on a cytotoxicity pre-tests)	Tea Tree Oil was negative with regard to the mutagenicity in the presence or absence of metabolic activation in tests with *Salmonella typhimurium* strains TA98, TA100 and TA102, respectively.	2	ECHA dissemination site (1989)[38]
Tea Tree Oil: *In Vitro* Mammalian Cell Gene Mutation Test in CHO Cells OECD 476 (1997) GLP	Tea Tree Oil 9.7% α-Terpinene, 17.8% γ-Terpinene, 2.6% 1,8-Cineole, 1.5% p-Cymene and 41.5% Terpinen-4-ol	Test system: Chinese hamster Ovary cells (CHO) Initial /confirmatory gene mutation assays Concentrations tested: - S9: 8/7, 19/17, 41/39 and 90 µg/mL + S9: 8/7, 19/17, 41/39 and 90 µg/mL (Concentration selection based on a cytotoxicity pre-tests)	There was no evidence of induction of gene mutations in any of the test item treated cultures either in the presence or absence of metabolic activation.	1	Anonymous (2015f)
In vitro mammalian micronucleus test (Similar to OECD 487) GLP not stated	Tea Tree Oil (from *Melaleuca alternifolia* leaves) Terpinen-4-ol (42.8%), γ-terpinene	Test system: human lymphocyte cultures Concentrations tested: 95, 182, 365 µg/mL (Concentration selection based on a cytotoxicity pre-tests, determined by reduction in mitotic index) No information on metabolic activation	None of the tested TTO concentrations caused significant increase in the observed frequencies of micronuclei when compared to those	2	Pereira, T.S., (2014)

[38] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2/?documentUUID=1ea9fce2-9169-4571-b7f3-8dbad1740e15

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
	(20.4%), p-cymene (9.6%), α-terpinene (7.9%), 1,8-cineole (3%), α-terpineol (2,8%) and α-pinene (2.4%)		in negative control.		
In vitro mammalian chromosomal aberration test (similar to OECD 473) GLP not stated	Tea Tree Oil (from *Melaleuca alternifolia* leaves) Terpinen-4-ol (42.8%), γ-terpinene (20.4%), p-cymene (9.6%), α-terpinene (7.9%), 1,8-cineole (3%), α-terpineol (2,8%) and α-pinene (2.4%)	Test system: human lymphocyte cultures Concentrations tested: 95, 182, 365 µg/mL (Concentration selection based on a cytotoxicity pre-tests, determined by reduction in mitotic index) No information on metabolic activation	No significant differences regarding the frequency of chromosome aberration were observed compared to those in negative control.	2	Pereira, T.S., (2014)

Table 31: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
In vivo micronucleus test of Australian Tea Tree Oil (*Melaleuca alternifolia*) Protocol based on OECD 474 (1997) GLP	Tea Tree Oil	The mutagenicity of Tea Tree Oil was investigated *in vivo* in somatic cells. The test item TTO was administered orally at 3 dose levels (1000 (10% w/w), 1350 and 1750 mg/kg) to 4 groups of 10 animals (5 males and 5 females). The vehicle (corn oil), as	The results of the *in vivo* mouse bone marrow micronucleus assay indicated that TTO did not increase the number of micro nucleated PCE up to and including cytotoxic doses as shown by a statistically significant depression of the ratio of polychromatic erythrocytes to total erythrocytes. Thus, TTO is not mutagenic *in vivo*. In addition, the results indicated that the	1	Anonymous (2005)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
		a control is tested parallel (20 animals treated with vehicle at 10.61 mL/kg). The positive control (DMBA) was administered by IP injection at 40 mg/kg (8.8 mg/mL). The animals treated with the test item and the control were sacrificed after 24 and 48 hours. 10 animals treated with the positive control were sacrificed 48 hours after administration.	highest dose of TTO (1750 mg/kg) was however toxic to the tested animals.		

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

The genotoxic potential of Teat Tree Oil has been investigated in several *in vitro* and *in vivo* studies. Following table summarizes the results.

Table 32: Summary of genotoxicity testing of Tea Tree Oil

Parameter	Concentration	Results	Reference
***In vitro* studies**			
Bacterial Reverse Mutation Test	50, 158, 500, 1581 and 5000 µg/plate (+/- S9)	Negative	Anonymous (2010b)
Bacterial Reverse Mutation Test	50 µg/plate (+/- S9)	Negative	ECHA dissemination site
Mammalian Cell Gene Mutation Test	8, 19, 41 and 90 µg/mL (+/- S9)	Negative	Anonymous (2015f)
In vitro mammalian chromosomal aberration test	Exp. 1 (3/20 h treatment): 9.76, 19.53, 39.06 and 58.59 µg /mL (+/- S9) Exp. 2 (20/28 h treatment): 4.88, 9.76, 19.53 and 39.06 µg /mL (- S9) Exp. 3 (3/28 h treatment: 9.76, 19.53, 39.06 and 58.59 µg /mL (+ S9)	Negative	ECHA dissemination site
In vitro mammalian cell gene mutation test	Assay 1: 100, 75, 50, 25, 10 and 5 µg/mL/70, 60, 40, 20, 10 and 5 µg/mL (+ S9/-S9) Assay 2: 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL/40, 30, 20, 10 and 5 µg/mL (+ S9/-S9)	Negative	ECHA dissemination site

Parameter	Concentration	Results	Reference
In vitro mammalian micronucleus test	95, 182, 365 and 548 µg/mL	Negative	Pereira *et al.* (2014)
In vitro mammalian chromosomal aberration test	95, 182, 365 and 548 µg/mL	Negative	Pereira *et al.* (2014)
***In vivo* studies**			
Mouse Micronucleus Test	1000 (10% w/w), 1350 and 1750 mg/kg bw	Negative	Anonymous (2005)

The potential of Tea Tree Oil to induce genotoxicity was investigated *in vitro* and *in vivo*. Bacterial gene mutation was negative under GLP and non-GLP conditions with and without metabolic activation. Tea Tree Oil was also tested negative for mammalian cell gene mutation in a guideline conform study. With respect to clastogenicity, two *in vitro* studies were performed. In both, a mammalian micronucleus test and a mammalian chromosomal aberration test, Tea Tree Oil did not show the potential to induce DNA damage *in vitro*. In order to determine whether Tea Tree Oil is able to induce genotoxicity *in vivo*, a mouse micronucleus assay in bone marrow was performed. The test followed an in-house experimental procedure which was based on the OECD GD 474 under GLP conditions. Bone marrow exposure of the absorbed Tea Tree Oil was proven as seen in the significant depression of the PCE and PCE+NCE ratio indicating bone marrow toxicity in high dose animals 48 h after dosing, and also inferred from systemic toxicity (wobbly gait, laboured breathing, rough coat) and ADME studies. Mice treated with the test item at any dose did not reveal an increase in the incidence of micronuclei (MPCEs) when compared with the negative vehicle control at 24 and 48 hours sampling times. The positive control DMBA induced statistically significant increase in the frequency of MPCEs compared to the vehicle control, demonstrating the validity of the test method. It can therefore be concluded that Tea Tree Oil is not clastogenic *in vivo*.
All in all, there are no indications, neither *in vitro* nor *in vivo* that Tea Tree Oil induces gene mutations or DNA damage. The substance can therefore be considered as non-genotoxic.

Photomutagenicity testing
According to Regulation (EC) No 1107/2009, a photomutagenicity testing with an active substance is required if the UV/VIS molar extinction coefficient (□) of the active substance is > 1000 L x mol^{-1} x cm^{-1} at 290-700 nm.
The UV spectra of the marker components of Tea Tree Oil have been measured. None of the Tea Tree Oil components noteworthily absorb at > 290 nm in neutral aqueous media (at pH 6). Accordingly, a photomutagenicity testing with the active substance Tea Tree Oil is not required.

For the evaluation of genetic toxicity of Tea Tree Oil several guideline- and GLP-studies were available - sufficient to evaluate the genotoxic potential of TTO. Therefore, studies found in open literature for the single terpene compound of Tea Tree Oil were only briefly summarized below.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 33:

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
In vitro						
γ-Terpinene	Single-cell gel electrophoresis (basic alkaline comet assay)	Human lymphocytes	0.00005 mM 0.0005 mM 0.005 mM 0.025 mM 0.05 mM 0.1 mM 0.2 mM 0.5 mM 1 mM	No increase in DNA strand breakage was observed at concentrations below 0.1 mM, but at higher concentration of 0.2 mM significant increase in DNA damage was seen.	2	Aydin, S., Basaran, A. A., Basaran, N. (2005)
γ-Terpinene	Single-cell gel electrophoresis (comet assay)	Human lymphocytes	0.00005 mM 0.0005 mM 0.005 mM 0.025 mM 0.05 mM 0.1 mM 0.2 mM 0.5 mM 1 mM	Concentrations above 0.1 mM significantly induced DNA damage in human lymphocytes, but at the lower concentrations no additional DNA strand breakage has been observed.	2	Aydin, S., Basaran, A. A., Basaran, N. (2005)
α-Terpinene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found to be free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
Limonene	Single-cell gel electrophoresis (alkaline comet assay) Micronucleus (MN) assays	Human lymphocytes and V79 cells(Comet assay) V79 cells (MN assay)	2000 µM 4000 µM 6000 µM 8000 µM 10000 µM 12000 µM 14000 µM 16000 µM 18000 µM 20000 µM	Limonene at a concentration below 10000µM has no exerted genotoxic affects in lymphocytes and in V79 cells.	2	Bacanli, M., Basaran, A. A., Basaran, N. (2015)
Limonene	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	0.04 – 0.15 µmol/plate	The test substance was lacking a mutagenic effect.	2	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)
Limonene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test) SMART assay	*Drosophila melanogaster* strains	0.011 mM 0.73 mM	The test substance was non-mutagenic at lowest concentration. Nevertheless, Limonene was mutagenic in the SMART assay at the highest concentration.	2	Fernández-Bedmar, Z., Anter, J., Cruz-Ares, S., Muñoz-Serrano, A., Alonso-Moraga, À., Pérez-Guisado, J. (2011)
Limonene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test) SMART assay	*Drosophila melanogaster* strains	1.5 µL/mL 2.5 µL/mL 5.0 µL/mL	Statistical analysis of the study did not show clearly negative activity in the case of Limonene leading to an inconclusive result at the highest dose. Negative mutagenic activity was proven at the lower doses.	2	Mademtzoglou, D., Akmoutsou, P. Kounatidis, I., Franzios, G., Drosopoulou, E., Vokou, D. Mavragani-Tsipidou, P. (2011)
α-Terpineol	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	1.1 – 4.3 µmol/plate	The test substance was lacking a mutagenic effect.	1	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)
α-Terpineol	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found to be free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
1.8-Cineole	Single-cell gel (comet) assay	Mouse lymphoma cells	1.25 µL/mL	1.8-Cineole did not induce DNA strand breaks. It is therefore not likely to increase the level of DNA damage on mammalian cells.	2	Ribeiro, D. A., Marques, M. E. A., Salvadori, D. M. F. (2006)
1.8-Cineole	Single-cell gel (comet) assay	Chinese hamster ovary cells	1.25 µL/mL	1.8-Cineole did not induce DNA breakage at 1.25 µL/mL concentration. The results suggest that 1.8-Cineole may not be a factor that increases the level of DNA lesions in mammalian cells	2	Ribeiro, D. A., Matsumoto, M. A., Marquez, M. E. A., Solvadori, D. M. F. (2007)
1,8-Cineole	Alkaline and neutral comet assay	Human coloretal cancer cell line HCT116 Hamster fibroblast cell lines AA8, RAD51D1, V79-2 and VC8	5 µM 50 µM 200 µM	No increase in DNA strand break formation was observed in response to 1,8-Cineole treatment.	2	Dörsam, B, Wu, C., Efferth, T., Kaina, B, Fahrer, B. (2015)
1,8-Cineole	Alkaline comet assay	Vero cell line obtained from the kidney of a normal adult African green monkey *E. coli* bacterial cells	5 µL/plate 7.5 µL/plate 10 µL/plate 15 µL/plate	The analysis of tail moment indicated no genotoxicity up to 10 µM. However, at higher concentrations the indication of genotoxicity was obtained.	2	Nikolić, B., Mitić-Ćulafić, D., Vuković-Gačić, B., Knežević-Vukčević, J. (2011)
1,8-Cineole	Mutagenicity assay	*E. coli* WP2 strains IC185 *trpE65* and its derivative IC202 *trpE65oxy R/*PKM101	0.05 mg/plate 0.1 mg/plate 0.5 mg/plate 1.0 mg/plate 1.5 mg/plate	1,8-Cineole was not mutagenic in IC185 or IC202 strain. In IC202 strain, but not in IC185 strain.	2	Mitić-Ćulafić, D., Žegura, B., Nikilić, B., Vuković-Gačić, B., Filipič, M. (2009)
	Single-cell gel electrophoresis (comet assay)	Human hepatoma cell line (HepG2) Human B lymphoid NC-NC cells	1 µh/mL	The test substance did not induce DNA damages.		
1,8-Cineole	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	1.5-6.0 µmol/plate	The test substance was lacking of mutagenic effect.	2	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
α-Pinene	Alkaline single-cell gel electrophoresis (comet assay)	Human lung epithelial A549 cells	1 mg/m³ 20 mg/m³ 1000 mg/m³ 1800 mg/m³	α-Pinene failed to induce DNA migration.	2	Gminski, R., Tang, T., Mersch-Sundermann, V. (2010)
α-Pinene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

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

The genotoxic properties of Tea Tree Oil were investigated with *in vitro* tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and with an *in vivo* test for DNA damage (mouse micronucleus test).
Tea Tree Oil was not genotoxic in the tests conducted under the test conditions used.
Overall, the weight of evidence indicates that Tea Tree Oil does not pose an *in vivo* mutagenic or genotoxic concern to humans.
No information is available on the genotoxicity of Tea Tree Oil in humans. Therefore, it clearly does not meet the criteria for classification in category 1A. Since Tea Tree Oil was negative in *in vivo* tests in mammals and there is no information on its mutagenicity in germ cells, classification in category 1B is not appropriate, as well.
Classification for germ cell mutagenicity category 2 is not appropriate as the *in vivo* study and the *in vitro* studies have shown that Teat Tree Oil is negative in all assays.

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

Tea Tree Oil does not need to be classified for germ cell mutagenicity. Data conclusive but not sufficient for classification.

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

There are no carcinogenicity studies with Tea Tree Oil available.
A waiver is provided to show that long-term studies with carcinogenicity testing were not necessary for Tea Tree Oil (see DRAR, Volume 3, B.6 (AS)) and is summarized in the following. Additionally, publicly available carcinogenicity data for single Tea Tree Oil components are presented in support of the assessment.

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

TTO/its components occur naturally and are degraded in edible crops and in the environment.
Consumers are exposed to TTO and its components from natural sources (edible crops and environment) and from their use in health care products and as food flavours. TTO had a long history of safe use in a wide range of cosmetic and human and animal care products (mouthwash, toothpaste, shampoo, deodorants, lotions, and antifungal treatment). TTO (*Melaleuca alternifolia* oil) is listed as an ingredient employed in cosmetic products.
Consumers are not exposed to TTO/its components from crops treated with TTO, due to the lack of residues on these treated crops.
Moreover, TTO/its components would be metabolized and cleared rapidly within 2-3 days if entered human body, as it was shown earlier by animal and human volunteers studies. In addition, TTO components were metabolized to innocuous metabolites which are rapidity cleared from the body.
It is noteworthy that due to the rapid clearance from the body, there is no potential for bioaccumulation and there is no specific target organ in which TTO (or components) is incorporated after absorption.
In case a small fraction of TTO/its components remains in the body, it is unlikely to cause any long-term effects, such as carcinogenicity.
It is further notable that TTO was tested negative in genotoxicity studies. It is therefore unlikely that carcinogenicity appears which is based on genotoxic mechanisms of action.
In contrast, several studies demonstrated that TTO and its main component terpinene-4-ol have anti-carcinogenic activities against various types of cancer, both *in vitro* and *in vivo*.

The following carcinogenicity studies with single TTO components were available:
Terpineol (Study information published on ECHA homepage[39])

- In a carcinogenicity study, female mice were given intraperitoneal injections of alpha-terpineol or beta-terpineol at 1900 and 9600 mg/kg bw in tricaprylin, 3 times a week for a total of 24 doses. Animals were then observed for mortality and bodyweights for 24 weeks after first injection and were all macroscopically necropsied after death or sacrifice. No dose-related increase was observed in tumour formation.

[39] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/22822/7/8 (accessed: 22.03.2021)

- **Under the test conditions, β terpineol was not considered to be carcinogenic to A/He mice** "alpha-Terpineol and beta-terpineol injected intraperitoneally to mice were not found to be carcinogenic."

1,8-Cineole (Eucalyptol) (Bhowal and Gopal (2015))

- In a study of the carcinogenic effects of toothpaste constituents including chloroform, eucalyptol, and peppermint oil, eucalyptol was given to groups of 52 male specific pathogen-free CFLP mice at a dose of 8 or 32 mg/kg per day by gavage, 6 days per week for 80 weeks. Control groups of 52 mice were either untreated or received a toothpaste base which lacked chloroform, peppermint, or eucalyptol (vehicle control). Animals were housed four per cage and given food and water *at libitum*. Mice were weighed weekly for the first 6 months and then every 2 weeks during the last 6 months of the study. The consumption of food was noted on a cage-by-cage basis. Animals were observed twice daily and those found dead or in a moribund condition during the study were gross inspected.
- At week 80, animals were killed and organ weights for the kidneys, adrenals, lungs, liver, and spleen were noted. All macroscopically identified tumours were examined histopathologically, along with tissues from the kidneys, liver, lungs and brain.
- No treatment-related changes were reported for the following parameters: food consumed, body weight, organ weights, and clinical signs of toxicity. Necropsy and organ weight measurements showed no treatment-related differences between control and test groups. Histopathological examination revealed **no notable differences between control, test, or vehicle control groups in the incidence or severity of tumours of the kidney, liver, lung, or malignant lymphoma.**
- Studies using the substrain of A mouse strain originating from Walter Heston [A/HE] primary lung tumour model was carried out for carcinogenicity of eucalyptol (12 g/kg/ 8 wk intermittent; Max tolerated dose) but it was observed to be **negative for tumour induction** (Bhowal and Gopal (2015).

Limonene (Jameson (1990) and IARC (1999))

- *Animal carcinogenicity data*

  D-Limonene was tested as a cancer-preventive agent in other experimental models with known carcinogens. It inhibited lung carcinogenesis in mice, preneoplastic stages of colon carcinogenesis in rats and pancreatic carcinogenesis in d-Limonene was tested for carcinogenicity by oral gavage in mice and rats and in several two-stage experiments with multi-organ carcinogens. In the tested animals, limonene significantly increased the incidence of renal tubular tumours (adenomas and carcinomas) and induced atypical renal tubular hyperplasia in male rats only, which normally synthesize α2u-globulin in the liver, but not in female rats or in mice of either sex. It consistently enhanced the incidences of renal tubular tumours and atypical renal tubular hyperplasia initiated by carcinogens in two-stage carcinogenesis assays in male rats of a strain conventionally used in bioassays, but not in a strain that lacks hepatic synthesis of α2u-globulin.

  D-Limonene was tested as a cancer-preventive agent in other experimental models with known carcinogens. **It inhibited lung carcinogenesis in mice, preneoplastic stages of colon carcinogenesis in rats and pancreatic carcinogenesis in hamsters.**
- *Relevant data*

  d-Limonene is metabolized in humans and experimental animals to a variety of metabolites, including perillic acid and d-limonene-1,2-diol. d-Limonene causes a male rat specific nephrotoxicity resulting from accumulation of the male rat-specific protein α2uglobulin. D-Limonene-1,2-epoxide binds reversibly to α2u-globulin. d-Limonene causes sustained cell proliferation in renal proximal tubular cells, and the dose–response relationships for tumours outcome, enhanced cell proliferation and other histopathological end-points typical of α2u-globulin nephropathy are similar. Female rats, male rats of strains that do not express this protein and other species are not susceptible to the nephrotoxic action of d-limonene.
  The few available data indicate that d-limonene and its 1,2-epoxide metabolite are not genotoxic
- *Evaluation*

  There is inadequate evidence in humans for the carcinogenicity of d-limonene. There is sufficient evidence in experimental animals for the carcinogenicity of d-limonene.
  In making its overall evaluation of the carcinogenicity to humans of d-limonene, the Working Group concluded that d-limonene produces renal tubular tumours in male rats by a on-DNA-reactive mechanism, through an α2u-globulin-associated response. Therefore, the mechanism by which d-limonene increases the incidence of renal tubular tumours in male rats is not relevant to humans. d-Limonene is not classifiable as to its carcinogenicity to humans (Group 3). (Jameson (1990) and IARC (1999)).

Overall, it is demonstrated that TTO and its main component terpinen-4-ol have anticarcinogenic potential. On the other hand, 1,8-cineole and terpineol (TTO terpene compounds), though through i.p. administration, were not carcinogenic in laboratory animals. While limonene induced renal tumours in male rat only, the underlying mechanism is very specific to this species and not relevant to other species and to humans.

The carcinogenicity studies cited cover the following TTO components:

- Terpineol which forms 7.4% of TTO in average (though its study carried out by IP administration). Terpinen-4-ol, which forms 44 % of TTO, is close analogue to Terpineol. Thus, around 51% of TTO components could be covered by the study of Terpineol.
- 1,8-cineole forms 5.2 % of TTO.
- The structural analogy of limonene is comparable to alpha- and gamma-terpinene, alpha-terpinolene, alpha-pinene and sabinene. All these form about 40% of TTO. Thus, around 40% of TTO components could be covered by the studies of Limonene.

In total, the carcinogenicity studies of 1,8-cineole, terpineol and limonene cover > 95% of TTO components. Hence, TTO is unlikely to be a carcinogen.

In conclusion, consumers are exposed to TTO and its components from natural sources and from their use in health care products and as food flavours. However, they are not exposed to TTO/its components from crops treated with TTO, due to the lack of residues on these treated crops. Absorbed TTO is rapidly cleared from the body, there is no potential for bioaccumulation and there is no specific target organ in which TTO (or components) is incorporated after absorption. A genotoxic mode of action for carcinogenicity is highly unlikely since all available *in vitro* and *in vivo* tests for genotoxicity of TTO were negative. Terpineol, 1,8-cineole and limonene do not show carcinogenic potential in publicly available studies which are relevant to humans. In contrast, it has been demonstrated that TTO and its main component terpinen-4-ol have anticarcinogenic potential. Based on the above, it is very unlikely that TTO has carcinogenic potential.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

From the above, there is no evidence that TTO or its components are carcinogenic in the studies summarized above. Thus a classification for carcinogenicity is not required for TTO.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Not classified – due to inconclusive data.

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

The reproductive toxicity of Tea Tree Oil has been investigated in rats, rabbits and dogs. A two-generation study in rats, is available to investigate the effects of Tea Tree Oil on sexual function and fertility. One developmental toxicity study in rats (oral) and one in rabbits (oral) are also available

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

The effect of Tea Tree Oil on sexual function and fertility has been investigated in a two-generation reproduction study in rats

Table 34: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	NOEL/NOAEL [mg/kg bw/day]	Results	Reliability score	Reference
Two generation study in the rat OECD 416 Oral (gavage) GLP Dose levels: Generation-P: 0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day Treatment related alterations were observed in the reproductive performance at 50 mg/kg bw/day (P generation). Hence, the high dose of 50 mg/kg bw/day was reduced to 38 mg/kg bw/day for the pups selected for F1 generation.	Tea Tree Oil Purity: 10.30 % α-Terpinene, 20.90 % γ-Terpinene, 1.53 % p-cymene and 42.36 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>25 mg/kg/day</u> <u>No effects observed</u> <u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) *More detailed results are presented in 33 – Table 37*	1	Anonymous (2017a)

Table 35: Summary table of repeated dose toxicity studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
28-day feeding study, rats (Wistar) OECD 407 (2008) Non-GLP Dose levels: 0, 62.5, 125, 250 mg/kg bw/day Deviations: not all required organs have been fixed for histopathological examination	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in clompliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	NOEL = 62.5 mg/kg body weight/day	<u>At 62.5 mg/kg bw/day</u> <u>No effects observed</u> <u>At 125 mg/kg bw/day</u> • Degenerative changes in testes • Oligospermia • Epididymal cell debris • Pale liver • Hepatocyte vacuolation • ↑Liver weight <u>At 250 mg/kg bw/day</u> ↓ absolute and relative weights of testes and epididymides • Small sized epididymides and testes • Degenerative changes in testes • Aspermia • Pale liver • Hepatocyte vacuolation • Zona fasciculata hypertrophy (adrenals) • ↑Liver weight • ↑Adrenal weight *More detailed results are presented in Table 38 – 40.*	2	Anonymous (2010b)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
90-days, feeding, rats (Wistar rats – HsdCpb) OECD 408 GLP Dose levels: 0, 30, 60, 120 mg/kg bw/day	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	Males: NOAEL = 30 mg/kg bw/day Females: NOAEL (= 60 mg/kg bw/day	<u>At 30 mg/kg bw/day</u> <u>No effects observed</u> <u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms <u>At 120 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms ↓ absolute and relative weights of testes and epididymides -degenerative changes in seminiferous tubules -cell debris in tubular lumen of testes and atrophic appearance -sertoli cell vacuolation -sperm granuloma -cell debris in epidydimal duct lumen • Spleen vacuolation (minimal degree) • Tubular dilatation in kidneys (minimal degree) *More detailed results are presented in Table 41 - Table 43*	1	Anonymous (2011b)
90-days, feeding, rats (Wistar rat - Hsd Han) OECD 408 GLP Dose levels: 0, 60 mg/kg bw/day	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	LOAEL = 60 mg/kg bw/d (effects on sperm reversible after recovery period)	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms - Sperm granuloma - Oligospermia, - Single cell necrosis, - Luminal cell debris - Degeneration/atrophy of seminiferous tubules More detailed results are presented in Table 44	1	Anonymous (2016a)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
90-days oral, dogs (Beagle) OECD 409 GLP Dose rates: 0, 30, 75/60, 180/120 mg/kg bw/day **(dose reduction from test day** 27 on due to signs of intoxication)	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Sesame oil Administration: gavage	NOAEL = 30 mg/kg bw/day	<u>At 30 mg/kg bw/day</u> <u>No effects observed</u> <u>At 75/60 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids <u>At 180/120 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids • Clinical signs (starting within 5 minutes after administration and lasting up to 20 minutes) • ↓Body weight/gain (180mg) • ↓Food consumption (180mg) *More detailed results are presented in Table 45 -* *47*	1	Anonymous (2018a)

Study 1: Anonymous (2017a) Tea Tree Oil: Two generation reproduction toxicity study in Wistar rats, OECD 416, GLP

Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following table:

Table 36: Body weight and food consumption

Parameters		**Concentration (mg/kg bwt/day)**			
		0	**10**	**25**	**50**
No. of Animals per Concentration		25	25	25	25
Net Body Weight Gain (g)	Males (Day 113)	326.87 ± 39.75	294.31* ± 45.69	309.11 ± 56.71	284.14* ± 45.81
	Females (Day 71)	125.99 ± 14.84	130.93 ± 23.39	135.60 ± 19.35	135.42 ± 26.28
Average Food consumption (g/rat/day)	Males (Week 10)	17.27 ± 1.20	11.92* ± 1.54	13.74* ± 1.80	13.00* ± 1.16
	Females (Week 10)	11.34 ± 0.87	8.32* ± 0.98	9.09* ± 1.00	9.42* ± 0.83
Maternal Body Weight Change during Gestation Period (g)		80.63 ± 14.09	66.53* ± 17.52	68.28* ± 12.83	57.86* ± 17.65
Maternal Food Consumption during Gestation Period (g/rat/day)		14.27 ± 1.29	11.49* ± 1.77	11.66* ± 1.29	12.23* ± 2.19
Maternal Body Weight Change during Lactation Period (g)		-9.50 ± 20.12	-23.96 ± 23.40	-21.20* ± 14.81	-15.06 ± 16.73
Maternal Food Consumption during Lactation Period (g/rat/day)		26,63 ± 4,51	18.88* ± 5.66	20.67* ± 2.98	23.08* ± 6.94
Mean Body Weight of Pups (F1-Generation) during Lactation Period on Day 21 (g)		26,61 ± 5,14	22.24* ± 4.96	22.47* ± 3.91	26.93* ± 5.81

*: Significantly different from the control group (p<0,05)

Table 37: Summary of clinical signs and mortality – Generation: P

Parameters	**Group**	**G1**		**G2**		**G3**		**G4**	
	Dose (mg/kg bwt/day)	**0**		**10**		**25**		**50**	
	Sex	M	F	M	F	M	F	M	F
	No. of rats	25	25	25	25	25	25	25	25
1. GENERAL CONDITION									
Salivation-Slight		0	0	0	0	20	10	23	23
Dehydration-Moderate		0	0	0	0	1	0	0	0
Posture-Recumbent		0	0	0	0	1	0	0	0
2. SKIN AFFECTIONS	(Sparse hair loss)	0	1	2	0	1	0	1	0
3. EYE AFFECTIONS		0	0	0	0	0	0	0	0
4. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
5. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
6. PRE-TERMINAL DEATHS (Total)									
Death during treatment		0	0	0	0	0	0	0	0
Death during gestation		NA	0	NA	0	NA	0	NA	0
Death during lactation		NA	0	NA	1	NA	0	NA	0
Dystocia deaths		NA	0	NA	0	NA	0	NA	0
Moribund sacrifice		0	0	0	0	0	0	0	0
Total mortality		0	0	0	1	0	0	0	0

NA: Not Applicable; M: Male; F: Female

Table 38: Summary of clinical signs and mortality – Generation: F1

Parameters	Group	G1		G2		G3		G4	
	Dose (mg/kg bwt/day)	0		10		25		38	
	Sex	M	F	M	F	M	F	M	F
	No. of rats	25	25	25	25	25	25	25	23
1. GENERAL CONDITION		0	0	0	0	0	0	0	0
2. SKIN AFFECTIONS		0	0	0	0	0	0	0	0
3. EYE AFFECTIONS		0	0	0	0	0	0	0	0
4. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
5. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
6. PRE-TERMINAL DEATHS (Total)									
Death during treatment		0	0	0	0	0	0	0	0
Death during gestation		NA	0	NA	0	NA	0	NA	0
Death during lactation		NA	0	NA	0	NA	0	NA	0
Dystocia deaths		NA	1	NA	0	NA	0	NA	0
Moribund sacrifice		0	0	0	0	0	0	0	0
Total mortality		0	1	0	0	0	0	0	0

NA: Not Applicable; M: Male; F: Female

Table 39: Summary of the sperm evaluation

	P-Generation			**F1-Generation**		
Concentration (mg/kg bwt/day)	**10**	**25**	**50**	**10**	**25**	**38**
No. of Animals per Concentration	25	25	25	25	25	25
Progressive motile sperms %	—	—	↓(15)	—	—	↓(16)
Motile sperms %	—	—	↓(12)			
Normal sperms %	—	—	↓(14)			
Abnormal sperms %	—	—	↑ (486)			
No. of sperms per cauda epididymis	—	↓(16)	↓(32)	—	—	↓(19)
No of sperms per gram of cauda epididymis	—	—	↓(19)	—	—	↓(18)

↑: Increased ↓: Decreased —: No Change
Values in parenthesis indicate percentage change

Table 40: Summary of survival data of pups, mating and fertility index

Parameters	**Concentration (mg/kg bwt/day)**			
	0	**10**	**25**	**50**
No. of Animals per Concentration	25	25	25	25
Male Fertility Index°	84	76	80	44*
Female Fertility Index°	92	84	84	56*
Mean No. of Corpora Lutea#	12.8	11.8	11.7	9.3*
Mean No. of Implantations#	11.1	10.5	10.1	6.7*
Gestation Length (Days)#	22.77 ± 0.53	22.75 ± 0.55	22.55 ± 0.51	22.45 ± 0.69*
Mean Litter Size#	10.0	8.7	9.0	7.0*
Mean Viable Litter Size	9.9	7.8	8.5	6.7*
Day 4 Survival Index	99.1	94.2*	91.1*	81.1*

*: Significantly different from the control group (p<0,05); #: Compared by Levens, ANOVA and Dunnett's test after transformation (√ x + ½); °: Compared by 'Z' test

The 2-generation reproduction toxicity study performed according OECD TG 416 and in GLP conditions.
For exposure of P generation Tea Tree Oil was mixed in refined groundnut oil and administered orally by gavage to Wistar rats at the dose levels of 10, 25 and 50 mg/kg bwt/d for the male and female rats of P generation. The vehicle or test item was administered to the male rats once daily at approximately the same time each day for 10 weeks prior to mating. The treatment was continued during mating and after completion of mating process until the necropsy. As in males, females received the vehicle or test item once daily at approximately the same time each day (varied by ± 2 hours) for 10 weeks prior to mating. Treatment was continued through mating, pregnancy and up to the weaning of F1 offspring, after which, parental females were sacrificed. F1 generation offspring were treated from weaning till they were sacrificed after obtaining F2 weanlings.
In P generation the pregnancy occurred in 23 (92%), 21 (84%), 21 (84%) and 14 (56%) out of 25 mated female rats in each group. Three dams in control, five dams at 10 mg/kg bwt/day, four dams at 25 mg/kg bwt/day and fourteen dams at 50 mg/kg bwt/day did not deliver litters. The pre-coital interval was longer (not statistically significant) at 50 mg/kg bwt/day, when compare to control group.
Treatment with TTO at 50 mg/kg bwt/day dose resulted in significantly lower male and female mating and fertility indices, associated with decrease in sperm motility, cauda epididymal sperm counts and increase in percentage of abnormal sperm counts, when compared to vehicle control.
The maternal data such as mean number of corpora lutea and implantations were significantly lower and percentage of pre-implantation loss were significantly higher at 50 mg/kg bwt/day, which in-turn resulted in significantly lower mean litter and viable litter sizes. The mean litter size was 10.0, 8.7, 9.0 and 7.0 in the control, 10, 25 and 50 mg/kg bw/d group. Mean litter size and mean viable litter size was significantly reduced at 50 mg/kg bw/d.
For the F1 generation, the top dose of 50 mg/kg bwt/d was reduced to 38 mg/kg bwt/day for the animals due to adverse effect on fertility in P generation. In F1 generation the pregnancy occurred in 25/25 females(100%), 24/25 females (96%), 24/25 females (96%) and 20/23 (87%) females in the 0 (vehicle control), 10, 25 and 38 mg/kg bwt/day dose groups, respectively. The pre-coital interval and the gestation length (average days to litter) was not altered by treatment at all the doses tested. The maternal data such as mean number of corpora lutea and implantations were comparable to the control group. The mean litter size was comparable in control and treatment groups. Statistically significant decrease in the percentage of progressively motile sperms at 38 mg/kg bwt/day was considered test item-related. This finding was also associated with the lower cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) at 38 mg/kg bwt/day. However microscopic examination of testes and epididymides did not reveal any associated changes.
Based on the results of this study it is concluded that TTO at dose of 50 mg/kg bw/d significantly affected fertility of rats, apparently of male rats, without inducing alteration of body weight, body weight gain or producing significant adverse effects in other internal organs. The No Observed Adverse Effect Level (NOAEL) for reproductive toxicity is considered to be 25 mg/kg bwt/day, under the test conditions and doses employed.

Anonymous (2010b) Tea Tree Oil: 28-Day Repeated Dose Toxicity Study in Wistar Rats (Non-GLP Study)
Tables providing more detailed numerical information are too extensive to be integrated in the summary table 32. Therefore they are presented in the following:

Table 41: Summary of the significant alterations in organ weights and organ ratios

Sex	**Male**				**Female**			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Epididymides								
– Absolute	-	-	-	↓(34)	NA	NA	NA	NA
– Relative	-	-	-	↓(28)	NA	NA	NA	NA
Testes								
– Absolute	-	-	-	↓(43)	NA	NA	NA	NA
– Relative	-	-	-	↓(39)	NA	NA	NA	NA
Liver								
– Absolute	-	-	-	-	-	-	↑(19)	↑(23)
– Relative	-	-	-	↑(14)	-	-	↑(18)	↑(32)
Adrenals - Relative	-	-	-	↑(18)	-	-	-	↑(18)

↑/↓: Statistically significant Increase/Decrease
Values in parenthesis indicate % change when compared to mean value of vehicle control
-: No statistical significance

Table 42: Summary of the gross findings

Sex	**Male**				**Female**			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Epididymides – small sized	0	0	0	2	NA	NA	NA	NA
Testes – small sized	0	0	0	2	NA	NA	NA	NA
Liver – Pale, diffuse	0	0	4	1	0	0	0	2

Table 43: Summary of the microscopic changes observed

Sex	**Male**				**Female**			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Adrenals								
– hypertrophy-zona fasciculata	0	0	0	0	0	0	0	0
– Minimal	-	-	-	3	-	-	-	2
Testes								
– degenerative changes bilateral	0	0	4	6	NA	NA	NA	NA
– Minimal	-	-	4	3	NA	NA	NA	NA
– Mild	-	-	-	3	NA	NA	NA	NA
Epididymides								
– aspermia	0	0	0	5	NA	NA	NA	NA
– oligospermia	0	0	2	1	NA	NA	NA	NA
– cell debris in lumen	0	0	4	0	NA	NA	NA	NA
Liver								
– hepatocyte vacuolation	0	0	2	6	1	0	1	5
– Minimal	-	-	2	4	1	-	1	5
– Mild	-	-	-	2	-	-	-	-

The study performed according to OECD TG 407 is acceptable with restriction. Tea Tree Oil given for 28 days by gavage at doses 62.5, 125 and 250 mg/kg bw/d did not produce major systemic toxicity besides increased weight of liver, pale liver, vacuolar degeneration of hepatocytes and the degenerative changes in the testes and aspermia/oligospermia in epididymis which were linked with decreased weights of testes and epididymis at 125 and 250 mg/kg bw/d. NOAEL of 62.5mg/kg bw/d can be established based on results of this study.

Anonymous (Anonymous 2011b) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats
Tables providing more detailed numerical information are too extensive to be integrated in the summary table 30. Therefore they are presented in the following:

Table 44: Summary the significant alterations ($p \leq 0.05$) in organ weights and organ ratios of male rats

In-life phase	**Treatment**				**Recovery**			
Group No.	G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)	0	30	60	120	0		120	
Sacrificed on day	91				15	29	15	29
Testes No. examined	10	10	10	10	10	10	10	10
Absolute	3.787	-	-	-	3.808	3.760	-	2.855↓ (24)
Ratios to body weight	0.970	-	-	-	0.974	9.967	-	0.717 ↓ (26)
Epididymides – No. examined	10	10	10	10	10	10	10	10
Absolute	1.497	-	-	-	1.561	1.521	-	1.154 ↓ (24)
Ratios to body weight	0.382	-	-	-	0.400	0.390	-	0.290 ↓ (26)

↑/↓: Significant Increase/Decrease of Mean values; Values in parenthesis indicate % change
Note: Data subjected to Shapiro-Wilk test, Levene's test, F-test (ANOVA) and Dunnet's 't' test

Table 45: Summary of the gross lesions found in male rats

In-life phase		**Treatment**				**Recovery**			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
Testes	No. examined	10	10	10	10	5	5	10	10
	Small and flaccid	0	0	0	1	0	0	0	4
	Small	0	0	0	3	0	0	0	0
	Flaccid	0	0	0	0	0	0	2	4
Epididymides	No. examined	10	10	10	10	5	5	10	10
	Abcess	0	0	0	6	0	0	4	1

Table 46: Summary of the microscopic changes observed in male rats

In-life phase		**Treatment**				**Recovery**			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
Testes	No. examined	10	10	10	10	5	5	10	10
Degenerative changes - seminiferous tubules		**0**	**0**	**0**	**8**	**0**	**0**	**9**	**8**
	Minimal	0	0	0	4	0	0	3	2
	Mild	0	0	0	2	0	0	4	4
	Moderate	0	0	0	1	0	0	2	2
	Marked	0	0	0	1	0	0	0	0
Sertoli cell vacuolation		**0**	**0**	**0**	**9**	**0**	**0**	**10**	**9**
	Minimal	0	0	0	7	0	0	6	5
	Mild	0	0	0	2	0	0	4	4
Sperm stasis		**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**
	Minimal	0	0	0	0	0	0	4	0
Epididymides	No. examined	10	10	10	10	5	5	10	10
Sperm granuloma		**0**	**0**	**0**	**4**	**0**	**0**	**6**	**1**
Cell debris in lumen		**0**	**0**	**1**	**7**	**0**	**0**	**9**	**9**
	Minimal	0	0	1	6	0	0	1	2
	Mild	0	0	0	0	0	0	6	5
	Moderate	0	0	0	0	0	0	1	2

In-life phase		Treatment				Recovery			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
	Marked	0	0	0	0	0	0	1	0
Oligospermia		**0**	**0**	**0**	**3**	**0**	**0**	**5**	**6**
Aspermia		**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Kidneys	No. examined	10	10	10	10	5	5	10	10
Dilatation of tubules		**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	Minimal	0	0	0	3	0	0	0	0
Spleen	No. examined	10	10	10	10	5	5	10	10
Vacuolation		**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**
	Minimal	0	0	0	5	0	0	0	0

This 90-day repeated dose toxicity study in rats with Tea Tree Oil was performed according to OECD TG 408 and in GLP conditions. Tea Tree Oil administered by gavage for 90 days (males) or 91 days (females) at doses of 30, 60 or 120 mg/kg bw/day did not induce significant changes in feed consumption, body weight, locomotor activity, haematology, blood coagulation, blood and urine chemistry parameters at any of the doses in either sex. In macroscopic examination no treatment related changes were seen at 30 and 60 mg/kg bw/d, while at 120 mg/kg bw/d only gross lesions were observed in testes (small testes with flaccid appearance, unilateral or bilateral abscess in epididymides). In histopathological examination the degenerative changes were seen at 120 mg/kg bw/d in seminiferous tubules of testes with vacuolisation of Sertoli cell, in epididymides at 120 mg/kg bw/d sperm granuloma, cell debris in lumen, oligospermia (in 3 out of 10 rats) or aspermia (in 1 out of 10 rats) were observed. In other internal organs of males only minimal degree of tubular dilatation in cortical area of kidneys or minimal degree of vacuolation in red pulp area of spleen at 120 mg/kg bw/d were found. In females no treatment related microscopic changes in all organs were observed except for two moribund sacrificed rats at 120 mg/kg bw/d.
In sperm examination no variation was seen in the sperm parameters of rats treated at 30 mg/kg bw/day. At 60 and 120 mg/kg bw/day, significant reduction in the sperm counts and motility were observed and these changes were associated with microscopic changes in the testes (germ cell degeneration and sertoli cell vacuolation) and epididymides (sperm granuloma and oligospermia) only at 120 mg/kg bw/day. Further, significant increase in the percent abnormal (sperms with headless tail, tailless head, bent tail and bent neck) sperms with corresponding decrease in the percent normal sperms was observed.
The results of this study indicate that testes and epididymis are target organs for TTO toxicity, and seminiferous epithelium and sperm cell are more sensitive to toxicity of TTO than somatic cells of rats.
Taking into account the effect in the most sensitive organs the NOAEL for TTO from this study is 30 mg/kg bw/day.

Anonymous (2016a) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats
Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following:

Table 47: Summary of the clinical pathology investigations, sacrifice and pathology

Gpe No.	Dose [mg/kg bw/day]	No. of rats per group		Clinical pathology investigations			Pathology			Sacrifice on day
		M	F	Haema-tology	Clinical Chemistry	Urina-lysis	Gross pathology	Organ weights	Histo-pathology	
G1	0	10	10	+	+	+	+	+	+	91
G2	60	10	10	+	+	+	+	+	+	91
G1R	0	10^1	5^1	+	+	+	+	+	a	147
G1R	0	10^2	5^2	+	+	+	+	+	a	175
G1R	0	10^3	5^3	+	+	+	+	+	a	203
G2R	60	10^1	5^1	+	+	+	+	+	a	147
G2R	60	10^2	5^2	+	+	+	+	+	a	175
G2R	60	10^3	5^3	+	+	+	+	+	a	203

+: Yes
a: Gross lesions and target organs

[1]: Rats sacrificed after completion of 8 weeks recovery period
[2]: Rats sacrificed after completion of 12 weeks recovery period
[3]: Rats sacrificed after completion of 16 weeks recovery period

The study performed in GLP conditions according to OECD TG 408, however with a major deviation, since only one dose was used, is considered reliable and results can be used for assessment of health hazard caused by TTO. This study is supplementary to the previous one (Anonymous 2011b). Tea Tree Oil administered by gavage for 90 days to female and male rats at dose of 60 mg/kg bw/day did not induce significant changes in food consumption and body weight or clinical parameters in either sex. In histopathological examination the degeneration/atrophy of seminiferous tubules were seen in testes and sperm granuloma/chronic active inflammation, oligospermia, single cell necrosis, luminal cell debris in epididymides. In sperm examination reduced epididymal sperm counts and vas deferens sperm motility with increased abnormal sperms were found in main group exposed at 60 mg/kg bw/day. The pathological changes in testes and epididymides and in sperm examinations observed in rats exposed for 90 days at 60 mg/kg bw/day were no longer seen 8, 12 and 16 weeks after cessation of exposure indicating a complete recovery of damaged organs and tissues.

Anonymous (2018a) Repeated dose 90-Day oral toxicity study of Tea Tree Oil in Beagle dogs - - according to OECD 409, GLP

Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following tables :

Table 48: Body weight over recovery period in repeated dose 90-day oral toxicity study

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	10.45	10.60	10.83	10.70
	SD	0.26	0.29	0.22	0.12
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	10.27	10.33	10.53	10.43
	SD	1.62	1.40	1.46	1.59
	N	3	3	3	3
	%Diff	-1.8	-2.5	-2.7	-2.5

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date			
		127	134	141	147
Group 1: Control	Mean	10.70	10.50	10.60	10.55
	SD	-	-	-	-
	N	2	2	2	2
Group 4: 180/120 mg/kg	Mean	10.00	9.80	10.20	10.10
	SD	-	-	-	-
	N	1	1	1	1
	%Diff	-6.5	-6.7	-3.8	-4.3

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	9.65	9.70	9.28	9.85
	SD	1.53	1.54	1.13	1.45
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	8.83	8.98	9.18	8.93
	SD	0.97	0.97	1.02	0.91
	N	4	4	4	4
	%Diff	-8.5	-7.5	-1.1	-9.4

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	9.65	9.70	9.28	9.85
	SD	1.53	1.54	1.13	1.45
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	8.83	8.98	9.18	8.93
	SD	0.97	0.97	1.02	0.91
	N	4	4	4	4
	%Diff	-8.5	-7.5	-1.1	-9.4

The summary of food consumption data is not provided due to its extents. Please refer to the original study report.

Table 49: Summary of sperm analysis compared to the respective values of the control animals in repeated dose 90-day oral toxicity study

Changes in sperm viability and motility compared to the control group 1 at the end of the treatment period (test week 13)				
Parameter	**Group 1 Control**	**Group 2 30 mg/kg**	**Group 3 75/60 mg/kg**	**Group 4 180/120 mg/kg**
Viability [%]:				
Alive / Dead	83.4 / 16.6	85.5 / 13.5	54.8 / 45.2**	63.1 / 36.9**
Motility [%]:				
Estimated motility	68.8	81.3	20.0**	43.0**
Progressive motility	54.5	61.0	9.7**	29.4**
Non-progressive motility	16.3	23.3	14.3	15.0
Immotility	29.3	15.8	76.0**	55.6**

**: statistically significant at $p \leq 0.01$ (chi^2-test)

Table 50: Body weight measurements (g) in repeated dose 90-day oral toxicity study

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date						
		-9 [a]	1 [a1]	8 [a1]	15 [a1]	22 [a1]	29 [a1]	36 [a1]
Group 1: Control	Mean	7.64	7.75	8.11	8.29	8.46	8.75	8.94
	SD	0.67	0.62	0.60	0.62	0.54	0.52	0.51
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	7.63	7.78	8.25	8.43	8.63	9.03	9.25
	SD	1.04	1.01	1.18	1.27	1.27	1.20	1.28
	N	4	4	4	4	4	4	4
	%Diff	-0.2	0.3	1.7	1.7	1.9	3.1	3.5
Group 3: 75/60 mg/kg	Mean	7.50	7.65	7.85	8.28	8.50	8.75	9.10
	SD	0.70	0.70	0.88	0.88	0.88	0.96	0.96
	N	4	4	4	4	4	4	4
	%Diff	-1.8	-1.3	-3.2	-0.2	0.4	0.0	1.8
Group 4: 180/120 mg/kg	Mean	7.61	7.80	7.78	7.29	7.33	7.46	8.21
	SD	0.73	0.63	0.80	0.82	1.04	1.45	0.98
	N	8	8	8	8	8	8	7
	%Diff	-0.3	0.6	-4.2	-12.1	-13.4	-14.7	-8.1

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date						
		43 [a]	50 [a]	57 [a]	64 [a]	71 [a]	78 [a]	85 [a1]
Group 1: Control	Mean	9.19	9.34	9.46	9.76	9.96	10.04	10.14
	SD	0.51	0.53	0.50	0.52	0.56	0.54	0.51
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	9.58	9.60	9.63	10.05	10.23	10.40	10.38
	SD	1.47	1.28	1.37	1.42	1.50	1.53	1.53
	N	4	4	4	4	4	4	4
	%Diff	4.2	2.8	1.7	2.9	2.6	3.6	2.3
Group 3: 75/60 mg/kg	Mean	9.13	9.38	9.48	9.73	9.98	10.13	10.10
	SD	0.97	0.90	0.86	0.90	0.91	0.95	0.91
	N	4	4	4	4	4	4	4
	%Diff	-0.7	0.4	0.1	-0.4	0.1	0.9	-0.4
Group 4: 180/120 mg/kg	Mean	8.67	8.66	8.89	9.27	9.60	9.80	9.84
	SD	0.89	0.88	0.89	0.94	1.05	1.13	1.24
	N	7	7	7	7	7	7	7
	%Diff	-5.6	-7.3	-6.1	-5.0	-3.6	-2.4	-2.9

Day(s) Relative to Start Date
91
10.19
0.52
8
10.43
1.53
4
2.3
10.20
0.91
4
0.1
9.93
1.28
7
-2.5

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date						
		-10 [a]	1 [a1]	8 [a1]	15 [a]	22 [a]	29 [a]	36 [a]
Group 1: Control	Mean	6.84	6.78	7.18	7.50	7.75	8.10	8.36
	SD	1.06	1.03	1.15	1.21	1.21	1.38	1.33
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	6.58	6.65	7.05	7.30	7.53	7.75	8.00
	SD	1.22	1.28	1.39	1.33	1.24	1.33	1.44
	N	4	4	4	4	4	4	4
	%Diff	-3.8	-1.8	-1.7	-2.7	-2.9	-4.3	-4.3
Group 3: 75/60 mg/kg	Mean	6.83	6.75	7.23	7.48	7.70	7.90	8.00
	SD	1.32	1.53	1.55	1.58	1.54	1.65	1.75
	N	4	4	4	4	4	4	4
	%Diff	-0.2	-0.4	0.7	-0.3	-0.6	-2.5	-4.3
Group 4: 180/120 mg/kg	Mean	6.88	7.11	7.39	7.23	7.35	7.36	7.69
	SD	0.99	0.87	0.95	0.89	1.01	1.03	1.00
	N	8	8	8	8	8	8	8
	%Diff	0.5	5.0	3.0	-3.7	-5.2	-9.1	-8.1

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date						
		43	50	57	64	71	78	85
Group 1: Control	Mean	8.44	8.48	8.80	8.79	8.94	9.04	9.23
	SD	1.34	1.47	1.62	1.55	1.53	1.57	1.56
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	8.28	8.45	8.63	8.88	9.00	9.10	9.15
	SD	1.42	1.50	1.48	1.53	1.45	1.45	1.30
	N	4	4	4	4	4	4	4
	%Diff	-1.9	-0.3	-2.0	1.0	0.7	0.7	-0.8
Group 3: 75/60 mg/kg	Mean	8.28	8.35	8.53	8.73	8.80	8.93	8.88
	SD	1.84	1.81	1.65	1.71	1.62	1.60	1.72
	N	4	4	4	4	4	4	4
	%Diff	-1.9	-1.5	-3.1	-0.7	-1.5	-1.2	-3.8
Group 4: 180/120 mg/kg	Mean	7.89	8.11	8.34	8.51	8.68	8.85	8.73
	SD	1.12	1.06	1.17	1.30	1.30	1.31	1.14
	N	8	8	8	8	8	8	8
	%Diff	-6.5	-4.3	-5.3	-3.1	-2.9	-2.1	-5.4

Day(s) Relative to Start Date
91
9.05
1.50
8
8.80
1.35
4
-2.8
8.73
1.42
4
-3.6
8.51
1.23
8
-5.9

[a] - Anova & Dunnett [a1] - Anova & Dunnett(Log)

The 90-day study of repeated dose toxicity in dogs performed according to OECD TG 409 and in GLP conditions is acceptable and results can be used for assessment of health hazard and risk caused by TTO. Tea Tree Oil administered by gavage to Beagle dogs (4 animals/sex/dose level) for 90 days at doses of 30, 60 or 120 mg/kg bw/day did not affect the feed consumption, body weight, hematological, biochemical and urine parameters at any of the doses in either sex. The highest dose was 180 mg/kg for first 4 weeks, but it was reduced to 120 mg/kg bw/d due to excessive toxicity. The mid dose was also reduced after first 4 weeks from 75 mg/kg to 60 mg/kg bw/d. In macroscopic and histopathological examinations no treatment related changes were seen at 30, 60 and 120 mg/kg bw/d after termination of exposure and at the end of 4-week and 8-week treatment free recovery periods, however size of right and left testicles were reduced in dogs given the highest dose in comparison with those of control animals. Exposure of male dogs to TTO at doses 75/60 and 180/120 mg/kg bw/day did not affect morphology and mean number of spermatozoa in ejaculate, but led to a decrease of percentage of alive spermatozoa and percentage of motile spermatozoa. No changes in viability or motility, in comparisons to the controls, were noted in dogs exposed at 30 mg/kg bw/d.

At the end of 4 and 8 weeks recovery periods the percentage motile and viable of spermatozoa in the group 180/120 mg/kg bw/day was not different from the concurrent control indicating a full recovery from alterations induced by TTO. The results of this study indicate that male reproductive organs are target organs for TTO toxicity, and sperm cell are more sensitive to toxicity of TTO than somatic cells of dogs. NOAEL in dogs for TTO established based on results of this study is 30 mg/kg bw/day.

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

In the 2-generation study treatment with TTO at 50 mg/kg bwt/day dose resulted in P generation significantly lower male and female mating and fertility indices, associated with decrease in sperm motility, reduced cauda epididymal sperm counts and increase in percentage of abnormal sperm counts, when compared to vehicle control. The mean number of corpora lutea and implantations were significantly lower and percentage of pre-implantation loss were significantly higher at 50 mg/kg bw/day, which in-turn resulted in significantly lower mean litter and viable liter sizes. These effects were not observed at dose of 10 and 25 mg/kg bw/d.
The highest dose 50 mg/kg bw/d was reduced to 38 mg/kg bw/d for animals selected for F1 generation due to adverse effect on fertility. In F1 generation statistically significant decrease in the percentage of progressively motile sperms at 38 mg/kg bwt/day was considered test item-related. This finding was also associated with the lower cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) at 38 mg/kg bwt/day.
Parental toxicity was limited to slight salivation for short time after dosing of TTO at 25 and 50 mg/kg. No treatment related changes of body weight and organ weight were observed. No treatment related histopathological changes were observed in parental animals (males and females) as well as in pups of P and F1 generations at all dose levels tested. The results of the 2-generation study indicate that TTO at dose of 50mg/kg bw/d by gavage have adverse effect on sperm count and sperm motility leading to reduced fertility of rats, while at dose 38 mg/kg bw/d TTO affects the motility and sperm counts without reducing fertility index in the exposed group. The No Observed Adverse Effect Level (NOAEL) for reproductive toxicity is considered to be 25 mg/kg bwt/day under the test conditions.

The adverse effect of TTO on testes and/or sperm count and motility was also noted in the repeated dose toxicity studies in rats given a test substance by gavage for 28 days at dose of 250 mg/kg bw/d, in rats given a test substance by gavage for 90 days at dose of 60 and 120 mg/kg, in dogs given a test substance by gavage for 90 days at dose of 60/75 mg kg/bw/d and 120 mg/kg bw/d. These effect were not observed in rats and dogs given for 90 days by gavage TTO at dose of 30 mg/kg, so this dose level cab be taken as No Observed Adverse Effect Level (NOAEL)

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

The adverse effects of TTO on fertility, testes, epididymides and sperm observed in two species (rats and dogs) in four acceptable studies at dose levels inducing slight or moderate general systemic toxicity provide some evidence meeting the classification criteria for reproductive toxicity of TTO in animals. It is noted that such effects were not reported in humans exposed to components of TTO at relatively high doses with food, although no targeted epidemiological studies were done, therefore there is some doubt whether these effects observed in animals are relevant for humans. Taking the above uncertainty into account DS is of the opinion that TTO warrants classification to subcategory Repr. 2 with hazard statement H361f - Suspected of damaging fertility. The highest dose level at which these effects were not observed in 2-generation study was 25 mg/kg bw/d, which considered as No Observed Adverse Effect Level (NOAEL) for reproductive toxicity.

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

The potential of Tea Tree Oil to adversely affect development has been investigated in rats and rabbits by the oral route.

Table 51: 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	Reliability score	Reference
Prenatal Developmental Toxicity Study in the rat OECD 414 GLP Wistar rats – HsdHan Females 24/group (owing to severe clinical signs and mortality doses were reduced)	Tea Tree Oil Purity: 8.18 % α-Terpinene, 1.80 % 1,8-Cineole, 14.23 % γ-Terpinene, 3.86 % p-cymene and 41.73 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 75, 150 and 300 mg/kg/day and 0, 30, 60 and 120 mg/kg/day.	<u>60 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake <u>120 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake ↓Fetal weight <u>150 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake <u>300 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake ↑Resorptions *More detailed results are presented in* - *50* NOAEL, maternal toxicity: 30 mg/kg/day NOAEL, fetal toxicity: 60 mg/kg/day	1	Anonymous (2012a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reliability score	Reference
Prenatal Developmental Toxicity Study in the rabbit Oral (gavage) OECD 414 GLP New Zealand white rabbits 24/group	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 15, 30, and 75 mg/kg/day	75 mg/kg day: ↑Post implantation loss *More detailed results are presented in Table 52.* NOAEL, maternal toxicity: 75 mg/kg/day NOAEL, fetal toxicity: 30 mg/kg/day NOAEL teratogenicity: 75 mg/kg/day	1	Anonymous (2018b)
Prenatal Developmental Toxicity Study in the rat Oral (gavage) OECD 414 GLP Wistar rats – HsdHan:WIST Up to 27 females/dose level	Melaleuca alternifolia, ext., Purity: 100% Content of terpinen-4-ol: 37% 0 mg/kg bw/day Group 1. Control (vehicle only - PEG 400). 20 mg/kg bw/day Group 2. Low dose. 100 mg/kg bw/day Group 3. Mid dose. 250 mg/kg bw/day Group 4. High dose. Vehicle: Polyethylene glycol 400 (PEG 400) Exposure: From days 5 to 19 of gestation (GD 5 to GD 19) (Daily treatment by oral gavage 7 days/week, at a similar time each day.)	Maternal animals: NOAEL: 20 mg/kg bw/day based on: (test mat.) Adverse effects at 100 and 250 mg/kg bw/day comprised clinical signs, reduced food consumption and reduced weight loss gains (with mortality at the high dose). Fetuses: NOAEL: 20 mg/kg bw/day based on: (test mat.) Reductions in foetal body weight were seen at 100 and 250 mg/kg bw/day. Increases in external and skeletal malformations were also seen in foetuses from the high dose group. All effects were secondary to maternal toxicity. Overall developmental toxicity: yes Lowest effective dose / concentration: 100mg/kg bw/day. Relation to maternal toxicity: Reproductive effects as a secondary non-specific consequence of other toxic effects.	1	ECHA disseminati on site (study report 2011)[40]

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

The developmental toxicity of Tea Tree Oil has been investigated in rats (oral, gavage) and rabbits (oral, gavage).

Study 1, Anonymous, (2012a), Prenatal developmental toxicity study of Tea Tree Oil in Wistar Rats by oral route, OECD 414, GLP.

[40] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/9/3

Reliability statement: The study is conducted in accordance with OECD 414. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

In the prenatal developmental toxicity study (Anonymous 2012a) in rats TTO was administered orally by gavage on GD 5- GD19 to pregnant Wistar rats at the initial tested doses of 75, 150 and 300 mg/kg/day and at the reduced doses of 30, 60 and 120 mg/kg/day. The doses were reduced on GD 8 (third day of treatment) due to mortality, at a dose of 300 mg/kg.
Maternal toxicity: At initial doses 150 and 300 mg/kg bw/d TTO induced clinical signs of dullness and incidence of mortality at 300 mg/kg bw/d, but no clinical signs were observed at doses 30, 60 and 120 mg/kg bw/d. Body weight gain and food consumption were reduced at 150/60 and 300/120 mg/kg bw/d. No gross visceral pathology was observed at necropsy in any treated groups.
Developmental toxicity: Mean number of corpora lutea, implantation, early resorption, late resorption, pre-implantation loss, post-implantation loss were not affected by TTO at any dose. Number (11 and 12) and percentage (47.8 and 57.1%) of dams with any resorption were higher at dose 150/60 and 300/120 mg/kg bw/d than in control group (6 and 25%).
Total number of life foetuses, mean litter size were unaffected, but the mean weight of foetuses in the groups 150/60 and 300/120 mg/kg bw/d were significantly reduced by respectively 4.6% and 15%. No major external, visceral or skeletal malformations were observed an no effect of TTO on incidence of minor external, visceral or skeletal anomalies was found in any exposed group. Increased incidence of delayed ossification of various bones was observed in 150/60 and 300/120 mg/kg bw/d. The No Observed Adverse Effect Level (NOAEL) for maternal and developmental toxicity of 30/75 mg/kg bwt/day can be derived due to mortality at dose of 300 mg/kg and reduced body weight gain and food consumption of dams and delayed ossification in foetuses at 150/60 and 300/120 mg/kg bw/d. The effects observed are not meeting classification criteria for developmental toxicity

Table 52: Body weights, body weight gain and food intake during a prenatal developmental toxicity study in the rat treated with Tea Tree Oil

Concentration (mg/kg Bwt/day)		**0**	**75/30**	**150/60**	**300/120**
No. of dams		24	23	23	21
Maternal body weight (g)					
Days of gestation	0	224.83 ± 15.96	223.72 ± 14.92	226.05 ± 15.40	225.66 ± 15.04
	3	233.87 ± 17.92	233.56 ± 18.16	235.71 ± 17.94	236.06 ± 17.90
	5	238.66 ± 18.14	239.00 ± 20.52	241.34 ± 20.95	241.75 ± 19.46
	8	244.78 ± 19.22	243.71 ± 22.73	237.08 ± 21.14	236.74 ± 21.52
	11	256.94 ± 21.03	257.99 ± 24.42	252.55 ± 21.22	248.67 ± 20.42
	14	269.40 ± 20.59	270.13 ± 25.15	262.00 ± 20.10	255.62 ± 22.06
	17	291.96 ± 23.50	293.50 ± 26.49	283.32 ± 22.48	274.30 ± 21.53↓
	20	317.11 ± 25.61	323.14 ± 28.39	308.11 ± 25.76	291.32 ± 25.37↓
Corrected Bwt. gain		18.24 ± 9.21	20.05 ± 13.04	9.33 ± 9.07↓	-4.40 ± 12.15↓
Body weight gain					
Period	Pre-treatment (days 0 – 5)	13.84 ± 5.70	15.28 ± 7.87	15.29 ± 7.46	16.10 ± 6.12
	Treatment (days 5 – 20)	78.45 ± 10.99	84.13 ± 13.04	66.77 ± 14.58↓	49.57 ± 15.40↓
	Throughout gestation (days 0 – 20)	92.28 ± 13.20	99.42 ± 17.12	82.06 ± 15.24	65.67 ± 16.70↓
Food intake (g/day/rat)					
Period (Days of gestation)	0 - 3	15.31 ± 2.82	16.86 ± 3.22	16.52 ± 1.62	16.70 ± 2.08
	3 - 5	16.68 ± 2.54	19.98 ± 3.61	19.09 ± 2.34	19.40 ± 2.42
	5 - 8	13.45 ± 2.52	13.12 ± 2.56	10.35 ± 3.27↓	11.63 ± 3.47
	8 - 11	14.44 ± 2.13	15.12 ± 2.64	13.72 ± 2.30	12.42 ± 2.43↓
	11 - 14	16.59 ± 1.74	16.86 ± 2.22	15.28 ± 1.87	13.39 ± 2.91↓
	14 - 17	16.25 ± 2.15	16.42 ± 2.26	14.74 ± 1.92	12.90 ± 1.96↓
	17 - 20	15.66 ± 2.44	16.19 ± 2.18	14.67 ± 2.15	12.05 ± 2.90↓

↑/↓: Significant Increase/Decrease of Mean values ($p \leq 0.05$)

Table 53: Litter data during a prenatal developmental toxicity study in the rat treated with Tea Tree Oil

Concentration (mg/kg Bwt/day)	**0**	**75/30**	**150/60**	**300/120**
No. of litters examined	24	23	23	21
No. of foetuses examined	134	134	126	118
FETUS ANOMALIES (Incidence and %)				
External observations				
Normal variant	- NIL -			
Minor anomalies				
Haemorrhagic patch on dorsal thoracic spine	0	1 (0.75%)	0	0
Fore limb flexed at wrist (+) (Rt/Lt/B)	0	0	0	1 (0.85%)
Small foetus	0	0	0	1 (0.85%)
Major malformations	- NIL -			
Visceral observations				
Normal variant				
Umbilical artery displaced	7 (5.22%)	6 (4.51%)	7 (5.51%)	7 (5.93%)
Liver median lobe extra lobation	1 (0.76%)	3 (2.26%)	2 (1.57%)	1 (0.85%)
Kidney renal pelvis dila. (Rt/Lt/B) (+)	1 (0.75%)	1 (0.75%)	0	0
Minor anomalies	- NIL -			
Major malformations	- NIL -			
NORMAL VARIANT PARAMETERS FOR WITH SIGNIFICANT ALTERATIONS ($p \leq 0.05$) **IN THE INCIDENCES** (%)				
Delayed skeletal ossification				
Stern: # 5	2.24	2.26	11.02↑	16.95↑
Stern: # 5, 6	0.75	0.00	6.30↑	16.95↑
Cervical centra 2/7	23.88	15.04	15.75	6.78 ↓
Cervical centra 6/7	13.43	10.53	26.77↑	36.44↑
Cervical centra 7/7	0.75	0.00	4.72	9.32↑
Caudal vertebral centra 1/4	17.16	12.78	24.41	60.17↑
Caudal vertebral centra 2/4	0.00	0.00	5.51↑	20.34↑
Caudal vertebral arch 1/2	11.94	6.77	16.54	57.63↑
Forelimb metacarpal 1/4	43.28	44.36	69.29↑	89.83↑
Forelimb proximal phalange 1/2	2.99	12.78↑	3.94	0.85
Forelimb proximal phalange 2/2	49.25	54.89	84.25↑	98.31↑
Forelimb distal phalange 1/4	14.18	11.28	35.43↑	39.83↑
Hind limb distal phalange 5/5	11.19	3.01↓	15.75	30.51↑
Incomplete/poor ossification				
Frontal, parietal and interparietal	0.00	0.00	0.00	4.24↑
Stern: #3	0.00	0.00	0.00	4.24↑
Stern: #4	0.00	0.00	1.57	8.47↑
Stern: #6	32.84	24.06	38.58	61.86↑

↑/↓: Significant Increase/Decrease of Mean values ($p \leq 0.05$)

Study 2, Anonymous, (2018b), Tea Tree Oil: Embryo-foetal developmental toxicity study by oral gavage in New Zealand White rabbits, OECD 414, GLP.

Reliability statement: The study is conducted in accordance with OECD 414. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).

In the second study, pregnant rabbits were treated orally by gavage at dose levels of 15, 30 and 75 mg/kg/day from GD 6 to 28.

Rabbits were observed for clinical signs, morbidity, mortality, body weight changes and food consumption. Caesarean section was performed for all the surviving rabbits on GD 29 and dams were examined for gross pathological changes. The uterus was removed by laparotomy, weighed and the contents were examined for number of implantation sites, early and late resorptions and number of foetuses. The number of corpora lutea in ovaries was counted. All the foetuses were sexed, weighed and examined for external malformations. All the live foetuses were examined for visceral and skeletal variations and malformations.

There was no mortality, clinical signs or gross necropsy findings in dams at any of the doses tested.

The group mean maternal body weights during the different days of gestation were comparable to the vehicle control group at all tested dose levels. However, as compared to the control, statistically significant decrease in net body weight gain during GD 6 – 9 was observed at 30 and 75 mg/kg/day. At 30 mg/kg/day, there was a 40 to 42% decrease in mean body weight gain during GD 6 to 29 and 0 to 29 respectively. The decrease in body weight gain

was statistically not significant. At 75 mg/kg/day there was significant decrease in body weight gain during treatment period GD 6 - 29 and for entire gestation period 0-29 which was 64 % and 54%, respectively. The decrease in body weight was considered non-adverse as the corrected body weight gain was comparable to vehicle control.

As compared to vehicle control, significant reduction in mean food consumption was observed following treatment at 30 and 75 mg/kg/day dose groups during intermittent periods of GD 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27 and 27 to 29 which was approximately 23 % to 45 % at 30 mg/kg/day and 28% to 63% at 75 mg/kg/day. Also during treatment period GD 6 - 29 and for entire period of gestation GD 0 - 29 there was significant reduction in food consumption which was 28 to 36% at 30 mg/kg/day and 34 to 43% for 75 mg/kg/day.

Table 54: Body weight gain, Food consumption and maternal data during the total gestation period (days 0-29) in a developmental toxicity study in rabbits with Tea Tree Oil

Dose (mg/kg/day)	**0**	**15**	**30**	**75**
No. of Pregnant rabbits	21	20	21	21
Mean body weight gain (kg)				
Pre-treatment period (d 0-6)	0.102 ± 0.09	0.051 ±0.07	0.065 ± 0.11	0.076 ± 0.07
Treatment period (d 6-29)	0.310 ± 0.19	0.284 ± 0.17	0.181 ± 0.20	0.113* ± 0.26
Total gestation period (d 0-29)	0.412 ± 0.21	0.335 ± 0.27	0.246 ± 0.25	0.189* ± 0.29
Corrected Body wt gain (kg)	-0.0036 ± 0.21	-0.043 ± 0.20	-0.137 ± 0.16	-0.199 ± 0.22
Food consumption				
Pre-treatment period (d 0-6)	147.56 ±18.06	154.32 ± 15.75	150.90 ± 14.23	145.86 ± 18.43
Treatment period (d 6-29)	130.14 ± 14.03	123.45 ± 19.26	82.71* ± 24.92	73.98* ± 24.21
Total gestation period (d 0-29)	133.75 ± 12.28	129.84 ± 15.81	96.82* ± 20.56	88.85* ± 20.96
Maternal Data (mean data)				
Gravid Uterine Weight (g)	346.04 ± 114.62	327.41 ± 94.88	317.54 ± 95.02	311.62 ± 110.41
No. of Corpora lutea	8.38 ± 1.66	7.80 ± 1.58	8.19 ± 1.29	8.90 ± 1.73
No. of Implantations	6.52 ± 2.18	6.25 ± 1.89	6.24 ± 2.00	6.76 ± 2.23
No. of Early Resorptions	6.52 ± 2.18	6.25 ±1.89	6.24 ± 2.00	6.76 ± 2.23
No. of Late Resorptions	0.24 ± 0.54	0.30 ± 0.47	0.33 ± 0.48	0.90 ± 1.79
No. of Pre-implantation Loss	1.86 ± 1.31	1.55 ± 1.32	1.95 ± 1.28	2.14 ± 1.31
No. of Post-implantation Loss	0.52 ± 0.81	0.65 ± 0.67	0.76 ± 0.89	1.76* ± 1.84
Dams with any Resorption	8	11	11	15
Dams with all Resorption	0	0	0	1
Maternal data (% per litter)				
Early resorptions	3.45 ± 7.92	6.31 ± 11.04	6.43 ± 10.41	14.30 ± 24.38
Late resorptions	4.61 ± 7.91	5.85 ± 11.99	6.78 ± 11.26	10.72 ± 14.58
Pre-implantation loss	23.87 ± 19.62	20.15 ± 17.19	25.00 ± 17.94	25.17 ± 16.77
Post-implantation Loss	8.06 ± 13.63	12.16 ± 14.74	13.22 ± 18.37	25.02 ± 23.87
Implantation index	76.13 ± 19.62	79.85 ± 17.19	75.00 ± 17.94	74.83 ± 16.77

*: Significantly different from the control group; Corrected Body wt gain = carcass weight - body weight on day 6

The maternal parameters comprising of gravid uterine weight, mean number of corpora lutea, implantations, early and late resorptions, pre and post implantation loss and dams with resorptions at the tested doses of 15 and 30 mg/kg/day treated groups were statistically comparable to the vehicle control group. At 75 mg/kg/day there was a significant increase in post implantation loss and this increase was considered treatment related as the value was higher than historical data.

Gross evaluation of placenta did not reveal any findings in any dams at any tested dose levels.

Table 55: Summary of Litter data

Parameters	Group No.	G1	G2	G3	G4
	Dose (mg/kg/day)	**0**	**15**	**30**	**75**
	No. of Pregnant rabbits	**21**	**20**	**21**	**21**
No. of litters		21	20	21	20
Total No. of foetuses		126	112	115	105
Mean litter size		6.0	5.6	5.5	5.0
Dead foetuses	Total No.	0	0	0	0
	%	0	0	0	0
Live foetuses	Total No.	126	112	115	105
	Mean weight (g) ± SD	38.84 ± 5.00	38.71 ± 4.98	37.71 ± 4.88	35.13 ± 6.33
Live male foetuses	Total No.	68	59	56	55
	Mean weight (g) ± SD	38.62 ± 4.46	39.55 ± 5.05	38.39 ± 5.35	35.97 ± 5.93
Live female foetuses	Total No.	58	53	59	50
	Mean weight (g) ± SD	38.32 ± 5.45	37.67 ± 5.93	36.37 ± 4.96	33.03 ± 7.31
Sex Ratio - Male: Female		1:0.85	1:0.9	1:1.05	1:0.91
(Percentage of number of males)		(54%)	(53%)	(49%)	(52%)

The litter parameters comprising total number of foetuses, mean foetal weight and number of live foetuses at all the treated groups were statistically comparable to the vehicle control group.
External, visceral and skeletal examination of foetuses revealed no signs of teratogenicity or developmental toxicity.
There were no gross pathological changes in any animal at any dose levels.
The post implantation loss observed within the second study (Anonymous, 2018b) at the highest dose tested can be considered as a consequence of an increased number of late resorptions. Even if a dam with a total loss due to early resorptions was considered by the study author as an outlier, the late resorptions still remain significantly increased while significance of post-implantation loss itself decreases.
In this study it was further reported that in the mid (30 mg/kg bw/day) and high (75 mg/kg bw/day) dose groups the food consumption of the dams was significantly decreased.
Compared to the animals of the control group, the TTO treated animals ate, in a dose dependent manner, significantly less from the sixth day of pregnancy on. Especially between days 6 - 18 (the critical time frame of organogenesis) only about half of the control values was consumed. Since TTO was administered by gavage, the effect cannot simply be attributed to reduced palatability. It can be assumed that consequently an energy deficiency situation has occurred in the dam, which in turn can have negative effects on the development of the foetus.
Concomitantly, as compared to the control, statistically significant decrease in net body weight gain during GD 6 – 9 was observed at 30 and 75 mg/kg/day. At the end of the treatment, all dosed animals showed weight loss (negative net body weight gains which is the terminal BW corrected for uterine weight). Clearly visible in the mid and high dose animal groups, though not significant anymore.
In this study peanut oil was used as the vehicle. Peanut oil can be considered as a vehicle with high caloric content that may cause an animal to consume less food with minimal or no net effect on body weight gain. This is likely the reason that the significant reduction of food consumption does not lead to a clear significant weight loss of the dams.
It has already been described several times that a decrease in maternal net body weight and concomitant reduction in food consumption usually indicate systemic toxicity.
Studies of dietary restrictions have demonstrated that reductions in food consumption of as little as 10% of the normal total dietary intake may be associated with increased prenatal death, dysmorphogenesis and/or growth retardation. Therefore, reduced maternal food intake may be an indication not only for maternal toxicity but also of secondary insult to the developing progeny.

Study 3, ECHA dissemination site, study report 2011, Tea Tree Oil: Oral Gavage Developmental Toxicity Study in the Hannover Wistar Rat, OECD 414, GLP.
A GLP compliant developmental toxicity study was conducted with Tea Tree Oil (TTO) in naturally mated, assumed pregnant Hannover Wistar female rats according to OECD Test Guideline 414, to evaluate the effect on dams and developing conceptuses after oral (gavage) administration during pregnancy. A control group which received PEG 400 only and three groups treated with TTO formulated in PEG 400 at 250, 100 and 20 mg/kg bw/day were included in the study. TTO formulated in PEG 400 was administered daily from gestation day (GD) 5 to GD19, where GD0 was considered the day of mating. Caesarean section and maternal necropsy with macroscopic examination were performed on GD20 in all the females surviving to termination. Placentas and foetuses were examined macroscopically and foetal body weight was measured. The gender of each foetus was

determined. Thereafter, approximately half of each litter was subjected to a visceral examination and the remaining foetuses were processed for skeletal examination.
At 250 mg TTO/kg bw/day, mortality occurred in 7/27 females between GD8 and GD11. Clinical signs in animals that died included noisy respiration, decreased activity and/or piloerection. Clinical signs in surviving animals included decreased activity, hunched back position, noisy respiration, piloerection, red spots on the tail and/or soft faeces. Treatment at 100 mg/kg bw/day resulted in no mortality but clinical signs such as noisy respiration, decreased activity, hunched back position, red spots and/or soft faeces were noted in 13/26 females. At 20 mg TTO/kg bw/day, there was no mortality. Noisy respiration or soft faeces were occasionally noted, but were considered not to be toxicologically relevant.
Severely reduced maternal body weight gain (-20% and -45% respectively, when compared to control) and food consumption were noted at 100 and 250 mg TTO/kg bw/day.
In females treated at 250 mg TTO/kg bw/day, bilateral enlarged adrenals were observed in all animals found dead and in 6/20 of animals that survived until scheduled necropsy. This finding was attributed to treatment. A single animal in the mid dose group also had bilateral enlarged adrenals.
At 250 mg TTO/kg bw/day, there was a higher number of late embryonic deaths and consequently postimplantation loss, leading to an overall higher total intrauterine mortality. Post-implantation losses were unchanged in the low and mid treatment groups. Post-implantation mortality was considered secondary to maternal toxicity.
Statistically lower mean gravid uterine weight was noted at 250 mg TTO/kg bw/day and lower terminal mean body weights when corrected for the gravid uterine weight, were noted at 100 and 250 mg TTO/kg bw/day. The corrected mean body weight gains were lower than the controls in the two highest dose groups. These adverse effects were considered to be related to TTO administration.
Most foetuses were viable and no effects related to TTO were noted in the mean number of viable foetuses/ group, or their sex distribution. The sex ratios were similar in the control and treated groups when evaluated per litter.
Adverse effects were noted in mean foetal weights at 100 and 250 mg TTO/kg bw/day, with a dose related pattern. The effects on foetal body weight were related to intrauterine growth retardation. External abnormalities such as local edema in the cervical area, generalized edema or short maxilla were noted in the high dose group. There was no statistically significant difference from control in the number of visceral malformations. A statistically higher number of visceral variations were noted in the 250 mg/kg bw/day dose group. These included dilated brain ventricles and displaced gonads associated with the intrauterine growth retardation were noted. In addition, variations such as small nasal conchae, close origin of brachiocephalic and carotid, dilated ureter or dilated renal pelvis were statistically increased at 250 mg/kg bw/day.
A statistically higher incidence of skeletal malformations unrelated to intrauterine growth retardation was noted in the 250 mg/kg bw/day group. This included displaced rib cartilages at the sternum, malformed vertebrae, and/or short, bent scapula, humerus or femur. Statistically higher numbers of skeletal variations, secondary to maternal toxicity, were noted at 100 and 250 mg/kg bw/day.
In summary, severe maternal toxicity was seen in dams from the 100 and 250 mg/kg bw/day groups, as evidenced primarily by clinical signs, reduced food consumption and reduced weight loss gains (and mortality at the high dose). Foetal abnormalities seen in the 100 and 250 mg/kg bw/day groups were secondary to maternal toxicity. The NOAEL for Tea Tree Oil for developmental toxicity (secondary to severe maternal toxicity) was 20 mg/kg bw/day. The NOAEL for maternal toxicity was 20 mg/kg bw/day.

Within the developmental toxicity study in rats (Anonymous , 2012a) there were no relevant findings other than maternal toxicity (reduced body weights and food intake). An increased number of resorptions occurred, however only at extremely high doses (300 mg/kg bw/day) in parallel with severe maternal toxicity and increased mortality. Furthermore, there are indications that TTO, if administered by gavage, shows pharmakokinetic (and then pharmacodynamic) properties other than after dietary administration: Short-term toxicity tests conducted with Tea Tree Oil revealed detrimental effects on sperm count and motility, at higher doses also linked to microscopical changes in tissue. These effects were demonstrated to be reversible within max. 8 weeks following exposure. It should be noted however, that all these studies, like the present study, were conducted with gavage as method of application, since most components of Tea Tree Oil have extremely high vapour pressure which makes dosing via mixing into food very difficult. However, data is available for bicyclic monoterpenes (α-Terpineol, a constituent of Tea Tree Oil and very similar to its main component Terpinen-4-ol) where reproductive studies were conducted both via gavage and via diet administration. It was demonstrated that after dietary administration of α-Terpineol sperm damage did not occur. Pharmacokinetic analysis confirmed that oral gavage at high doses clearly resulted in much higher systemic exposure than expected, leading to biologically non-relevant effects that should not be considered for classification purposes (for details please refer to 10.10.2). Assuming that the unexpected high systemic exposure is the trigger of sperm damage, it is not unlikely that comparably this overexposure would have a negative impact on the already fragile developmental course, additionally to the affection by maternal food and energy deficiency.

Then the converse conclusion would also be admissible, that after a dietary administration, which is the more relevant one for humans, the degree of late resorption would be lower, if not completely absent.
Overall, the increase in post-implantation loss at the highest tested dose is due to an increased number of late resorptions. This kind of developmental impairment might be caused by reduced food consumption, especially if in parallel the net weight gains of the dams were reduced indicating maternal toxicity. Peak concentrations of TTO in blood after gavage administration may potentiate the effects already induced by maternal food energy deficiency. Late resorptions may occur to a lesser extent if TTO is administered via food which is the most relevant route for humans.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Category 1A: *Known human reproductive toxicant*. The classification of a substance in Category 1A is largely based on evidence from humans.
Category 1B: *Presumed human reproductive toxicant*. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide **clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects**, or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
Category 2: *Suspected human reproductive toxicant*. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects **shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.**
There is no data on humans to inform on the developmental toxicity of Tea Tree Oil, and so classification in category 1A is not appropriate.
Since there is no clear evidence of an adverse effect in the absence of other toxic effects, no classification in category 1B or category 2 for toxicity on development is warranted.
The effects observed in both developmental studies do not indicate that TTO developmental toxicity in rats and rabbits meets classification criteria for this health hazard. Main developmental parameters were not affected therefore they are not considered as significant adverse effects warranting classification for developmental toxicity.

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

The potential of Tea Tree Oil to elicit adverse effects on or via lactation has been investigated in a two-generation study in rats (see also section 10.10.1).

Table 56: 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	NOEL/NOAEL [mg/kg bw/day]	Results	Reliability score	Reference
Two generation study in the rat OECD 416 GLP	Tea Tree Oil Purity: 10.30 % α-Terpinene, 20.90 % γ-Terpinene, 1.53 % p-cymene and 42.36 % Terpinen-4-ol (in compliance with ISO specification) Administration: Oral (gavage) Dose levels: Generation-P:	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P)	1	Anonymous (2017a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	NOEL/NOAEL [mg/kg bw/day]	Results	Reliability score	Reference
	0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day Treatment related alterations were observed in the reproductive performance at 50 mg/kg bw/day (P generation). Hence, the high dose of 50 mg/kg bw/day was reduced to 38 mg/kg bw/day for the pups selected for F1 generation.		↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) *More detailed results are presented in -Table 37 (Section 10.10.1)*		

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

The potential of Tea Tree Oil to elicit adverse effects on or via lactation has been investigated in a two-generation study in rats (see also section 10.10.1).

Study 1: Anonymous (2017a) Tea Tree Oil: Two generation reproduction toxicity study in Wistar rats, OECD 416, GLP

Reliability statement: The study is conducted in accordance with OECD 416. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restrictions (reliability score: 1).Within the study, post-natal survival of pups was not affected by Tea Tree Oil exposure: there were no effects on lactation or viability indices in either generation. The only toxic effect on pups which might be due to substance transfer via milk was seen on bodyweight of the pups of the F1 generation (i.e. F2 litter). During lactation, treatment with Tea Tree Oil significantly reduced mean body weights on Days 1 and 4 in male pups and on Days 1, 4 and 7 in female pups and combined sex at 38 mg/kg bw/day, which is the highest dose tested within this generation. The bodyweights of the P generation pups (i.e. F1 litter) were however not affected even though the dams received 50 mg/kg bw/day. At the end of the lactation period (21 day), body weights recovered and were no longer different from control animals indicating that the body weight reduction, even if treatment related, should not be considered as a severe toxic effect. There was no indication of impaired nursing behaviour.
The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

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

According to the Regulation (EC) No 1272/2008 (CLP) Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:
"(a) human evidence indicating a hazard to babies during the lactation period; and/or
(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."
No data is available to address criteria (a) and (c). The reduced body weight gain of F1 pups during the initial days of lactation is not considered to "*provide clear evidence of adverse effect in the offspring due to transfer in the milk*".

No classification for reproductive toxicity concerning effects on or via lactation is proposed.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Adverse effects on sexual function and fertility
Based on all provided data Tea Tree Oil TTO warrants classification to subcategory Repr. 2 with hazard statement H361f - Suspected of damaging fertility.

Adverse effects on development
No classification is proposed. Data conclusive but not sufficient for classification.

Adverse effects on or via lactation
No classification is proposed. Data conclusive but not sufficient for classification.

2.6.7 Summary of neurotoxicity

Not deemed required, because Tea Tree Oil has no similar or related structure to those capable of inducing neurotoxicity. Tea Tree Oil does not induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity. No effects were observed in other (sub-) chronic studies which are indicative for neurotoxicity.

2.6.8 Summary of other toxicological studies

There is no reason to perform additional studies on Tea Tree Oil

2.6.8.1 Toxicity studies of metabolites and impurities

As the components of Tea Tree Oil are naturally present in edible and medicinal crops, plant metabolites are not relevant for the evaluation.
Consequently, studies on the toxicity of the possible metabolite of Tea Tree Oil were not conducted and are not deemed necessary.

2.6.8.2 Supplementary studies on the active substance

Tea Tree Oil does not induce specific indications of potential immunotoxicity in toxicity studies. No effects were observed in other (sub-) chronic studies which are indicative for immunotoxicity.

2.6.9 Summary of medical data and information

No hazardous incident had occurred with workers in the production facilities of Tea Tree Oil and its formulated products.
The reported in the open literature effects of TTO in humans are related to skin irritation and sensitization after dermal contact. Following ingestion of TTO, neurological effects such as ataxia, unresponsiveness and drowsiness may occur. After ingestion of half a cup of pure TTO coma and disturbances of consciousness were reported as well. The reactions after ingestion and symptoms initially observed were reversible and recovery was demonstrated. Fresh TTO seems to be better tolerated so a date of minimum durability should be considered.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 57: 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	Two generation study, OECD 416	TTO	↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1)	25 mg/kg/day	38 mg/kg day	Anonymous (2017a)

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

The natural exposure to components of TTO with food, mostly fruits and vegetables, is relatively high up to 12.5 mg/kg bw/d, (with TMDI for aliphatic and aromatic hydrocarbon monocyclic monoterpenes: 0.814 mg/kg bw/d; for aromatic unsaturated tertiary alcohols monocyclic monoterpenes: 8.878 mg/kg bw/d; for bicyclic monoterpenes 0.868 mg/kg bw/d, for polycyclic sesquiterpenes 1.99 mg/kg bw/d (Anonymous, KCA 6.9/01. Since they are natural plant products, the toxicity of none of these terpenes has not been evaluated in line with requirements of Commission Regulation (EU) No 283/2013 and Regulation (EC) No 1272/2008. The potential effects of the current human exposure to any of these terpenes were not assessed based on the results of targeted epidemiological studies, which would be extremely difficult, if possible at all, thus there are no toxicological endpoints which could be used for calculation of ADI. Although the natural exposure to components of TTO is relatively high, the proportion and occurrence of various terpenes in fruits and vegetables are very different from their proportion and occurrence in TTO, therefore the intake of terpenes with natural food is qualitatively and quantitatively different from their potential intake from of TTO. Therefore, it is proposed to based ADI on results of TTO toxicity studies in animals. Taking into account the lowest No Observed Adverse Effect Level (NOAEL) for reproductive toxicity of 25 mg/kg bw/d derived in the 2-generation study in rats, an ADI of 0.25 mg/kg bw/d can be set using an assessment factor of 100.

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

The results of acute toxicity and repeated-dose toxicity studies do not indicate acute toxic effects which would require setting Acute Reference Dose for TTO, therefore it is proposed that no ARfD is needed.

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

Based on the lowest No Observed Adverse Effect Level (NOAEL) for reproductive toxicity of 25 mg/kg bw/d derived in in 2-generation study in rats, an **AOEL** of 0.25 mg/kg bw/d can be set using an assessment factor of 100.

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

The results of acute toxicity and repeated-dose toxicity studies do not indicate acute toxic effects which would require setting Acute Acceptable Operator Exposure Level for TTO, therefore it is proposed that no AAOEL is needed.

2.6.11 Summary of product exposure and risk assessment

Operator

Estimations of potential operator exposure have been performed for Tea Tree Oil for all intended uses and the following predictive models.

- EFSA model (2015)

- ECPA model (2010) for greenhouse

For the inhalation exposure assessment during and after application in the greenhouse the results of the study to monitor operator and worker inhalation exposure to Timorex Gold (Tea Tree Oil) in greenhouse crops were used (B.6.4.1.2/01 / KCP 7.2.1.2/01).

No AAOEL is needed for TTO since it does not pose a risk of acute poisoning due to application as an active substance in plant protection products, thus according to EU Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (SANTE-10832-2015 rev. 1.7; 24 January 2017; EFSA Journal 2014;12(10):3874) only longer term risk assessment for operator is required.

Outdoor

Since both, potential and actual exposure of operator applying BM 608 on tomato and grape in accordance with GAP at a rate of 445 g a.i./ha are well below AOEL of 0.25 mg/kg bw/d (25% of AOEL or less), the exposure of operator applying BM 608 outdoor in accordance with GAP does not pose an unacceptable risk.

Indoor

The potential exposure of operator wearing T-shirt and shorts and applying BM 608 on tomato in a greenhouse using knapsack sprayer in accordance with GAP at a rate of 445 g a.i./ha equal 0.39 mg/kg bw/d is above AOEL of 0.25 mg/kg bw/d (156% of AOEL), therefore the risk is considered unacceptable. However, using PPE such as protective coverall, gloves and mask A1P2 reduces exposure of operator to 0.01395 mg/kg bw/d, thus to 5.6% of AOEL, therefore the risk of operator wearing these PPE is acceptable.

Resident

The exposure to TTO by all routes (inhalation, dermal and oral for a child) calculated with EFSA model (2015) amounted for a child resident to 0.028 mg/kg bw/d, and for adult resident – 0.009 mg/kg bw/d. Thus, the exposure of a child resident corresponded to 11.2% of AOEL equal to 0.25 mg/kg bw/d, and exposure of adult resident was equal to 3.6% of AOEL. Therefore, application of product BM 608 does not pose an unacceptable risk for residents.

Since no AAOEL is proposed for TTO, thus according to EU guidance (SANTE-10832-2015 rev. 1.7 24 January 2017) there is no needed to evaluate exposure of bystander, since it is acute type of exposure. Nevertheless, it is noted that the bystander exposure calculated with EFSA model (2015) is below 10% of AOEL for TTO equal to 0.25 mg.kg bw/d).

Worker

Outdoor

The exposure of worker to TTO when reaching, picking tomato for 8 hours on the outdoor field treated 3 times with 7-day intervals with BM 608 at 0.445 kg a.s./ha calculated with EFSA model (2015) using default DFR amounted to 0.070 mg/kg bw/d (28% of AOEL equal 0.25 mg/kg bw/d) for worker wearing work wear with arms, body and legs covered or 0.016 mg/kg bw/d (6.4%) of AOEL equal 0.25 mg/kg bw/d) for worker wearing work wear arms, body and legs and gloves. Thus, the exposure of worker reaching or picking tomato for 8 hours and wearing work cloths covering arms, body and legs does not pose an unacceptable risk.

For worker harvesting for 8 hours grapes on the outdoor field treated 4 times with 10-day intervals with BM 608 at 0.445 kg a.s./ha calculated with EFSA model (2015) using default DFR the exposure was not acceptable, therefore the new DFR of 0.234 µg/cm2/kg a.s./ha was derived based on the measured residues decrease within 24 hours after application.

Using new justified DRF for estimation of exposure of worker to TTO when harvesting grapes for 8 hours on the outdoor field treated 4 times with 10 day intervals with BM 608 at 0.445 kg a.s./ha calculated with EFSA model (2015) this exposure amounts to 0.025 mg/kg bw/d for worker wearing work wear with arms, body and legs covered (10% of AOEL equal 0.25 mg/kg bw/d) or 0.074 mg/kg bw/d for worker not wearing work wear with arms, body and legs and gloves – potential exposure (29.64%) of AOEL equal 0.25 mg/kg bw/d). Thus, the exposure of worker harvesting grapes outdoor for 8 hours and wearing T-shirt and shorts or work cloths covering arms, body and legs does not pose an unacceptable risk.

Indoor

The potential exposure of worker to TTO when reaching, picking tomato for 8 hours in the greenhouse was calculated in to steps: step 1 – dermal exposure by EFSA model (2015) and step 2 due to high volatility of TTO components, based on measurement of actual inhalation exposure in the greenhouse on the day of application. The total potential dermal and inhalation exposure of worker picking up tomato for 8 hours in the greenhouse first day after application amounted to 0.2417 mg/kg bw/d, while of worker wearing work wear: arms, body and legs covered + gloves to 0.0608 mg/kg bw/d, both below AOEL of 0.25 mg/kg bw/d (respectively 96.7% and 24.3% of AOEL). Thus, the exposure of worker harvesting tomato in the greenhouse for 8 hours and wearing T-shirt and shorts or work cloths covering arms, body and legs does not pose an unacceptable risk.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Plant matrices

Stability of analyte residues of Terpinen-4-ol and 1,8-Cineole in sample extracts is verified by the acceptable fortification recovery data in grape and tomato for at least 13 months at -20°C (Meseguer, C., 2017).
The residues of γ-Terpinene have been found to be not stable in tomato cucumber and grape when stored for above 45 days at -20°C based on results of both submitted studies (Oppilliart, S., 2010 and Meseguer, C., 2017).
These data are appropriate to support frozen storage stability of two 'lead components' of TTO (Terpinen-4-ol and 1,8-Cineole) in tomato and grape for up to 13 months, these accommodate the time that samples were stored in the residue trials studies.
Content of γ-Terpinene declined to 48% after 7 months and 45% after 13 months in tomato and 20% after 6 months and 13% after 13 months in grape. Therefore, for dietary risk assessment, the residues of γ-Terpinene should be multiplied with a factor of 2.2 for tomato and 7.7 for grape to account for the loss of residues.

Animal matrices

No studies submitted.
No residue definition has been proposed for TTO, then assessment of residue stability is not relevant.

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

Plant matrices

In a greenhouse plant metabolism trial with the components of Tea Tree Oil on tomatoes, no major metabolite was identified. On fruits the constituents of TTO dissipate and no uptake and metabolization occurs. This can most likely be accounted to the significantly lower number of stomata on fruit. Only catabolism products from the plant are transferred into the fruit.

Very low levels of radioactivity and none of the measured compounds p-Cymene, 1,8-Cineole, γ-Terpinene, Terpinene-4-ol, (+)-Aromadendrene and (-)-Globulol were detectable in the tomato fruits in metabolism study, following two over the top post emergence foliar applications with [4-^{14}C]-terpinene-4-ol and five additional major compounds (γ-Terpinene, p-Cymene, 1,8-Cineole, (+)-Aromadendrene and (-)-Globulol) of Tea tree oil at BBCH 87 and BBCH 88. Raw agricultural commodities (RAC) were harvested at BBCH 88 (tomato plant and fruit), nine days after first application and one day after second application. Due to the low residue levels in tomato fruit, characterization and identification of the components of the residue was not required. This indicates that there was no significant uptake of major components of TTO, or metabolites, from the plant into the fruit.
Considering the naturally occurring levels of TTO components in the variety of plants species (plants used for medicinal or seasoning purpose, as well as edible crops) and low residue levels in tomato fruit further plant metabolism data are not required.
The metabolism study of TTO in grape (intended use) was not submitted but the metabolism study of TTO in tomato (fruiting vegetable) can be considered applicable for grape taking into account that both crops belong to the category fruits (code: F) and that the same application conditions (method, rate, timing, PHI) are intended for uses in grape and tomato.

Animal matrices

No metabolism data are required.

2.7.3 Definition of the residue

For Tea Tree Oil, no residue definition is needed due to the fact that the intended uses give rise to a no residue situation. The plant metabolism study demonstrated that no compounds of Tea tree oil and no significant metabolites are occur in the edible part of the fruiting crops. All study results (plant metabolism study and residue trials) demonstrate that after application the residues will quickly decline to natural background levels. Natural background levels of the substances are summarized under point B.7.7. in the DAR.
This conclusion is also shared by the RMS in the DAR for Tea Tree Oil (September 2008) which is confirmed in the Review Report from 1 August 2008 (SANCO/2609/08 rev.1). Furthermore, Commission Regulation 839/2008 of 31 July 2008 included the extract of Tea Tree into Annex IV of Regulation 396/2005. Substances listed there in do not

require setting of an MRL and consequently, a residue definition is also not necessary. Since dietary intake from natural occurrence clearly exceeds the dietary exposure due to uptake from treated crops and as no additional residues are expected due to the use application of Tea tree oil to edible crops, Tea Tree oil is proposed to be kept in Annex IV of Regulation 396/2005.

No residue definition for plant or animal products is proposed.

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

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)
Grape	EU-S	Terpinene-4-ol: 1N: 3 x <0.01 (LOQ), 0.025*; 2N: < 0.01 (LOQ), 0.05*	GAP: 4 x 0.445 kg TTO along the crop cycle
		γ-Terpinene: 1N: 6 x <0.01 (LOQ), 2N: 2 x < 0.01 (LOQ)	
		1,8-Cineole: 1N: 6 x <0.01 (LOQ), 2N: 2 x < 0.01 (LOQ)	
Tomato	Indoor	Terpinene-4-ol: 1N: 0.01, 3 x < 0.01 (LOQ); 2N: 2 x < 0.01 (LOQ)	GAP: 4 x 0.445 kg TTO along the crop cycle
		γ-Terpinene: 1N: 4 x <0.01 (LOQ), 2N: 2 x < 0.01 (LOQ)	
		1,8-Cineole: 1N: 4 x <0.01 (LOQ), 2N: 2 x < 0.01 (LOQ)	
Tomato	EU-S	Terpinene-4-ol: 1N: 3 x < 0.05 (LOQ), 2N: 3 x < 0.05 (LOQ)(3 applications) 1N: 4 x < 0.01 (LOQ), 2N: 2 x < 0.01 (LOQ); 0.01 (4 applications)	GAP: 3 x 0.445 kg TTO along the crop cycle
		γ-Terpinene: 1N: 3 x < 0.05 (LOQ), 2N: 3 x < 0.05 (LOQ)(3 applications) 1N: 4 x < 0.01 (LOQ), 2N: < 0.01 (LOQ); 0.01 (4applications)	
		1,8-Cineole: 1N: 3 x < 0.05 (LOQ), 2N: 3 x < 0.05 (LOQ) (3 applications) 1N: 4 x < 0.01 (LOQ), 2N: 2 x < 0.01 (LOQ); 0.01 (4 applications)	

Grape
A total of 14 new residue trials conducted in 2014, 2015 and 2016 are available to evaluate the residue behaviour of lead components of TTO in grape grown in southern Europe after application of Timorex Gold (TTO 222.5 g as/L EC), in support of the critical GAP.
Four studies (trials no. S16-04716-01, S16-04716-02, S16-04716-03 and S16-04716-04) fully comply with the proposed GAP (4 treatments and the application rate <±25%), thus the minimum number of residue trials to support the GAP in grapes was achieved taking into account that no residues of the three components Terpinen-4-ol, γ-Terpinene and 1,8-Cineole above the LOQs (=0.01mg/kg) were detected after one hour after the last treatment in any of the trials.
A sufficient number trials, comparable with the proposed GAP, have been conducted to confirm the residue situation in grape in southern Europe.
The trials with two or three applications and the trials conducted with the 2-N GAP-rate can be used to support the GAP.

Tomato - outdoor
A total of 12 new residue trials conducted in 2015 and 2016 are available to evaluate the residue behaviour of lead components of TTO in tomato grown outdoor in southern Europe after application of Timorex Gold (TTO 222.5 g as/L EC), in support of the critical GAP.

Four studies (trials no. S16-04714-01, S16-04714-02, S16-04714-03 and S16-04714-04) comply with the proposed GAP (the application rate per crop and per season < ±25% of GAP rate with increased number of applications by not more than 25% -from 3 to 4), thus the minimum number of residue trials to support the GAP in outdoor tomato was achieved taking into account that no residues of the three components Terpinen-4-ol, γ-Terpinene and 1,8-Cineole above the LOQs (=0.01mg/kg) were detected after one hour after the last treatment in any of the trials.
A sufficient number trials, comparable with the proposed GAP, have been conducted to confirm the residue situation in outdoor tomato in southern Europe.
The trials with three applications with application rate > ±25% of GAP rate and the trials conducted with the 2-N GAP-rate can be used to support the GAP.

Tomato - indoor
A total of 8 new residue trials conducted in 2008 and 2016 are available to evaluate the residue behaviour of lead components of TTO in greenhouse tomato grown in Europe after application of Timorex Gold (TTO 222.5 g as/L EC), in support of the critical GAP.
Four studies (trials no. S16-04715-01, S16-04715-02, S16-04715-03 and S16-04715-04) fully comply with the proposed GAP (4 treatments and the application rate <±25% of GAP rate), thus the minimum number of residue trials to support the GAP in greenhouse tomato was achieved taking into account that no residues of the three components Terpinen-4-ol, γ-Terpinene and 1,8-Cineole above the LOQ (=0.01 mg/kg) were detected after one hour after the last treatment in any of the trials.
A sufficient number trials, comparable with the proposed GAP, have been conducted to confirm the residue situation in greenhouse tomato.
The trials with one or two applications and the trials conducted with the 2-N GAP-rate can be used to support the GAP.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish
No feeding studies are required.

2.7.6 Summary of effects of processing
No data on processing are required.

2.7.7 Summary of residues in rotational crops
No data on the magnitude of residues in rotational crops are required.

2.7.8 Summary of other studies
No other studies are required.

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

As neither MRLs nor a residue definition are proposed due to a no residue situation in all intended crops after application of TTO according to the GAP, TMDI calculations are not required. However, dietary exposure calculations were carried out and are summarized in the following in order to demonstrate that dietary exposure from natural occurrence is substantially higher than dietary exposure from application to crops following the intended uses. Consumer exposure is therefore considered to be acceptable. This conclusion is drawn in analogy to the Guidance Document on botanical active substances (ENV/JM/MONO(2017)69, p. 29 where it is stated that "It is acknowledged that if the proposed botanical active substance is considered to be the same material that is reasonably expected to be a, or to become a component of, food, this provides considerable reassurance for consumer exposure. Food grade material is difficult to define however, and therefore the applicant is asked to provide a reasoned case/evidence to the way the material complies with relevant food legislation, confirming that technical material is the same as that which is supplied to the food industry, and explaining the extent to which the material is used in food." Most of the TTO main components (4-Carvomenthenol (Terpinen-4-ol), α-Pinene, α-Terpinene, p-Cymene, 1,8-Cineole, γ-Terpinene, Terpinolene and α-Terpineol) are included in the US Code of Federal Regulations lists, as permitted flavoring substances for direct addition to food for human consumption (https://www.law.cornell.edu/cfr/text/21/172.515). Based on this fact and their natural occurrence in food significantly exceeding exposure from treated crops, the consumer exposure is considered to be acceptable.

Data point:	KCA 6.9/01
Report author	Anonymous
Report year	2018
Report title	Tea Tree Oil- Dietary exposure of Consumers to components of Tea tree oil: natural exposure and exposure after application of TTO to crops
Report No	-
Document No	-
Guidelines followed in study	None, expert statement
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	In this expert statement, information from literature was compiled and used for dietary exposure calculations using EFSA PRIMo rev. 3(2018). It is deemed to provide supportive information.

Aim

The aim of this evaluation is to calculate the natural exposure of consumers to the components contained in Tea tree oil from food sources and to compare these results to the exposure via crops which were previously treated with Tea tree oil. For evaluation of dietary exposure, the TMDI and the IESTI are calculated for the components contained in Tea tree oil.

Data

Natural occurrence

A literature review was conducted in order to evaluate the natural level of these components in food commodities (Anonymous, 2016[41]). A summary of the residues in edible crops is given in Table 2.7.9-1 (detailed information were provided in Appendix 1 to KCA 6.9/01). When a range of results was given, mean values were used to assess the natural exposure of consumers. In case more than one value was available per crop (e.g. due to different cultivars), the highest value was chosen for intake calculations.

Metabolism in plants

A metabolism study was conducted for the following compounds of Tea tree oil in tomato (Rieder, 2018, report no. S17-01154): [^{14}C] Terpinene-4-ol, γ-Terpinene, p-Cymene, 1,8-Cineole, Aromadendrene and Globulol. The results suggest that:

- On the surface, the constituents of Tea tree oil dissipate rapidly due to the high vapour pressures. About 89 % of Terpinene-4-ol dissipated from the plant surface - the amount of radioactivity recovered in the tomato samples after the second application was 11.6%
- In the plant (green parts), p-Cymene (vapour pressure = 219 Pa) was detected. However, the level in the control plant was above the p-Cymene level in the treated plants. Therefore, it is likely that the residues of p-Cymene are naturally occurring in tomato plants and are not due to the treatment with Tea tree oil.
    - The uptake of the TTO components tested occurs through the stomata where they are rapidly metabolized, most likely catabolized. This is supported by the fact no residues of the tested substances could be detected (except of p-Cymene which apparently is inherently present in the green parts of tomato). Metabolites could be detected, but only in the green plant parts.
- In and on the fruit (edible part of the crop), no lead components or metabolites were detected and a very low TRR and no individual peaks in HPLC analysis were measured, leading to the conclusion that:
    - On fruits the constituents of TTO dissipate and no uptake and metabolization occurs. This can most likely be accounted to the significantly lower number of stomata on fruit.
    - Only catabolism products from the plant are transferred into the fruit.

The catabolism products from the plant are transferred into the fruit.

[41] Anonymous (2016). Plant terpenoids – occurrence, diversity, biological function and biosynthesis, with a special focus on Tea tree (Melaleuca alternifolia) essential oil compounds. Position paper. Prepared by Registration Department, Stockton Group, February 2016 [31 pp]

Table 2.7.9-1 Natural occurrence of TTO-components in unprocessed food commodities (mean values [mg/kg]). Values used for dietary exposure calculations marked in bold.

	Terpinen-4-ol	**α-Terpinolene**	**p-Cymene**	**Limonene**	**α-Terpinene**	**γ-Terpinene**	**α-Terpineol**	**α-Pinene**	**1,8-Cineole**	**Sabinene**	**δ-Cadinene**	**Aromadendrene**	**Globulol**	**Viridiflurol**
Grapefruit (Pomelo)	-	-	-	**1138**	**21.6**	-	-	-	-	-	-	-	-	-
Grapefruit (Redblush)	-	-	-	547	7.8	-	-	-	-	-	-	-	-	-
Citrus (EFSA)	-	-	-	-	-	-	-	-	-	-	**1000**	-	-	-
Grape (berries)	**0.039**	-	-	**0.004**	**0.002**	**0.005**	**0.012**	**0.008**	-	-	-	-	-	-
Black currant (EFSA)	-	-	-	**0.3**	-	-	-	-	-	**1.9**	-	-	-	-
Raspberry	**0.114**	-	**0.013**	**0.001**	**0.014**	**0.011**	**0.032**	**0.024**	-	**0.017**	-	-	-	-
Carrot refrigerated	-	-	0.046	0.082	**0.025**	0.295	-	0.094	-	0.312	-	**0.009**	-	-
Carrot frozen	-	-	0.043	0.06	0.018	0.171	-	0.068	-	0.124	-	0.002	-	-
Carrot (EFSA)	-	-	-	1.6	-	-	-	-	-	**5.2**	-	-	-	-
Carrot (orange)	-	**3.465**	**0.057**	**0.236**	0.010	**0.569**		**0.180**		0.023				
Tomato	-	-	**0.005**	-	-	**0.019**	-	-	-	-	-	-	-	-
Corn	-	-	-	-	-	-	**1259**	**39.5**	-	**82.7**	-	**34.3**	**170**	**26**
Black pepper	**400**	-	-	-	-	-	-	-	-	-	-	-	-	-

Residues from application to crops

The results from residue trials conducted with Tea tree oil, formulated as Timorex Gold (according to point 7.3 of this document) were used for intake calculations.

An overview on the final GAP with the relevant information is presented in Table 2.7.9-2 and a short summary of highest relevant residues is given in Table 2.7.9-3

Table 2.7.9-2 Intended GAP for the formulation BM-608 / Timorex Gold (222.5 g TTO/L)

Crop and/or situation	**F, G or I**	**Application**				**Application rate per treatment (kg a.s./ha)**	**PHI (days)**
		Method, kind	**Growth stage and season**	**Number min/max.**	**Interval between applications**		
Tomato	G	Foliar sprayer	Along crop cycle	4	7	0.334	-
Tomato	G	Foliar sprayer	Along crop cycle	4	7	0.445	-
Tomato	F	Foliar sprayer	Along crop cycle, from spring till autumn	4	7	0.334	-
Tomato	F	Foliar sprayer	Along crop cycle, from spring till autumn	4	10	0.445	-
Grape	F	Foliar sprayer	Along crop cycle, from spring till autumn	3	7	0.334	-
Grape	F	Foliar sprayer	Along crop cycle, from spring till autumn	3	7	0.445	-

Table 2.7.9-3 HR values [mg/kg]from residue trials conducted with Tea tree oil (all residues < LOQ max. 12 h after application)

	Terpinen-4-ol	**γ-Terpinene**	**1,8-Cineole**
Tomato Greenhouse (4 x 445 g TTO/ha, 11 h after appl.)	< 0.01	< 0.01	< 0.01
Tomato Field (3 x 445 g TTO/ha, 12 h after appl.)	< 0.05	< 0.05	< 0.05
Grapes Field (4 x 445 g TTO/ha, 12 h after appl.)	< 0.01	< 0.01	< 0.01

Grouping of components

The components contained in Tea tree oil can be grouped according to their chemical structure as several components have very similar structures. Consequently, their chemical properties are comparable. To be highlighted, the vapour pressure of a component has substantial influence on the magnitude of residues on the plant surface. This is especially true for Tea tree oil which is a mixture of mainly highly volatile compounds.

Details on chemical structure and component groups are given in Appendix 3 if the original report (KCA 6.9/01). A short overview is given in Table 2.7.9-4 where the components are grouped and their respective vapour pressure is listed. It becomes obvious that nearly all compounds are volatile[42] and the magnitude of vapour pressure of the compounds in the allocated groups is comparable. Consequently, their dissipation on the crops will be similar, as well

Table 2.7.9-4 Chemical grouping of components contained in Tea tree oil and their vapour pressures (VP) [Pa]

Monocyclic monoterpenes				**Bicyclic monoterpenes**		**Polycyclic sesquiterpenes**					
Aliphatic and aromatic hydrocarbons [a)]		**Alicyclic and aromatic saturated & unsaturated tertiary alcohols** [b)]				**Cadinane group**		**Aromadendrene group**			
										Alcohols	
Substance	VP	Substance	VP	Substance	VP	Substance	VP	Substance	VP	Substance	VP
γ-Terpinene [a)] α-Terpinene [a)] α-Terpinolene [a)] Limonene [a)] p-Cymene [a)]	145 145 222 192 219	Terpinen-4-ol [b)] α-Terpineol [b)]	5.7 5.6	1.8-Cineole [c)] α-Pinene [a)] Sabinene [a)]	208 536 981	δ-Cadinene [a)]	2.51	Aromadendrene [d)] Ledene [d)]	5.27 2.72	Globulol [d)] Viridiflurol [b)]	4.95×10^{-3} 4.95×10^{-3}

a) Chemical group no. 31 Aliphatic and aromatic hydrocarbons
b) Chemical group no. 6 Aliphatic, alicyclic, and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols
c) Chemical group no. 16 Aliphatic and aromatic hydrocarbons
d) not allocated

[42] EPPO classifications of volatility from plants: Vapour pressure (Pa): highly volatile: $\geq 10^{-3}$, medium volatile between 10^{-3} and 10^{-5}, low or non volatile: $< 10^{-5}$. EPPO (2003), Environmental risk assessment scheme for plant protection products, Chapter 3: Air, Bulletin OEPP/EPPO Bulletin, 33, 115-129. (accessed 18.01.2018)

Exposure calculations

Exposure calculations were carried out using the excel tool EFSA PRIMO rev. 3[43]. The exposure is expressed in mg/kg bw/day. TMDI (Theoretical Mean Daily Intake) and IESTI (International Estimated Short Term Intake) were modelled.

Exposure from natural occurrence
For the calculation of intake levels (TMDI and NESTI) from natural exposure, mean values of the available data were used in order to avoid an overestimation and obtain conservative results.

Exposure from treated crops
Sufficient residue information is available for all intended uses. The results of the storage stability study (Meseguer, 2017[44]) show that the residues of Terpinen-4-ol and 1,8-Cineole were stable in grape and tomato for at least 13 months at -20°C. The residues of γ-Terpinene have been found to substantially decline in both plant matrices when stored at -20 °C (target temperature) its content was 48 % after 6 months and 45% after 13 months. The times between sampling and extraction were > 30 days in all provided residue trials. The residues of γ-Terpinene were therefore corrected by a factor of 2.2 to account for the loss of residues. This approach is considered to provide sufficiently conservative results. Consequently, the input values for PRIMO calculations are as follows:

Table 2.7.9-5 Corrected input values [mg/kg] deduced from residue trials conducted with Tea tree oil (all residues < LOQ max. 12 h after application)

	Terpinen-4-ol	γ-Terpinene	1,8-Cineole
Tomato Greenhouse (4 x 445 g TTO/ha, 11 h after appl.)	< 0.01#	< 0.02#	< 0.01#
Tomato Field (3 x 445 g TTO/ha, 12 h after appl.)	0.05*	0.11*	0.05*
Grapes Field (4 x 445 g TTO/ha, 12 h after appl.)	0.01*	0.02*	0.01*

not relevant for risk assessment, highest residue values originate from field application
* as < is not considered by the PRIMO tool for TMDI and IESTI calculations, it is not displayed here

RMS: Content of γ-Terpinene declined to 48 % after 7 months and 45 % after 13 months in tomato and 20 % after 6 months and 13 % after 13 months in grape. Therefore, for dietary risk assessment, the residues of γ-Terpinene should be multiplied with a factor of 2.2 for tomato and 7.7 for grape to account for the loss of residues. Therefore, the input value of γ-Terpinene in grape of 0.08 mg/kg (instead of 0.02 mg/kg) should be used for calculations of consumer exposure.
According to RMS calculations consumer exposure from residues after application of TTO conducted for γ-Terpinene using the excel tool EFSA PRIMO rev. 3 (see table 2.7.9-6), the highest TMDI is 0.00048 mg/kg bw/d (instead of 0.00042 calculated by the applicant) and the highest contributor is still tomato. Thus, conclusion that for γ-Terpinene the natural exposure significantly exceeds the expected exposure from application of Tea tree oil is still valid.

[43] Available online: https://www.efsa.europa.eu/de/efsajournal/pub/5147 (accessed: 31.01.2018)
[44] Meseguer, C. (2017) Tea tree oil – Storage stability study of Tea tree oil in frozen crops. Report no. S15-03445, Eurofins Agroscience Services Chem SAS, France.

Table 2.7.9-6 Additional estimation of the dietary intake for input value in grape of 0.08 mg/kg (instead of 0.02 mg/kg) for γ-Terpinene using EFSA PRIMo rev.3

European Food Safety Authority

EFSA PRIMo revision 3.0; 2017/12/11

gamma-terpinene (F)

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	**0,25**	ARfD (mg/kg bw):	**na**
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%	GEMS/Food G06	0,48	0,2%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,2%
	0,1%	RO general	0,36	0,1%	Tomatoes	0,1%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	PT general	0,32	0,1%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,1%
	0,1%	GEMS/Food G07	0,26	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	FR adult	0,25	0,1%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,1%
	0,1%	GEMS/Food G15	0,24	0,1%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	GEMS/Food G08	0,24	0,1%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	NL toddler	0,23	0,0%	Table grapes	0,0%	Tomatoes		Grapefruits		0,1%
	0,1%	DE child	0,22	0,0%	Table grapes	0,0%	Tomatoes	0,0%	Wine grapes		0,1%
	0,1%	GEMS/Food G11	0,21	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	GEMS/Food G10	0,21	0,1%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	DE women 14-50 yr	0,17	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	IT toddler	0,17	0,1%	Tomatoes	0,0%	Table grapes		Grapefruits		0,1%
	0,1%	IE adult	0,16	0,0%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,1%
	0,1%	DE general	0,16	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	FR child 3 15 yr	0,15	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	DK adult	0,15	0,0%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,1%
	0,1%	NL child	0,14	0,0%	Table grapes	0,0%	Tomatoes	0,0%	Wine grapes		0,1%
	0,1%	UK vegetarian	0,14	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,1%
	0,1%	UK adult	0,14	0,0%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,1%
	0,1%	IT adult	0,14	0,1%	Tomatoes	0,0%	Table grapes		Grapefruits		0,1%
	0,0%	ES adult	0,12	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,0%
	0,0%	PL general	0,12	0,0%	Tomatoes	0,0%	Table grapes		Grapefruits		0,0%
	0,0%	NL general	0,11	0,0%	Wine grapes	0,0%	Tomatoes	0,0%	Table grapes		0,0%
	0,0%	ES child	0,11	0,0%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,0%
	0,0%	FI adult	0,09	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,0%
	0,0%	UK toddler	0,09	0,0%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,0%
	0,0%	SE general	0,08	0,0%	Tomatoes		Grapefruits		Grapefruits		0,0%
	0,0%	FI 3 yr	0,08	0,0%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,0%
	0,0%	DK child	0,07	0,0%	Tomatoes	0,0%	Table grapes		Grapefruits		0,0%
	0,0%	FR toddler 2 3 yr	0,07	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,0%
	0,0%	LT adult	0,07	0,0%	Tomatoes	0,0%	Table grapes		Grapefruits		0,0%
	0,0%	FI 6 yr	0,06	0,0%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,0%
	0,0%	UK infant	0,04	0,0%	Tomatoes	0,0%	Table grapes	0,0%	Wine grapes		0,0%
	0,0%	FR infant	0,01	0,0%	Tomatoes	0,0%	Wine grapes	0,0%	Table grapes		0,0%
	0,0%	IE child	0,01	0,0%	Tomatoes	0,0%	Table grapes		Grapefruits		0,0%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of gamma-terpinene (F) is unlikely to present a public health concern.

Extrapolation of residue values

For the calculation of exposure via residues (originating from residue trials after application of Tea tree oil to several crops), HR (highest residue) values from fruiting vegetables residue trials were applied. However, the available residue trials do not cover every component or even every component group. Therefore, theoretical residues for each component not addressed in the residue trials were modelled based on their content in Tea tree oil as outlined in the following.

For TMDI-calculations the mean content from the 5-Batch-results (see Table 1.2.3-1 of Vol. 4 C), the maximum application rate and data from Baril et al (2005)[45] as outlined in EFSA Journal 2009; 7(12):1438[46] (see Table 2.7.9-7) was used. For TMDI calculations the mean residues were applied (RUD = x mg a.i./kg a.i. applied) and a multiple application factor (MAF = 2.0 for 3 applications with 7 days interval) from the same guidance document was applied, as well, with regard to conservativity.

For IESTI calculations the mean content + 2 x SD from 5-batch-results, i.e. 95th%ile as used in conjunction with the 90th%ile RUD and a multiple application factor (MAF = 2.0 for 3 applications with 7 days interval – worst case for tomato, MAF = 2.2 for 4 applications with 7 days interval – worst case for grape) from the same guidance document was applied, as well, with regard to conservativity.

The corresponding residue values used per crop and component (calculated as RUD x MAF x % contained in TTO mixture) for the highest intended application rate (i.e. 3 x 0.445 kg a.i./ha for Tomato and 4 x 0.445 kg/ha for grape).

Table 2.7.9-7 RUD values used for extrapolation of residues in crops after application of Tea tree oil

Crop	**Crop stage**	**RUD mean**	**Standard deviation**	**90th%ile RUD**	**n**	**Source**
Tomato	Fruiting period	12.8	14.6	30.6	86	Baril et al. (2005)
Berries*	Fruiting period	8.3	7.2	16.7	9	Baril et al. (2005)

* Used as surrogate for grapes

The extrapolated residue values for components of TTO not measured in the residue trials used as input for TMDI and IESTI calculations are presented in Table 12 of the original document (KCA 6.9/01).

Results

Consumer risk assessment

The calculated exposure from natural occurrence is consistently higher than the exposure (measured and conservatively extrapolated) from application to crops. In order to obtain comparative results, the exposure in mg/kg bw(/day) was summed up and compared per component group. An overview is presented in the following tables, for more detailed results (PRIMo 3 modelling), please refer to the original document (KCA 6.9/01).

Table 2.7.9-8 TMDI - Comparison of natural exposure and exposure from direct application of TTO in crops [mg/kg bw/day]

	TMDI Exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application calculated	Difference Nat. exp. and appl. exp.
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	0.00079	0.00042	-	
	α-Terpinene	0.015	-	0.007	
	α-Terpinolene	0.00476	-	0.002	
	Limonene	0.793	-	0.001	
	p-Cymene	0.00008	-	0.001	
	Sum	**0.814**	**0.0114**		**Factor: 71.3**
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.00468	0.00019	-	
	α-Terpineol	8.873	-	0.004	
	Sum	**8.878**	**0.0042**		**Factor: 2119**
	1,8-Cineole	-	0.00019	-	

[45] Baril A., Whiteside M. and C. Boutin, 2005. Analysis of a database of pesticide residues on plants for wildlife risk assessment. Environmental Toxicology and Chemistry, Vol. 24, No. 2, pp. 360–371.

[46] EFSA (2009) Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA; Appendix A. EFSA Journal 2009; 7(12):1438. [2 pp.]. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu

	TMDI Exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application calculated	Difference Nat. exp. and appl. exp.
Bicyclic monoterpenes	α-Pinene	0.279	-	0.003	
	Sabinene	0.589	-	0.0001	
	Sum	**0.868**	**0.0033**		**Factor: 264**
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	**0.372**	-	**0.0001**	**Factor: 3720**
Polycyclic sesquiterpenes, Aromadendrene group	Aromadendrene	0.242	-	0.0001	
	Ledene	-	-	0.0001	
	Sum	**0.242**	**0.0002**		**Factor: 1210**
Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	1.198	-	0.00001	
	Viridiflurol	0.183	-	0.00001	
	Sum	**1.381**	**0.00002**		**Factor: 69050**

For every component group the natural exposure exceeds the expected exposure from application of Tea tree oil by a magnitude of at least 70. Please note that the expected residues for the components not tested in the residue trials will actually be even lower due to the high vapour pressure of most of the components. Only Globulol and Viridiflurol are moderately volatile and for these the natural occurrence is ca. 70000 x higher than the conservatively modelled residues in crops.

Table 2.7.9-9 IESTI - Comparison of natural exposure and exposure from direct application of TTO in crops [mg/kg bw/day]

	IESTI – Dietary exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application calculated (highest IESTI value, tomato)	Difference Nat. exp. and appl. exp.
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	1.696	0.006	-	
	α-Terpinene	0.036	-	0.189	
	α-Terpinolene	0.220	-	0.051	
	Limonene	89.333	-	0.024	
	p-Cymene	0.0036	-	0.027	
	Sum	**91.289**	**0.297**		**Factor: 307**
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.0017	0.003	-	
	α-Terpineol	8.480	-	0.119	
	Sum	**8.482**	**0.122**		**Factor: 69.5**
Bicyclic monoterpenes	1,8-Cineole	-	0.003	-	
	α-Pinene	0.266	-	0.081	
	Sabinene	0.557	-	0.002	
	Sum	**0.823**	**0.086**		**Factor: 9.6**
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	**52.00**	**-**	**0.002**	**Factor: 26000**
Polycyclic sesquiterpenes, Aromadendrene group	Aromadendrene	0.231	-	0.006	
	Ledene	-	-	0.002	
	Sum	**0.231**	**0.008**		**Factor: 28.9**
Alcohols	Globulol	1.145	-	0.001	

	IESTI – Dietary exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application calculated (highest IESTI value, tomato)	Difference Nat. exp. and appl. exp.
	Viridiflurol	0.175	-	0.001	
	Sum	**1.320**	**0.002**		**Factor: 660**

For every component group the natural exposure exceeds the expected exposure from application of Tea tree oil by a magnitude of at least factor 9.6. Please note that the expected residues for the components not tested in the residue trials (Table 2.7.9-9) will actually be even lower due to the high vapour pressure of most of the components. Only Globulol and Viridiflurol are moderately volatile and for these the natural occurrence is ca. 200x higher than the conservatively modelled residues in crops.

Refined consumer risk assessment

As mentioned above, most of the components contained in Tea tree oil have a very high vapor pressure and are highly volatile. They are thus expected to rapidly dissipate from the plant surface, which is supported by residues >LOQ of the measured compounds (Terpinen-4-ol: 5.7 Pa, γ-Terpinene: 145 Pa and 1,8-Cineole: 208 Pa). According to EPPO (2003)42, all components except of Globulol and Viridiflurol are classified as volatile, whereas the latter two are medium volatile. For refined calculations, it is therefore assumed that the residues of all volatile TTO compounds can be based on and deduced from the residue value of the measured components. As a < -value cannot be entered in the modelling spreadsheet, a residue of 0.05 (i.e. LOQ) is used for exposure calculation from measured components. Based on the content of 44.25% of Terpinen-4-ol in TTO, the residue for non-measured compounds is calculated, taking into account the % occurrence in the mixture. Based on the residue result of Terpinen-4-ol being < LOQ (0.05 mg/kg for tomato) and on the mean content of 44.25 % Terpinen-4-ol in the mixture, residues extrapolated to 100% account for 0.112 mg/kg. The residue value of tomato (0.05 mg/kg, applied as conservative assumption for both commodities – tomato and grapes) was prorated with the % occurrence of the non-measured compounds in the mixture. Based on the outcome of the plant metabolism study this approach was considered reasonable for both, tomato and grapes. The corrected residues of γ-Terpinene (see point "Exposure from treated crops") were not considered for the extrapolation of the other components as it has a far higher vapour pressure than Terpinen-4-ol and thus is supposed to evaporate markedly faster than the latter. For Globulol and Viridiflurol this refinement is not applied due to the lower volatility. The respective input values for these two components are taken over from point "Extrapolation of residue values" in order to maintain a conservative approach.

Table 2.7.9-10 Refined TMDI - Comparison of natural exposure and exposure from direct application of TTO in crops [mg/kg bw/day]

	Refined TMDI Exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application extrapolated	Difference Nat. exp. and appl. exp.
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	0.00079	0.00042	-	
	α-Terpinene	0.015	-	0.0001	
	α-Terpinolene	0.00476	-	0.00001	
	Limonene	0.793	-	0.00001	
	p-Cymene	0.00008	-	0.00001	
	Sum	**0.814**	**0.00055**		**Factor: 1480**
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.00468	0.00023	-	
	α-Terpineol	8.873	-	0.00004	
	Sum	**8.878**	**0.00027**		**Factor: 32882**
Bicyclic monoterpenes	1,8-Cineole	-	0.00023	-	
	α-Pinene	0.279	-	0.00003	
	Sabinene	0.589	-	0.0000005	
	Sum	**0.868**	**0.00023**		**Factor: 3774**
Polycyclic sesquiterpenes,	δ-Cadinene	**0.372**	-	0.0000005	**Factor: 744000**

	Refined TMDI Exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application extrapolated	Difference Nat. exp. and appl. exp.
Cadinane group					
Polycyclic sesquiterpenes, Aromadendrene group	Aromadendrene	0.242	-	0.000001	
	Ledene	-	-	0.0000005	
	Sum	**0.242**	**0.000002**		**Factor: 121000**
Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	1.198	-	0.00001	
	Viridiflurol	0.183	-	0.00001	
	Sum	**1.381**	**0.00002**		**Factor: 69050**
Overall sum		**12.554**	**0.0011**		**Factor: 11705**

For every component group the natural exposure exceeds the expected exposure from application of Tea tree oil by a magnitude of at least 1480 when taking into account the volatility of the TTO components, where appropriate. When summing up the values from all components, the natural exposure exceeds the expected exposure from application of Tea tree oil by a factor of >11000. It is therefore considered that the chronic exposure from application of Tea tree oil is negligible when compared to the natural exposure to the single components.

Table 2.7.9-11 Refined IESTI - Comparison of natural exposure and exposure from direct application of TTO in crops [mg/kg bw/day]

	Refined IESTI – Dietary exposure level [mg/kg bw/day]				
		Natural exposure	Exposure from application measured	Exposure from application calculated (highest IESTI value, grapes)	Difference Nat. exp. and appl. exp.
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	1.696	0.006	-	
	α-Terpinene	0.036	-	0.001	
	α-Terpinolene	0.220	-	0.0003	
	Limonene	89.333	-	0.0001	
	p-Cymene	0.0036	-	0.0001	
	Sum	**91.289**	**0.0075**		**Factor: 12172**
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.0017	0.003	-	
	α-Terpineol	8.480	-	0.0006	
	Sum	**8.482**	**0.0036**		**Factor: 2356**
Bicyclic monoterpenes	1,8-Cineole	-	0.003	-	
	α-Pinene	0.266	-	0.0004	
	Sabinene	0.557	-	0.00001	
	Sum	**0.823**	**0.0034**		**Factor: 242**
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	**52.00**	**-**	**0.00001**	**Factor: 5200000**
Polycyclic sesquiterpe0.027+0.004+nes, Aromadendrene group	Aromadendrene	0.231	-	0.00003	
	Ledene	-	-	0.00001	
	Sum	**0.231**	**0.00004**		**Factor: 5775**
Alcohols	Globulol	1.145	-	0.001	
	Viridiflurol	0.175	-	0.001	
	Sum	**1.320**	**0.002**		**Factor: 660**
Overall sum		**154.44**	**0.0166**		**Factor: 9304**

For every component group the natural exposure exceeds the expected exposure from application of Tea tree oil by a magnitude of at least 242 when taking into account the volatility of the TTO components, where appropriate. When summing up the values from all components, the natural exposure exceeds the expected exposure from application of Tea tree oil by a factor of > 9300. It is therefore considered that the acute exposure from application of Tea tree oil is negligible when compared to the natural exposure to the single components.

Discussion and conclusion

Many of the components contained in Tea tree oil also occur in edible crops. Consequently, consumers are naturally exposed to these components. The magnitude of this exposure was calculated for each component where data is available. No information is available on natural occurrence of 1,8-Cineole and Ledene in crops consumed in the EU. Thus, natural exposure from food consumption is deemed to be negligible.

Magnitude of residues based on residue trials and vapour pressure

Due to structural similarities, the TTO-components can be sorted into chemical groups. As the chemical structure of the components of each group is very similar, their physical-chemical properties are also likely to be alike. This shows to be true with regard to vapour pressure (see point B.7.7. in the DAR). As the constituents of Tea tree oil are mostly classified as highly volatile, their dissipation from the crop after application is supposed to be very fast. This is confirmed by the results of residue trials conducted on representative crops with the marker compounds γ-Terpinene (VP = 145 Pa), Terpinen-4-ol (VP = 5.7 Pa) and 1,8-Cineole (VP = 208 Pa). They rapidly dissipate from the crop which leads to residues < LOQ within max. 12 hours.

This leads to the conclusion that even a DT_{50} = 6 h on plants for these three compounds is a vast overestimate and thus sufficiently conservative. Consequently, the half-life on plants of the other compounds with VP ≥ 5 Pa can also be assumed to be $DT_{50} \leq 6$ h. The polycyclic sesquiterpenes δ-Cadinene and Ledene have vapour pressures around 2.5 Pa. Being half as high as the vapour pressure of Terpinen-4-ol, for which residues were determined, DT_{50} = 12 h can confidently still be considered to be conservative, as DT_{50} = 6 h for substances with VP ≥ 5 Pa is already a vast overestimate. Even a DT_{50} of 12 h on plants will lead to residues < LOQ at the time of crop consumption, even if this takes place 12 or 24 h after application of the product.

The substances Globulol and Viridiflurol cannot be covered by this argumentation. With a vapour pressure of 4.95 x 10^{-3} they are considered as moderately volatile. However, their mean content in Tea tree oil is < 0.1%, i.e. 0.03%. Therefore, residues on plants will be negligible.

Magnitude of residues based on extrapolation

With regard to a conservative approach for assessment of dietary exposure, a second approach was applied, as well. The magnitude of theoretical residues of the not-measured components of Tea tree oil was calculated taking into account the highest intended application rate, a multiple application factor (which is, based on the high vapour pressure and thus extremely low DT_{50} on plants strictly speaking not necessary), the % occurrence of the components in the TTO mixture and a RUD value set by EFSA for the exemplary crops. For the not-measured components, these theoretical residues were used to perform TMDI and IESTI calculations using EFSA PRIMO. The measured residues (at max. 12 h after application) were also used for TMDI and IESTI calculations.

Comparison of exposure from natural occurrence and from application onto crops

The dietary exposure from natural occurrence and exposure from application of Tea tree oil (measured and conservatively extrapolated), respectively, were summed up for each component group and compared with each other. The results demonstrate that the exposure from application of TTO is substantially lower than the natural exposure to the constituents of Tea tree oil.

Under conservative assumptions, i.e. irrespective of the high vapour pressure and the thereby caused rapid dissipation from the crop, for TMDI the magnitudes reach at least factor 70. For Globulol and Viridiflurol, belonging to the Polycyclic Sesquiterpenes (Aromadendrene group - Alcohols) which have the lowest vapour pressure of all relevant components in the mixture, the intake from natural occurrence is at least 69000 x higher than the theoretical exposure from application of TTO.

Likewise, when applying a very conservative approach, for IESTI, the exposure from natural occurrence is at least 9 x higher than from application of TTO according to the intended GAP. For Globulol and Viridiflurol, natural exposure is at least 660 x higher than exposure from application to crops.

When the dietary exposure calculations are refined with regard to modelling a realistic exposure taking into account the high vapour pressure of most components on the edible part of fruiting crops and the PHI of 3 days, the natural exposure exceeds the exposure from application by several magnitudes: chronic (TMDI) calculations demonstrate that exposure from natural occurrence is at least 1400 x higher and in sum of all components even > 11000 x higher. Acute (IESTI) calculations demonstrate that exposure from natural occurrence is at least 240 x higher and in sum > 9300 x higher than exposure from application of TTO to the intended crops.

This gives a very wide margin of safety and supports the assumption, that an exposure to these compounds from the application of TTO to crops is negligible.

With natural exposure being far higher than can be from application, it is concluded that a consumer risk assessment - as opposed to exposure calculations – does not make much sense. Since no residues are expected above the natural background level of Tea tree oil components, no additional risk is expected due to the use of Tea tree oil as a plant protection product on edible crops. Therefore, the obtained exposure values (mg/kg bw/(day)) are not compared to any ADI or ARfD.

RMS: The applicant has carried out detailed consumer risk assessment by comparison of exposure from natural occurrence and from application onto crops components of Tea tree oil. The magnitude of exposure was estimated for each component where data was available. The results demonstrate that consumer exposure to each component of TTO from application of product BM608 is substantially lower than the natural exposure from food to the constituents of Tea tree oil. However, content and proportion of various natural terpenes from food is not exactly the same as in residues of TTO after crop treatment. Nevertheless, sum of estimated exposure from direct application of TTO in crops for all components of TTO (according to Table 7.7-7) is 0.01912 mg/kg bw/day (0.0114+0.0042+0.0033+0.0002+0.00002) and represents 7.7% of ADI value = 0.25 mg/kg bw/d proposed by RMS (see Volume 3 Part B.6). The result indicates that there is no unacceptable chronic risk to human health from the consumption of commodities treated with TTO according to the uses considered.
IESTI calculation is not required because an ARfD was not deemed necessary (see Volume 3 Part B.6).

Tomatoes and grapes do not form part of animal diets and consequently, intake calculations for animals (dietary burden calculations) are not required in the context of the supported uses.

2.7.10 Proposed MRLs and compliance with existing MRLs

For Tea Tree Oil, no residue definition is needed due to the fact that the intended uses give rise to a no residue situation. As no residue definition is required, the setting of MRLs is also not necessary. This is confirmed in the DAR for Tea Tree Oil (September 2008) and the Review Report from 1 August 2008 (SANCO/2609/08 rev.1). According to EFSA Journal 2013; 11(3):3141, residues of Tea Tree Oil are not expected to occur in any plant or animal commodity.
Furthermore, Commission Regulation 839/2008 of 31 July 2008 included the extract of Tea Tree into Annex IV of Regulation 396/2005. Substances listed there in do not require setting of an MRL and consequently, a residue definition is also not necessary.

Since dietary intake from natural occurrence clearly exceeds the dietary exposure due to uptake from treated crops and as no additional residues are expected due to the use application of Tea tree oil to edible crops, Tea Tree oil is proposed to be kept in Annex IV of Regulation 396/2005.

RMS: Since the residues of individual components of TTO arising from consumption of fruits and vegetables containing naturally some of these components may be quite high it is not possible to set MRLs based on the existing data.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

No import tolerances considered.

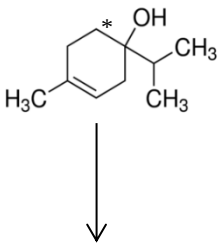
2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

Soil degradation

The route and rate of degradation of the main TTO constituents selected on the basis of their physico-chemical properties and concentration in TTO (Terpinene-4-ol, γ-Terpinene, p-Cymene, 1,8-Cineole, Aromadendrene and Globulol) were investigated in two regulatory studies performed in line with OECD 307 (Bloß, 2018a and 2018b, Vol. 3CA, B.8.1.1.1/01 and B.8.1.1.1/02).

No metabolites >5% AR formed from Terpinene-4-ol in LUFA 2.4 soil over the whole study period. Based on that the following metabolic pathway was proposed:



[^{14}C]Terpinene-4-ol

↓

CO_2, non-extractable residues and minor metabolites

It is, however, noted that Terpinene-4-ol was the only labelled compound used in the study and potential formation of metabolites was not determined for other main constituents of TTO included in the study. This was most probably due to the fact that Terpinene-4-ol is the leading constituent of TTO, representing 44.3% of the whole mixture. Nevertheless, other compounds are also present in quantifiable amounts (e.g. γ-Terpinene and 1,8-Cineole are present at 18.6 and 4.7%, respectively). Although in some of the literature studies degradation of terpenes resulted with rearrangement of the molecule and formation of other terpenes, some authors detected metabolites formed when microorganisms utilised terpenes as the carbon and energy source (Harder & Probian, 1995; Madhava & Renganathan, 1984; Yoo et al., 2001; Yoo & Day, 2002). Therefore it may be questioned if the route of degradation of the leading constituents has been sufficiently investigated.

In addition to that the rate of degradation of Terpinene-4-ol was investigated in only one soil (LUFA 2.4), while DT_{50} values of other tested compounds in this soil were clearly shorter comparing to other soils (LUFA 2.1, LUFA 2.2 and LUFA 6S). Therefore it may be expected that DT_{50} of Terpinene-4-ol in these other soils would be also longer.

The obtained soil degradation data were subjected kinetic analysis in line with FOCUS (2014) recommendations. The degradation pattern of all tested compounds was sufficiently described with SFO kinetics, although some improvement of visual fits was observed when either FOMC of DFOP was employed.

The actual DT_{50} values were normalised by the RMS for moisture content (correction for temperature was not necessary).
Summary of actual and normalised values is provided in table below.

Table 2.8.1-1: Summary of soil degradation data for selected TTO constituents

Compound	Soil / soil type	pH [1)]	t [ºC] / % MWHC	actual DT_{50} / DT_{90} [d]	DT_{50} at 20ºC and pF2 [d]	Geomean DT_{50} normalised [d]	Chi^2 [%]	Kinetic model
Terpinene-4-ol	LUFA 2.4 loam	7.2	20ºC / 50%	0.42 / 1.39	0.41	0.41	15.9	SFO
p-Cymene	LUFA 2.4 loam	7.2	20ºC / 50%	0.04 / 0.12	0.04	0.16	4.12	SFO
	LUFA 2.1 loamy sand	5.3	20ºC / 50%	0.14 / 0.52	0.14		15.6	SFO
	LUFA 2.2 sandy loam	5.7	20ºC / 50%	0.53 / 1.78	0.53		13.3	SFO
	LUFA 6S clay loam	6.9	20ºC / 50%	0.25 / 0.83	0.23		9.7	SFO
1,8-Cineole	LUFA 2.4 loam	7.2	20ºC / 50%	0.10 / 0 14	0.10	0.30	4.97	SFO
	LUFA 2.1 loamy sand	5.3	20ºC / 50%	0.39 / 1.30	0.39		8.08	SFO
	LUFA 2.2 sandy loam	5.7	20ºC / 50%	0.68 / 2.25	0.68		14.7	SFO
	LUFA 6S clay loam	6.9	20ºC / 50%	0.31 / 1.02	0.29		8.76	SFO
γ-Terpinene	LUFA 2.4 loam	7.2	20ºC / 50%	0.04 / 0 33	0.04	0.09	9.94	SFO
	LUFA 2.1 loamy sand	5.3	20ºC / 50%	0.09 / 0.31	0.09		11.3	SFO
	LUFA 2.2 sandy loam	5.7	20ºC / 50%	0.11 / 0.36	0.11		13.0	SFO
	LUFA 6S clay loam	6.9	20ºC / 50%	0.15 / 0.49	0.14		8.94	SFO
Aromadendrene	LUFA 2.4 loam	7.2	20ºC / 50%	2.94 / 9.75	2.91	4.96	19.7	SFO
	LUFA 2.1 loamy sand	5.3	20ºC / 50%	3.60 / 11.96	3.60		8.84	SFO
	LUFA 2.2 sandy loam	5.7	20ºC / 50%	7.75 / 25.71	7.75		11.5	SFO
	LUFA 6S clay loam	6.9	20ºC / 50%	3.73 / 12.38	3.47		13.8	SFO
Globulol	LUFA 2.4 loam	7.2	20ºC / 50%	6.29 / 21.0	6.23	6.37	8.91	SFO
	LUFA 2.1 loamy sand	5.3	20ºC / 50%	10.9 / 36.29	10.9		11.1	SFO
	LUFA 2.2 sandy loam	5.7	20ºC / 50%	7.75 / 25.79	7.75		11.7	SFO
	LUFA 6S clay loam	6.9	20ºC / 50%	6.96 / 23.17	6.68		8.01	SFO

[1)] In $CaCl_2$

In order to determine the potential dependence between degradation and soil pH, correlation coefficients have been calculated for compounds for which data on 4 soils are available. Obtained results are provided in table below.

Table 2.8.1-2: Correlation between soil pH and degradation

Compound	Soil type	pH	actual DT50 [d]	Correlation coefficient
γ-Terpinene	LUFA 2.1 (loamy sand)	5.3	2.22	-0.202
	LUFA 2.2 (sandy loam)	5.7	2.57	
	LUFA 6S (clay loam)	6.9	3.56	
	LUFA 2.4 (loam)	7.2	0.861	
p-Cymene	LUFA 2.1 (loamy sand)	5.3	3.37	-0.427
	LUFA 2.2 (sandy loam)	5.7	12.8	
	LUFA 6S (clay loam)	6.9	5.96	
	LUFA 2.4 (loam)	7.2	0.995	
1,8-Cineole	LUFA 2.1 (loamy sand)	5.3	9.43	-0.740
	LUFA 2.2 (sandy loam)	5.7	16.3	
	LUFA 6S (clay loam)	6.9	7.36	
	LUFA 2.4 (loam)	7.2	2.39	
Aromadendrene	LUFA 2.1 (loamy sand)	5.3	86.5	-0.484
	LUFA 2.2 (sandy loam)	5.7	186	
	LUFA 6S (clay loam)	6.9	89.5	
	LUFA 2.4 (loam)	7.2	70.5	
Globulol	LUFA 2.1 (loamy sand)	5.3	262	-0.875
	LUFA 2.2 (sandy loam)	5.7	186	
	LUFA 6S (clay loam)	6.9	167	
	LUFA 2.4 (loam)	7.2	151	

Based on calculated correlation coefficients, clear negative correlation between soil pH and degradation could be observed for 1,8-Cineole and Globulol. For remaining compounds the correlation was weak.
Dependency between degradation and soil pH could not be checked for main TTO constituent, Terpinene-4-ol, since its degradation was investigated in single soil only.

Overall, all investigated TTO constituents degraded rapidly in all soils tested. Information on degradation of particular TTO compounds obtained from the literature data are in good agreement with findings of both regulatory studies. Summary of literature data regarding degradation in soil is provided in table below.

Table 2.8.1-3: Literature data on biodegradation of terpene components

Substance	Study/endpoint type	Results	Reference
Ready biodegradability			
Limonene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 182 Max. degr. rate (mg/L/h): 0.044 Norm. degr. rate (h^{-1}): not measured Readily biodegradable	Vol. 3CA, B.8.1.1.5/01 Misra et al. (1996)
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: > 75 mg/L, complete degradation within 70 h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.9 mg/L/h - as individual substance: 1.9 mg/L/h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)
α-Pinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 200 Max. degr. rate (mg/L/h): 0.029 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	Vol. 3CA, B.8.1.1.5/01 Misra et al. (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 1.1 mg/L/h - as individual substance: 2.1 mg/L/h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)

Substance	Study/endpoint type	Results	Reference
	Liquid degradation by two bacterial species (*Pseudomonas fluorescens* and *Alcaligenes xylosoxidans*) tested singly and as consortium	Complete degradation (<0.l mg/L remaining) in 36 h by the consortium. After a 10-h lag period, a maximum rate of degradation of 3.6 mg/L/h was observed. The *A. xylosoxidans* isolate was shown to degrade α-Pinene to below 5 mg/L in the test system in 36 hours, achieving a maximum degradation rate of 3.6 mg/L/h. The *R. fluorescens* isolate showed little degradation of α-pinene until 36 h into the experiment and had a maximum degradation rate of 1.2 mg/L/h.	Vol. 3CA, B.8.1.1.5/03 Kleinheinz et al. (1999)
γ-Terpinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 168 Max. degr. rate (mg/L/h): 0.039 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	Vol. 3CA, B.8.1.1.5/01 Misra et al. (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.8 mg/L/h - as individual substance: 1.8 mg/L/h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)
α-Terpinolene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 174 Max. degr. rate (mg/L/h): 0.053 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	Vol. 3CA, B.8.1.1.5/01 Misra et al. (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.6 mg/L/h - as individual substance: 1.5 mg/L/h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)
α-Terpineol	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 94 Max. degr. rate (mg/L/h): > 0,10 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	Vol. 3CA, B.8.1.1.5/01 Misra et al. (1996)
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: 210 mg/L, complete degradation within 48 h	Vol. 3CA, B.8.1.1.5/02 Misra & Pavlostathis (1997)
Biotransformation			
Limonene	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	75 % of Limonene consumed, metabolite formed (traces): α-Terpinene	Vol. 3CA, B.8.1.1.5/05 Harder & Probian (1995)
α-Terpineol	Metabolism by *Pseudomonas incognita*	P. incognita degrades α-Terpineol by at least three routes: - via oleuropeic acid - aromatization of α-Terpineol - formation of limonene	Vol. 3CA, B.8.1.1.5/09 Madhava & Renganathan (1984)
	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	Decrease of 52 % after 7.5 d, remained constant until study termination (30 d)	Vol. 3CA, B.8.1.1.5/04 Pavlostathis & Misra (1999)
α-Pinene	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	No significant degradation	Vol. 3CA, B.8.1.1.5/04 Pavlostathis & Misra (1999)
	Bioconversion by *Pseudomonas* sp. strain PIN	Total bioconversion in 40 h: 33.5 % Formed products: Limonene, *p*-Cymene, *p*-Cymene-8-ol, α-Terpinolene, terpineol, camphor, terpinene-4-ol and borneol (all < 10 %)	Vol. 3CA, B.8.1.1.5/07 Yoo et al. (2001)

Substance	Study/endpoint type	Results	Reference
	Bacterial metabolism by *Pseudomonas* sp. strain PIN	Substrate: α-Pinene (1 % v/v) Biomass: 103.13 g/L Maximum yield: 1056 mg/L Specific yield (cells): 10.24 Productivity: 22.00 mg/l h Formed metabolites: *p*-Cymene, Limonene, Terpineol, Terpinolene, Borneol	Vol. 3CA, B.8.1.1.5/08 Yoo & Day (2002)
	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	67 % of Limonene consumed, metabolites formed (traces): α-Terpinene, Cymene, Limonene, Eucalyptol	Vol. 3CA, B.8.1.1.5/05 Harder & Probian (1995)
α-Pinene, p-Cymene 1,8-Cineole (=Eucalyptol), Terpinen-4-ol, α-Terpineol, Terpinolene, α- and γ-Terpinene	Mediterranean conditions; flowers of lavender oil.	Main substances were analysed monthly and after 12 months; p-Cymene and 1,8-Cineole were abundant in litter, and after 16 months 1,8-cineole. According to these investigations, the half-life rate of terpenes in litter is between 210 and 240 days under Mediterranean conditions. It should be noted that these are to be seen as worst case conditions for the Northern and Central Europe.	Vol. 3CA, B.8.3.4/20 Hassiotis (2010); submitted under KCA 5.5/20

No study on anaerobic degradation of TTO in soil has been submitted. The Applicant justified that TTO constituents are rapidly degraded under anaerobic conditions. However, results of the literature study by Pavlostathis & Misra (1999, Vol. 3CA, B.8.1.1.5/04) demonstrated that α-Pinene was stable under anaerobic conditions, while degradation of α-Terpineol was rapid within first 8 days of incubation (52% decrease in concentration), but remained stable over the remaining days of the incubation period (from day 8 to 30). This may indicate that degradation of TTO under anaerobic conditions will be slower comparing to aerobic conditions and for some compounds may not occur at all.
Furthermore, in the literature study by Harder & Probian (1995, Vol. 3CA, B.8.1.1.5/05) the anaerobic degradation of some TTO constituents (Limonene, 1,8-Cineole and α-Pinene) resulted with formation of other monoterpenes due to rearrangement of the molecules, which could lead to increase in concentration of monoterpenes following application of TTO as a pesticide. Although the study was not performed in soil, the species used for anaerobic degradation of tested compound was *Pseudomonas citronellolis*, which was isolated from the forest soils.
Therefore it may be questioned if the waiver of anaerobic degradation studies provided by the Applicant is sufficient.

No study on photolysis of TTO in soil has been submitted. Given the high vapour pressure of majority of TTO constituents it is not expected that the degradation pattern due to photolysis would be significantly different than this observed in aerobic degradation studies and most of losses of the substances from soil are expected to be due to volatilisation.

Soil mobility

Soil sorption of TTO constituents selected on the basis of their physico-chemical properties and concentration in TTO (Terpinene-4-ol, γ-Terpinene, p-Cymene, 1,8-Cineole, Aromadendrene and Globulol) was investigated in one regulatory study (Göcer, 2018, Vol. 3CA, B.8.1.2.1/01). Due to high volatility of tested compounds the Koc values were determined using HPLC method, in line with OECD 121. The obtained Koc values are presented in table below.

Table 2.8.1-4: Summary of K_{OC} values derived for selected TTO constituents using HPLC method

Test item	Mean log Koc of two runs	Koc [mL/g]
Terpinene-4-ol	1.95	89.1
γ-Terpinene	3.36	2291
p-Cymene	3.13	1349
1,8-Cineole	1.77	58.9
(+)-Aromadendrene	3.24	1738
(-)-Globulol	>5.0 (5.05)	112202

No studies specifically addressing mobility of terpenes in soil were found during the literature search, but in 2 publications (Maurer et al., 2008; Asensio et al., 2008; concentration of selected terpenes in various soil layers was investigated and demonstrated that these compounds are find mainly in the litter layer or organic layer just beneath the litter, while concentration of terpenes decreases with depth and no or very low residues are being found in mineral horizons (at ~20 cm). On this basis it may be concluded that terpenes do not migrate in the soil profile and are present mainly in the first (organic) soil horizons.

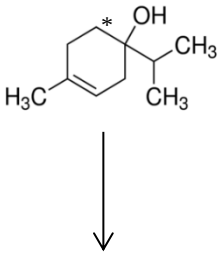
2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

No hydrolysis study was provided in support of the evaluation, but in opinion of the RMS such a study is not required based on the non-polar chemical structures and high vapour pressure leading to rapid volatility from surface water bodies. The same conclusion was taken during the first EU review of TTO (see EFSA Journal 2012;10(2):2542).

The photodegradation in water was also not investigated. In general, the RMS is of the opinion that in absence of conjugated double bonds, the photochemical degradation of TTO components in aquatic systems is rather not expected. However, based on results of the literature study by Goldberg et al. (1992, Vol. 3CA, B.8.2.1.2/02), photodegradation could be possible for some compounds under respective environmental conditions. For this reason relevant regulatory study should have been performed. Alternatively, more detailed justification for waiving of the study should be provided.

In the regulatory study TTO turned out to be readily biodegradable with 87% biodegradation observed already on day 7 (Fiebig, 2010, Vol. 3CA, B.8.2.2.1/01). These results were contrary to results of the literature study by Jenner et al. (2011, Vol. 3CA, B.8.2.2.1/03), in which δ-cadinene (one of TTO constituents) was not readily biodegradable since removal of ThOD was <60% after 28 days. However, in this literature study only single constituent was included, which is present in TTO at very low concentration and for this reason the biodegradation of the whole mixture cannot be predicted using data obtained for this single compound.

The route and rate of degradation of the main TTO constituents selected on the basis of their physico-chemical properties and concentration in TTO (Terpinene-4-ol, γ-Terpinene, p-Cymene, 1,8-Cineole, Aromadendrene and Globulol) in water/sediment systems were investigated in one regulatory study performed in line with OECD 308 (Bloß, 2018c, Vol. 3CA, B.8.2.2.3/01). No metabolites >5% AR formed from Terpinene-4-ol in water and sediment phase over the whole study period. Based on that the following metabolic pathway was proposed:



[^{14}C]Terpinene-4-ol

CO_2, non-extractable residues and minor metabolites

It is, however, noted that Terpinene-4-ol was the only labelled compound used in the study and potential formation of metabolites was not determined for other main constituents of TTO included in the study. This was most probably due to the fact that Terpinene-4-ol is the leading constituent of TTO, representing 44.3% of the whole mixture. Nevertheless, other compounds are also present in quantifiable amounts (e.g. γ-Terpinene and 1,8-Cineole are present at 18.6 and 4.7%, respectively). Therefore it may be questioned if the route of degradation of the leading constituents has been sufficiently investigated.
The kinetic evaluation was performed in general in line with recommendations of FOCUS Degradation Kinetics (2014) at Level P-I, which could be accepted, but it has to be noted that most of TTO constituents is highly volatile, while the procedure for Level P-I (an P-II as well) described in the FOCUS guidance is relevant only for non-volatile compounds undergoing losses by degradation. Described procedure could be also relevant for slightly volatile substances provided that results are corrected for volatile losses.

Since Terpinene-4-ol was the only labelled substance used in the study, quantification of organic volatiles formed during the study was not possible for other investigated highly volatile compounds (γ-Terpinene, p-Cymene, 1,8-

Cineole and Aromadendrene) and for this reason it is not known what are proportions of degradation and dissipation due to volatilisation of these compounds.
The kinetic assessment was validated by the RMS as if the compounds were non-volatile, but for reasons indicated above the derived DT_{50}/DT_{90} values cannot be considered to be fully reliable.

Due to relatively low vapour pressure ($4.95x10^{-3}$ Pa) results obtained for Globulol may be considered as more reliable, since losses of this compound from the test systems via volatilisation are expected to be minor.

Due to labelling of Terpinene-4-ol it was possible to determine the extent of losses of this compound via volatilisation. In Pfalz w/s system no losses due to volatilisation were observed at 0 and 2 HAT (hours after treatment), while at 6, 24 and 48 HAT the organic volatiles were formed at <5% AR. Formation of organic volatiles was slightly higher, but exceeded 10% AR only at the test termination (552 HAT), but was still <10% AR for Terpinene-4-ol. In case of Humsterbach w/s system no losses due to volatilisation were observed at 0, 2 and 6 HAT, while at 24, 48, 96 and 552 HAT organic volatiles were found at <5% AR. Organic volatiles >5% AR were found only at 158 and 336 HAT at 8.2 and 5.4% AR, respectively, and were characterised mainly by Terpinene-4-ol. Although no correction for volatilisation was made prior to kinetic evaluation, based on formation of CO_2 and losses via volatilisation <10% AR, the RMS is of the opinion that the DT_{50}/DT_{90} values derived for Terpinene-4-ol sufficiently describe degradation of this compound in water/sediment systems.

With regard to highly volatile compounds for which losses for volatilisation were not measured, the RMS is of the opinion that although obtained results are not reliable to describe degradation of these compounds in water sediment/systems, they may be used to characterise the overall behaviour of these compounds with rapid dissipation from the water phase (within hours to days) and not significant partitioning to sediment.

Summary of the derived DT_{50}/DT_{90} in both water/sediment systems is provided in tables below.

Table 2.8.2-1: Overview of best-fit degradation data of selected TTO components in Pfalz water/sediment system

1WS Pfalz					
Test item	**Test system**	**Best fit kinetic model**	**DT_{50} [h]**	**DT_{90} [h]**	**Chi^2 [%]**
[^{14}C]Terpinene-4-ol	Water phase	SFO	130	432	8.52
	Sediment extract	No kinetic calculation available for sediment extract, due to insufficient number of data points.			
	Total system	SFO	196	652	7.93
γ-Terpinene	Water phase	SFO	1.38	4.59	0.706
	Sediment extract	SFO	17.4	57.9	24.5
	Total system	SFO	1.49	4.95	2.22
p-Cymene	Water phase	SFO	1.42	4.72	1.55
	Sediment extract	SFO	41.5	138	17.7
	Total system	SFO	1.61	5.34	5.6
1,8-Cineole	Water phase	SFO	44.2	147	13.2
	Sediment extract	No acceptable fit			
	Total system	SFO	50.7	169	13.5
(+)-Aromadendrene	Water phase	SFO	16.8	55.9	22.1
	Sediment extract	SFO	149	495	25.1
	Total system	SFO	23.4	77.7	23.8
(-)-Globulol	Water phase	SFO	124	411	11.5
	Sediment extract	No acceptable fit			
	Total system	SFO	206	684	18.1

Table 2.8.2-2: Overview of best-fit degradation data of selected TTO components in Humsterbach water/sediment system

2WS Humsterbach					
Test Item	**Test System**	**Best Fit Kinetic Model**	**DT_{50} [h]**	**DT_{90} [h]**	**Chi^2 [%]**
[^{14}C]Terpinene-4-ol	Water phase	SFO	75.8	252	7.4
	Sediment extract	SFO	151	501	3.84
	Total system	SFO	104	345	6.95
γ-Terpinene	Water phase	SFO	1.07	3.54	3.71
	Sediment extract	SFO	26.9	89.5	28.9
	Total system	SFO	1.16	3.87	8.6
p-Cymene	Water phase	SFO	1.42	4.71	1.13
	Sediment extract	SFO	21.2	70.4	36.8
	Total system	SFO	1.6	5.31	6.81
1,8-Cineole	Water phase	SFO	13.3	44.2	5.92
	Sediment extract	No kinetic calculation available for sediment extract, due to insufficient number of data points.			
	Total system	SFO	15.2	50.4	7.36
(+)-Aromadendrene	Water phase	SFO	7.06	23.5	8.48
	Sediment extract	SFO	46.2	154	21.8
	Total system	SFO	8.38	27.8	12.7
(-)-Globulol	Water phase	SFO	33.5	111	9.28
	Sediment extract	SFO	73.2	243	9.92
	Total system	SFO	84	279	12.4

2.8.2.1 Rapid degradability of organic substances

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Table 2.8.2.1-1: Summary of relevant information on rapid degradability

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td>Tea Tree Oil (TTO): ready biodegradability – CO_2 in sealed vessels (Headspace Test)

OECD 310</td><td>Tea Tree Oil:
Purity: α-Terpinene: 10.433 % (w/w), 1,8-Cineole: 3.277 % (w/w), γ-Terpinene 21.667 % (w/w), p-Cymene 2.277 % (w/w), Terpinene-4-ol 40.067 % (w/w)

The ready biodegradability of Tea Tree Oil was determined with a non-adapted sludge over a test period of 28 days in the Headspace Test according to OECD 310.</td><td>In the toxicity control treatments (test medium + reference item + TTO), the test substance TTO did not have any toxic effect on the microorganisms.</td><td>1</td><td>Fiebig, S., (2010)</td></tr>
<tr><td colspan="5">Aerobic soil degradation</td></tr>
<tr><td>[^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene from Tea Tree Oil
Aerobic Degradation in one Soil at 20 °C in the Dark
OECD 307, GLP</td><td>The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p Cymene, 1,8-Cineole, (-)-Globulol/Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark using one soil of European origin (2.4). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene , 1,8-Cineole, ( ) Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 768 hours (32 days).

Results:
<table>
<tr><th>Test Item</th><th>Kinetic Model</th><th>DT_{50} [hours]</th><th>DT_{90} [hours]</th><th>Chi^2 Error [%]</th><th>Visual Assessment*</th></tr>
<tr><td>[^{14}C]Terpinene-4-ol</td><td>SFO</td><td>10</td><td>33.3</td><td>15.9</td><td>+</td></tr>
<tr><td>gamma-Terpinene</td><td>SFO</td><td>0.861</td><td>2.86</td><td>4.12</td><td>+</td></tr>
<tr><td>p-Cymene</td><td>SFO</td><td>0.995</td><td>3.3</td><td>4.97</td><td>+</td></tr>
<tr><td>1,8-Cineole</td><td>SFO</td><td>2.39</td><td>7.95</td><td>9.94</td><td>+</td></tr>
<tr><td>(-)-Globulol</td><td>SFO</td><td>151</td><td>503</td><td>8.91</td><td>+</td></tr>
</table></td><td>[^{14}C]Terpinene-4-ol degraded to CO_2, non-extractable residues and minor metabolites.</td><td>1</td><td>Bloß, K. (2018a)</td></tr>
</table>

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Relia-bility score</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td>(+)-Aromadendrene</td><td>SFO</td><td>70.5</td><td>234</td><td>19.7</td><td>+</td></tr>
</table>
* Visual Assessment: + = good, o = moderate, - = poor
</td><td></td><td></td><td></td></tr>
<tr><td>gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene - Aerobic Degradation in Three Soils at 20 °C in the Dark

OCED 307
GLP</td><td>The rate of degradation of gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene was investigated under aerobic conditions at 20 ± 2 °C in the dark using three soils of European origin (LUFA 2.1, LUFA 2.2 and LUFA 6S). The study was performed over a period of 32 days.

Results:
<table>
<tr><th>Test Item</th><th>Soil</th><th>Best Fit Kinetic Model</th><th>DT_{50} [h]</th><th>DT_{90} [h]</th><th>Chi^2 Error [%]</th><th>Visual Assessment *</th></tr>
<tr><td rowspan="3">p-Cymene</td><td>LUFA 2.1</td><td>FOMC</td><td>3.02</td><td>26.8</td><td>4.92</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>12.8</td><td>42.6</td><td>13.3</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>5.96</td><td>19.8</td><td>9.7</td><td>+</td></tr>
<tr><td rowspan="3">1,8-Cineole</td><td>LUFA 2.1</td><td>SFO</td><td>9.43</td><td>31.3</td><td>8.08</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>16.3</td><td>54</td><td>14.7</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>7.36</td><td>24.5</td><td>8.76</td><td>+</td></tr>
<tr><td rowspan="3">gamma-Terpinene</td><td>LUFA 2.1</td><td>DFOP</td><td>1.78</td><td>14.5</td><td>2.8</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>DFOP</td><td>1.63</td><td>13.4</td><td>0.962</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>3.56</td><td>11.8</td><td>8.94</td><td>+</td></tr>
<tr><td rowspan="3">(+)-Aromadendrene</td><td>LUFA 2.1</td><td>FOMC</td><td>81.9</td><td>361</td><td>8.8</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>186</td><td>617</td><td>11.5</td><td>o</td></tr>
<tr><td>LUFA 6S</td><td>FOMC</td><td>63.4</td><td>779</td><td>12.1</td><td>+</td></tr>
<tr><td rowspan="3">(-)-Globulol</td><td>LUFA 2.1</td><td>SFO</td><td>262</td><td>871</td><td>11.1</td><td>o</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>186</td><td>619</td><td>11.7</td><td>o</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>167</td><td>556</td><td>8.01</td><td>o</td></tr>
</table>
* Visual Assessment: + = good, o = moderate, - = poor</td><td>In the aerobic soil degradation study, 10 minor transformation products evolved. None of the metabolites reached 5 % AR. Therefore, no studies on metabolites are required.</td><td>1</td><td>Bloß, K. (2018b)</td></tr>
<tr><td colspan="5">Adsorption and desorption of the active substance</td></tr>
<tr><td>Estimation of Adsorption-Coefficient on Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC)

OECD 121
GLP</td><td>The aim of this study was the determination of the adsorption coefficient of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene on soil and on sewage sludge (K_{OC}) by high-performance liquid chromatography (HPLC) method according to EC method C.19 (440/2008) and OECD guideline 121.

Results:
<table>
<tr><th>Test Item</th><th>Mean retention time of two runs [min]</th><th>Absolute deviation [min]</th><th>Mean log K_{OC} of two runs</th><th>Absolute deviation of log K_{OC}</th></tr>
<tr><td>Terpinene-4-ol</td><td>3.40</td><td>0.01</td><td>1.95</td><td>0.00</td></tr>
<tr><td>gamma-Terpinene</td><td>6.06</td><td>0.03</td><td>3.36</td><td>0.01</td></tr>
<tr><td>p-Cymene</td><td>5.46</td><td>0.02</td><td>3.13</td><td>0.01</td></tr>
<tr><td>1,8-Cineole</td><td>3.19</td><td>0.01</td><td>1.77</td><td>0.00</td></tr>
<tr><td>(-)-Globulol</td><td>5.74</td><td>0.01</td><td>3.24</td><td>0.00</td></tr>
<tr><td>(+)-Aromadendrene</td><td>13.88</td><td>0.08</td><td>> 5.0 (5.05)*</td><td>0.00</td></tr>
</table></td><td></td><td>1</td><td>Göcer, M. (2018)</td></tr>
</table>

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td></td><td>* above the determination limit of the method (5.0 according to OECD 121) but within the calibration range (log K_{OC} of the highest reference standard 4,4'-DDT is 5.63)</td><td></td><td></td><td></td></tr>
<tr><td colspan="5">Fate and Behaviour in Water and Sediment</td></tr>
<tr><td>Hydrolytic degradation

Expert statement
Photolysis

Expert statement</td><td>Due to their high vapour pressure and rather low water solubility, especially of the terpene hydrocarbons, most of the TTO constituents will volatilise from surface water within a very short time period after application. This is indicated by the high Henry constants and vapor pressures of the constituents. As terpenes do not persist in water, no testing of hydrolysis is required. This conclusion is supported by calculations according to the German EVA model.
Estimations of the photochemical oxidative degradation and experimental data show that all TTO constituents rapidly degrade in the gas phases under the influence of light.
Based on their low solubility in water, their high volatility and high reactivity under the influence of light, it may be assumed that the persistence time of the different TTO constituents in flora, soil and surface waters is rather low. Furthermore, based on the fact that the TTO constituents react rapidly with OH, NO_3 radicals and O_3 their residence time in the troposphere is also considered to be short.
Further experimental data on hydrolysis and photolysis of TTO and its components are therefore not deemed necessary.</td><td></td><td></td><td>Weißmann, N. (2007)</td></tr>
<tr><td>None, statement

Direct photochemical degradation</td><td>None of the Components, with the exception of p-Cymene (max. 8% of total), contains conjugated double bonds. Accordingly, none of these substances have significant absorption at 290 nm or above.
Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) ≥ 290 nm. Therefore, no studies on direct photochemical degradation are deemed necessary.</td><td></td><td></td><td></td></tr>
<tr><td>[^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil
Aerobic Degradation and Metabolism in two Water/Sediment Systems
OECD 308
GLP</td><td>Results:
<table>
<tr><th>W/S system</th><th>Component</th><th>DT$_{50}$ [h]</th><th>DT$_{90}$ [h]</th><th>Chi²</th></tr>
<tr><td rowspan="6">Pfalz Hanhofen</td><td>Terpinene-4-ol</td><td>196</td><td>652</td><td>7.93</td></tr>
<tr><td>g-Terpinene</td><td>1.49</td><td>4.95</td><td>2.22</td></tr>
<tr><td>p-Cymene</td><td>1.61</td><td>5.34</td><td>5.6</td></tr>
<tr><td>1,8-Cineole</td><td>50.7</td><td>169</td><td>13.5</td></tr>
<tr><td>(-)-Globulol</td><td>206</td><td>684</td><td>18.1</td></tr>
<tr><td>(+)-Aromadendrene</td><td>23.4</td><td>77.7</td><td>23.8</td></tr>
<tr><td rowspan="6">Humsterbach</td><td>Terpinene-4-ol</td><td>104</td><td>345</td><td>6.95</td></tr>
<tr><td>g-Terpinene</td><td>1.16</td><td>3.87</td><td>8.6</td></tr>
<tr><td>p-Cymene</td><td>1.6</td><td>5.31</td><td>6.81</td></tr>
<tr><td>1,8-Cineole</td><td>15.2</td><td>50.4</td><td>7.36</td></tr>
<tr><td>(-)-Globulol</td><td>84</td><td>279</td><td>12.4</td></tr>
<tr><td>(+)-Aromadendrene</td><td>8.38</td><td>27.8</td><td>12.7</td></tr>
</table></td><td>No metabolites > 10% were observed in either soil or water/sediment study. The rapid metabolism of Tea Tree Oil observed both in soil and water/sediment, and the fact that Tea Tree Oil is readily biodegradable, indicates that Tea Tree Oil components is used as a readily available energy source.</td><td>1</td><td>Bloß, K. (2018a)</td></tr>
</table>

2.8.2.1.1 Ready biodegradability

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

The rapid degradability of TTO was tested in an OECD 310 GLP study.

Study 1, Fiebig, S., 2010, Tea Tree Oil (TTO): ready biodegradability – CO_2 in sealed vessels (Headspace Test), OECD 310, GLP.

Reliability statement: The study was conducted in line with OECD 310 and meets the respective validity criteria reported therein. The composition of the test substance reflects the ISO 4730:2004. Moreover, the method is considered suitable according to point 11 in OECD 310, since complete aerobic degradation could be demonstrated although some of the components of the active substance exceed the Henry's law constant criterion of maximum 50 (Pa x m³)/mol. The study also includes an analytical method fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 310 were identified. Hence, the study is considered reliable (reliability score: 1).

The test item was tested at a nominal dose of 10 mg C/L in triplicates.
The biodegradation was followed by TIC analysis of the produced CO_2 by the respiration of bacteria.

Tea Tree Oil was readily biodegradable under the test conditions. The 10% level was reached after 2 days. The 60% pass level was reached within the 10-d window after 5 days. The maximum biodegradation came to 106% after 28 days. Thus, TTO is readily biodegradable by the terms of this test.
In accordance with the EC Directives on dangerous preparations 1272/2008, substances are considered rapidly degradable in the environment if one of the following criteria holds true:
(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:
(i) tests based on dissolved organic carbon: 70 %;
(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.

These levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10 % of the substance has been degraded, unless the substance is identified as an UVCB or as a complex, multi- constituent substance with structurally similar constituents.
It is obvious from the OECD test on Ready Biodegradability that Tea Tree Oil can be regarded as readily biodegradable. This finding is further supported by Literature data on biodegradation of terpene components (see following table).

Table 2.8.2.1.1-1: Literature data on biodegradation of terpene components

Substance	Study/endpoint type	Results	Reliability score	Reference
Ready biodegradability				
Limonene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 182 Max. degr. rate (mg/L/h): 0.044 Norm. degr. rate (h^{-1}): not measured Readily biodegradable	2	Misra *et al.* (1996)
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: > 75 mg/L, complete degradation within 70 h	2	Misra & Pavlostathis (1997)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.9 mg/L/h - as individual substance: 1.9 mg/L/h	2	Misra & Pavlostathis (1997)
α-Pinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 200 Max. degr. rate (mg/L/h): 0.029 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra et al. (1996)
	Fate in wetland sediment	2004: 12240 ng/g 2005: 7890 ng/g (36 % decrease) 2006: 2640 ng/g (ca. 70 % decrease)	2	Palma-Fleming et al. (2013)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 1.1 mg/L/h - as individual substance: 2.1 mg/L/h	2	Misra & Pavlostathis (1997)

Substance	Study/endpoint type	Results	Reliability score	Reference
	Liquid degradation by two bacterial species (*Pseudomonas fluorescens* and *Alcaligenes xylosoxidans*) tested singly and as consortium	Complete degradation (<0.l mg/L remaining) in 36 h by the consortium. After a 10-h lag period, a maximum rate of degradation of 3.6 mg/L/h was observed. The *A. xylosoxidans* isolate was shown to degrade α-Pinene to below 5 mg/L in the test system in 36 hours, achieving a maximum degradation rate of 3.6 mg/L/h. The *R. fluorescens* isolate showed little degradation of α-pinene until 36 h into the experiment and had a maximum degradation rate of 1.2 mg/L/h.	2	Kleinheinz et al. (1999)
γ-Terpinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 168 Max. degr. rate (mg/L/h): 0.039 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra et al. (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.8 mg/L/h - as individual substance: 1.8 mg/L/h	2	Misra & Pavlostathis (1997)
α-Terpinolene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 174 Max. degr. rate (mg/L/h): 0.053 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra et al. (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.6 mg/L/h - as individual substance: 1.5 mg/L/h	2	Misra & Pavlostathis (1997)
α-Terpineol	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 94 Max. degr. rate (mg/L/h): > 0,10 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra et al. (1996)
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: 210 mg/L, complete degradation within 48 h	2	Misra & Pavlostathis (1997)
δ-Cadinene	Persistency and biodegradation, experimental data (OECD 301F test) vs. Catalogic estimation models	OECD test: > 60 % degradation in 28 days → not persistent Kinetic model: primary half-life 8 days, not persistent	1	Jenner et al. (2011)
Biotransformation				

Substance	Study/endpoint type	Results	Reliability score	Reference
Limonene	Biotransformation of limonene by *Pseudomonas putida*	Optimal degradation conditions at 30°C, pH 5 and 120 days. The bioconversion products were identified as perillyl alcohol and p-menth-1-ene-6,8-diol, and under optimum conditions the yields were 36% and 44%, respectively (a rate kinetic model indicated corresponding limiting yields of 44% and 56%). No further degradation of the products was observed using these bacteria.	2	Chatterjee & Bhattacharyya (2001)
	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	75 % of Limonene consumed, metabolite formed (traces): α-Terpinene	2	Harder & Probian (1995)
α-Terpineol	Metabolism by *Pseudomonas incognita*	P. incognita degrades α-Terpineol by at least three routes: - via oleuropeic acid - aromatization of α-Terpineol - formation of limonene	2	Madhava & Renganathan (1984)
	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	Decrease of 52 % after 7.5 d, remained constant until study termination (30 d)	2	Pavlostathis & Misra (1999)
p-Cymene	p-Cymene catabolic pathway in *Pseudomonas putida* F1	*P. putida* F1 utilizes p-Cymene by an 11-Step pathway through p-Cumate to isobutyrate, pyruvate, and acetyl CoA.	2	Eaton (1997)
α-Pinene	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	No significant degradation	2	Pavlostathis & Misra (1999)
	Bioconversion by *Pseudomonas* sp. strain PIN	Total bioconversion in 40 h: 33.5 % Formed products: Limonene, *p*-Cymene, *p*-Cymene-8-ol, α-Terpinolene, terpineol, camphor, terpinene-4-ol and borneol (all < 10 %)	2	Yoo et al. (2001)
	Bacterial metabolism by *Pseudomonas* sp. strain PIN	Substrate: α-Pinene (1 % v/v) Biomass: 103.13 g/L Maximum yield: 1056 mg/L Specific yield (cells): 10.24 Productivity: 22.00 mg/l h Formed metabolites: *p*-Cymene, Limonene, Trepineol, Terpinolene, Borneol	2	Yoo & Day (2002)
	Degradation by *Bacillus pallidus* BR425	Metabolites formed (all < 10 %): *β*-Pinene, Limonene, Pinocarveol, Pinocarvone, Myrtenol, Myrtenal, Carveol, Carvone	2	Savithiry et al. (1998)
	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	67 % of Limonene consumed, metabolites formed (traces): α-Terpinene, Cymene, Limonene, Eucalyptol	2	Harder & Probian (1995)

Substance	Study/endpoint type	Results	Reliability score	Reference
α-Pinene, p-Cymene 1,8-Cineole (=Eucalyptol), Terpinen-4-ol, α-Terpineol, Terpinolene, α- and γ-Terpinene	Mediterranean conditions; flowers of lavender oil.	Main substances were analysed monthly and after 12 months; p-Cymene and 1,8-Cineole were abundant in litter, and after 16 months 1,8-cineole. According to these investigations, the half-life rate of terpenes in litter is between 210 and 240 days under Mediterranean conditions. It should be noted that these are to be seen as worst case conditions for the Northern and Central Europe.	2	Hassiotis (2010)

Reliability statement: The literature studies from which the data listed in the above table are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria as set out in the EFSA guidance on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable or reliable with restrictions (supporting information).

2.8.2.1.2 BOD5/COD

No data available.

2.8.2.2 Other convincing scientific evidence

2.8.2.2.1 Aquatic simulation tests

No data available.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

TTO components are present in other environmental compartments due to the emission of terpenes and their redistribution (e.g. p-Cymene, gamma-Terpinene, alpha-Terpinene and Limonene) from rangeland and other crops. Emission from these natural sources results in background levels of terpene components in soil, water and sediment.
As these findings are not relevant for classification and labelling the respective information is not presented in the frame of the present assessment.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

No data available.

2.8.2.2.4 Soil and sediment degradation data

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

> *Study 1, Bloß, K. 2018, [^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil - Aerobic Degradation and Metabolism in two Water/Sediment Systems, OECD 308, GLP.*

Reliability statement: The study was conducted in line with OECD 121 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to paragraph ‚applicability of the method' OECD 121, since it is considered particularly useful for volatile substances. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 121 were identified. Hence, the study is considered reliable (reliability score: 1).

The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p-Cymene , 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark using two different water/sediment systems (1WS Pfalz and 2WS Humsterbach). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene, 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 552 hours (23 days).
No metabolites > 10% were observed in this study.

> *Study 2, Bloß, K. 2018, [^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil - Aerobic Degradation in one Soil at 20 °C in the Dark, OECD 307, GLP.*

Reliability statement: The study was conducted in line with OECD 307 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to point 5 in OECD 307, since the analytical method was able to provide evidence that the tested substances could be kept in soil under the experimental conditions of the test and complete aerobic degradation could be demonstrated. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 307 were identified. Hence, the study is considered reliable (reliability score: 1).

The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p-Cymene , 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark in one soil of European origin (2.4). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene , 1,8-Cineole, ( ) Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 768 hours (32 days).
[^{14}C]Terpinene-4-ol degraded to CO_2, non-extractable residues and minor metabolites. The DT_{50} values ranged from 0.9 to 151 hours.

> *Study 3, Bloß, K. 2018b, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene - Aerobic Degradation in Three Soils at 20 °C in the Dark, OCED 307, GLP.*

Reliability statement: The study was conducted in line with OECD 307 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to point 5 in OECD 307, since the analytical method was able to provide evidence that the tested substances could be kept in soil under the experimental conditions of the test and complete aerobic degradation could be demonstrated. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 307 were identified. Hence, the study is considered reliable (reliability score: 1).

The rate of degradation of gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene was investigated under aerobic conditions at 20 ± 2 °C in the dark using three soils of European origin (LUFA 2.1, LUFA 2.2 and LUFA 6S). The study was performed over a period of 32 days.
In the aerobic soil degradation study, 10 minor transformation products evolved. None of the metabolites reached 5 % AR. The DT_{50} values ranged from 1.6 to 262 hours.
The results from the water-sediment study in two different aquatic systems and from the soil degradation studies indicate that the components of Tea Tree Oil degrade rapidly in the tested environmental compartments.

2.8.2.2.5 Hydrolysis

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

An expert statement concluded on following concerning hydrolysis and photochemical degradation of Tea Tree Oil:

Due to their high vapour pressure and rather low water solubility, especially of the terpene hydrocarbons, most the TTO constituents will volatilise from surface water within a very short time period after application. This is indicated by the high Henry constants and vapor pressures of the constituents.
Estimations of the photochemical oxidative degradation and experimental data show that all TTO constituents rapidly degrade in the gas phases under the influence of light.
Based on their low solubility in water, their high volatility and high reactivity under the influence of light, it may be assumed that the persistence time of the different TTO constituents in flora, soil and surface waters is rather low. Furthermore, based on the fact that the TTO constituents react rapidly with OH, NO_3 radicals and O_3 their residence time in the troposphere is also considered to be short.

These findings support the overall conclusion that Tea Tree Oil can be considered as rapidly biodegradable in light of hydrolysis.

2.8.2.2.6 Photochemical degradation

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

None of the Components, with the exception of p-Cymene (max. 8% of total) and α-terpinene (max. 13% of total), contains conjugated double bonds. Accordingly, none of these substances have significant absorption at 290 nm or above.
Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) ≥ 290 nm. Since p-Cymene has 3 conjugated double bonds (aromatic ring) and α-terpinene only 2 conjugated double bonds, it is therefore clear that α-terpinene also will not absorb at wavelength (λ) ≥ 290 nm since absorption at higher wavelengths requires more conjugated double bonds.

Therefore, no studies on direct photochemical degradation are deemed necessary.

2.8.2.2.7 Other / Weight of evidence

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Table 2.8.2.2.7-1: Adsorption and desorption of the active substance

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td colspan="5">Adsorption and desorption of the active substance</td></tr>
<tr><td>Estimation of Adsorption-Coefficient on Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC)
OECD 121
GLP</td><td>The aim of this study was the determination of the adsorption coefficient of Terpinene-4-ol, gamma-Terpinene, p Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene on soil and on sewage sludge (KOC) by high-performance liquid chromatography (HPLC) method according to EC method C.19 (440/2008) and OECD guideline 121.

Results:
<table>
<tr><th>Test Item</th><th>Mean retention time of two runs [min]</th><th>Absolute deviation [min]</th><th>Mean log K_{OC} of two runs</th><th>Absolute deviation of log K_{OC}</th></tr>
<tr><td>Terpinene-4-ol</td><td>3.40</td><td>0.01</td><td>1.95</td><td>0.00</td></tr>
<tr><td>gamma-Terpinene</td><td>6.06</td><td>0.03</td><td>3.36</td><td>0.01</td></tr>
<tr><td>p-Cymene</td><td>5.46</td><td>0.02</td><td>3.13</td><td>0.01</td></tr>
<tr><td>1,8-Cineole</td><td>3.19</td><td>0.01</td><td>1.77</td><td>0.00</td></tr>
<tr><td>(-)-Globulol</td><td>5.74</td><td>0.01</td><td>3.24</td><td>0.00</td></tr>
<tr><td>(+)-Aromadendrene</td><td>13.88</td><td>0.08</td><td>> 5.0 (5.05)*</td><td>0.00</td></tr>
</table>
* above the determination limit of the method (5.0 according to OECD 121) but within the calibration range</td><td></td><td>1</td><td>Göcer, M. (2018)</td></tr>
</table>

Study	Method / Results	Remarks	Reliability score	Reference
	(log K_{OC} of the highest reference standard 4,4'-DDT is 5.63)			
None, statement Direct photochemical degradation	None of the Components, with the exception of p-Cymene (max. 8% of total), contains conjugated double bonds. Accordingly, none of these substances have significant absorption at 290 nm or above. Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) $\geq$ 290 nm.			

Mobility in soil:

Study 1, Göcer, M. 2018, Estimation of Adsorption-Coefficient on Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC), OECD 121, GLP.

Reliability statement: The study was conducted in line with OECD 121 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to paragraph ‚applicability of the method' OECD 121, since it is considered particularly useful for volatile substances. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 121 were identified. Hence, the study is considered reliable (reliability score: 1).
As for the components of Tea Tree Oil, the batch equilibrium method cannot be applied due to fast degradation, the HPLC (high-performance liquid chromatography) method was considered as a possible alternative.
For the components of Tea Tree Oil, mean log K_{OC} values of 1.77 – 5.05 were derived from two measurements, corresponding to K_{OC} values of 58.9 for 1,8-Cineole to 112201 for (+)-Aromadendrene.

Direct photochemical degradation
None of the components of TTO would be expected to be transported in the gaseous phase over large distances or to accumulate in the air.

2.8.3 Summary of fate and behaviour in air

The vapour pressure of the majority of TTO constituent is high indicating significant losses of these compounds via volatilisation. However, the DT_{50} values for each TTO constituent calculated using Episuite 4.11 ranged from 0.04 to 1.3 days, demonstrating that these compound dissipate rapidly from the air and for this reason the long-term effects of TTO emission are not expected. Performed calculations were confirmed by DT_{50} values for p-Cymene and Limonene, calculated and experimentally measured by Dash & Rajakumar, 2014 (Vol. 3CA, B.8.3.1/02) and Dash & Rajakumar, 2014 (Vol. 3CA, B.8.3.1/03), which were in good agreement with values calculated by the Applicant.

In addition to that numerous literature studies investigating emissions from natural sources (mainly vegetation and soil) were provided demonstrating that TTO constituents are released from many plant species (including crops, such as tomatoes).

Although available literature studies report emissions from gram or number of the investigated plant species, there are some data showing emissions from the surface unit (m^2 or ha). Examples are listed below:

- emission of terpenes estimated from forests: 480 mio tons/year,
- emission of terpenes from pastures: 5.72-12.81 kg/ha/year,
- emission of monoterpenes from leaves of beech tree : 76-144 µg/m^2/s,
- emission of total terpenoid from *Fagus sylvatica* branches : 54 µg/m^2/h,
- emission of monoterpenes (Limonene, α-Pinene, camphene, myrcene, β-Pinene, α-phellandrene, 3-carene, α-Terpinene and cymene) from soil in *Picea sitchensis* forest : mean 13-199 µg/m^2/h.

It is especially easy to compare emission from pastures at 5.72-12.81 kg/ha/year with the cumulative TTO application rate (1.78 kg/ha/year), which shows that the natural emission from plants is much higher than this after application of BM 608.
Summary of natural emissions of TTO constituents found in literature studies is provided in tables below.

Table 2.8.3-1: Natural emission of TTO components

<table>
<tr><th>Plants</th><th>Natural Emission –TTO main components</th><th>References</th></tr>
<tr><td>Forests, rangeland</td><td>Worldwide emission of terpenes estimated from forests area: 480 mio tons/years
United States: 25 to 50 mio tons/year
From rangeland: 5.72 – 12.81 kg /ha per year.</td><td>Vol. 3CA, B.8.3.4/22
Yatagai (1984)

Vol. 3CA, B.8.3.4/23
Kesselmeier et al. (1996),

Vol. 3CA, B.8.3.4/24
Geron. et al. (2000)</td></tr>
<tr><td>Cultivated tomato
(S. lycopersicum) and wild tomatoes
(S. pennellii) infested by B. tabaci</td><td>p-Cymene: 0.01 ± 0.01 and 1.63 ±0.77
γ-Terpinene: 0.01 ± 0.01 up to 5.88±2.50
α-Terpinene: 0.008 – 0.99±0.72.
(µg/24 hour/10 gram fresh weight)</td><td>Vol. 3CA, B.8.3.4/25
Bleeker. et. al. (2009)</td></tr>
<tr><td>Tomatoes
(L. esculentum)
[5 days after infestation by spider mite)</td><td>
<table>
<tr><th>Compounds</th><th>Total emission by control plants µg/plant/5 days</th><th>Total emission by spider mite infested plants µg/plant/5 days</th></tr>
<tr><td>α-Terpinene</td><td>0.0003±0.0003</td><td>0.03±0.03</td></tr>
<tr><td>p-Cymene</td><td>1.1±0.8</td><td>0.2±0.1</td></tr>
<tr><td>Limonene</td><td>5.0±3.0</td><td>3.4±1.8</td></tr>
</table>
</td><td>Vol. 3CA, B.8.3.4/26
Kant et al. (2004)</td></tr>
</table>

Table 2.8.3-2: Additional data on natural emission of TTO components from the open scientific literature

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Field measurement of volatile organic compounds (VOC)	Vosges forest (north-east France) Spring	*Abies alba* branch	Cuvette technique	In [**µg/g_{dw}/h**] α-Pinene: 0.17 Limonene: 0.32 Camphene: 0.14 1,8-Cineole (Eucalyptol): 0.26	Monoterpenes emissions of *A. alba*	Vol. 3CA, B.8.3.4/05 Moukhtar et al., (2006)
Litter bag experiment, measurement of volatile and extractive compounds	Pine needles from Kopna Góra arboretum in Puszcza Knyszyska Forest (north-eastern Poland), spruce needles from Norway spruce plantation Autumn	*Pinus sylvetris* and *Picea abies* needle litter in first stage of decompo-sition	Litter bag experiment, solid-phase microextrac-tion (SPME) and GC-MS	Emission rates [**µg/g_{dw}/h**] α-pinene: 0.06 – 5.13 ß-pinene: trace – 0.24 Limonene: trace – 0.16 Terpinolene: 0.01–0.02 Myrcene: 0.05* ß-phellandrene: 0.12* *only in larch litter		Vol. 3CA, B.8.3.4/14 Isidorov et al. (2010)
Inventory of VOC exchange (especially monoterpenes) between soil and atmosphere	Typical Mediterranean mountain environment, Southern Catalonia Spring 2003- Spring 2004		Sampling with dynamic PVC soil cuvette system,	Normalized soil exchange rates [**µg/m²/h**] α-pinene: -4.82 – 2.42 ß-pinene: nd – 0.12 Limonene: -3.35 – 1.67 Camphene: nd-7.56		Vol. 3CA, B.8.3.4/06 Asensio et al. (2006)
Study emission rate of two branches over two vegetation periods	A stand of 40 years old Scots pines (*Pinus sylvestris* L.) in Hartheim Forest, near Freiburg, Southern Germany All: March 1998-October 1999	Branches of *Pinus sylvestris*	Branch enclosure technique, adsorption tubes with Carbotrap and Tenax TA, thermodesorption coupled with GC/MS	Emission rates [**pmol/m²/s**] varying diurnally, seasonally and between trees: α-pinene: 5.1 – 1288.7 ß-pinene: 1.02 – 450.6 3-carene: 10.2 – 424.1	14 sesquiterpenes in spring, in summer and fall only 1,8-cineol and camphor; Monoterpenes: α-, β-pinene, 3-carene ~ 90% of main compounds	Vol. 3CA, B.8.3.4/07 Holzke, C. et al., 2006.

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Isoprene and monoterpene emission rates from perennial bioenergy crops (short-rotation coppice willow, *Miscanthus*) and annual arable crops (wheat, oilseed rape)	Arable fields in England and Scotland: Lincolnshire: fields with SRC willow (Salix spp.), *Miscanthus* x giganteus, wheat, oilseed rape; Perthshire: SRC willow Fife: willow Lincolnshire: April 2010-August 2012 Perthshire: September 2011-August 2012 Fife: March – August 2012	Leaves of willow, *Miscanthus*, wheat and oilseed rape	Cylindrical enclosure chambers, GC-MS analysis	In [**µg/g/h**] Willow: α-pinene: <LOD -803 δ-3-carene: <LOD – 268 ß-pinene: <LOD – 125 limonene: <LOD – 80.4 *Miscanthus*: α-pinene: <LOD – 20.8 (only 2010) Wheat: α-pinene: <LOD – 422 limonene: <LOD – 104 Oilseed rape: not significant	Willow: isoprene, α-pinene, ß-pinene, δ-3-carene-emission rates vary with season, correlation between isoprene, α-pinene, δ-3-carene emission rates and PAR and air temperature, correlation between isoprene, α-pinene, ß-pinene, limonene and soil moisture and soil temperature Miscanthus: no measurable emissions of ß-pinene, limonene and δ-3-carene, α-pinene lower than willow, correlation with season Wheat and oilseed rape: low to nondetectable isoprene emissions, emission rates of α-pinene > δ-3-carene > limonene, ß-pinene (not present in oilseed rape)	Vol. 3CA, B.8.3.4/15 Morrison et al. (2015)
Main tree species according to CORINE IV land cover classes from nurseries		A set of 4-year-old tree species	Leaf enclosure method	Basal emission factors [**µg/g_{dw}/h**]: α-pinene: 0.11 – 2.0 ß-pinene: 0.05 – 0.4 Sabinene: 0.01 – 9.33 Limonene: 0.05-0.44 Γ-terpinene: 0.01 –0.06 α-terpinolene: 0.01 α-terpineol: 0.01 – 0.04 γ-terpineol: 0.04 *p*-cymene: 0.01 – 0.04 1,8-cineol: 0.01 – 0.12	Isoprene, α-and ß-pinene, sabinene most important compounds; annual biogenic isoprene and monoterpene fluxes in 2006: ~ 31.3 Gg and 37.7 Gg, resp.;	Vol. 3CA, B.8.3.4/27 Kemper Pacheco et al. (2014).

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Catabolism of ^{14}C-labelled geraniol to $^{14}CO_2$ in soils	Rhizosphere of *Populus tremula* trees at 3 different sites in Slovenia: a) 487 m asl, ~3500 mm annual rainfall, bordering Alpine meadow and forest b) 833 m asl, ~1400 mm annual rainfall, old hedge bordering meadowland c) 445 m asl, ~1000 mm annual rainfall, garrigue/scrubland near coast	Soil/root system up to 200 cm from the tree trunk	Cylindrical soil enclosure on soil surface, air samples with Carbotrap, GC-FID analysis, respirometry to measure catabolism	Limonene (varied throughout sampling day): 0.7 – 18.5 **µg/m²/h** Other monoterpenes < LOD	Emission rate Limonene: 0.7 – 18.5 µg $m^{-2}h^{-1}$ Other monoterpenes: < LOD Degradation rate of geraniol varying with maximum at 100 cm from tree trunk and minimum at 200 cm	Vol. 3CA, B.8.3.4/28 Owen et al. (2007)
BVOC emissions from *Abies alba* under ambient conditions	Trees from Vielsalm Forest (Belgian Ardennes forest) Spring – Autumn	Branches of *Abies alba*	Dynamic branch enclosure technique, GC analysis	Emission rates [**µg/g_{dw}/h**]: Isoprene (standardized): max. (June) = 27 and min. (October) = 4.6 Monoterpene (non-standardized): α-pinene = 0.742 camphene = 0.917 ß-pinene = 0.429 limonene = 0.512	27 compounds, mainly isoprene, α-pinene, ß-pinene, camphene and limonene, relative contribution (%) varies with season	Vol. 3CA, B.8.3.4/08 Pokorska et al., (2012)
	Boreal forest region, trees from a pre-alpine region in Southern Germany Autumn	Branches of Silber birch (*Betula pendula* L.), Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and European larch (*Larix decidua* L.)	Dynamic cuvette system, PTR-MS, GC-MS	Exchange rate [**nmol/m²/s**]: α-pinene: ~0.05 – 6.25 Camphene: ~0.02 – 0.5 Sabinene + ß-pinene: ~ 0.04 – 4.25 Myrcene: ~0.19 – 0.5 Cymene: ~ 0.02 Limonene: ~0.03 –0.25 1,8-cineole: ~0.04 Isoprene: ~0.09-0.7		Vol. 3CA, B.8.3.4/09 Ghirardo et al. (2010)

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Explore and quantify the soil VOC exchange rates, especially monoterpenes	Mediterranean shrubland (Garraf National Park, Catalonia, Spain Various seasons	Petrocalcic Calcixerept soil	Flow-through chamber method and infrared analyser system, GC-MS, PTR-MS	Exchange rate **[nmol/m²/s]:** α-pinene: ~ -0.0025-0.0025 limonene: ~ -0.0015-0.0025 ß-pinene: 0.000004 – 0.000171 ß-myrcene: -0.000022 – 0.0003 M123 (sesquiterpenes): 0.04-0.25 M137 (mono-, sesquiterpenes): 0.14 – 0.62 M155 (linalool, 1,8-cineole): 0.0-0.16	No seasonal effects on total monoterpene or individual monoterpene exchange rates	Vol. 3CA, B.8.1.5/02 Asensio et al. (2008)
Impact of elevated temperature and elevated ozone on VOC synthesis and emissions	Finnish forest, boreal zone Summer-autumn	Norway spruce seedlings (3-years-old)	Headspace collection technique with polyethylene terephthalate (PET) cooking bags, GC-MS	Emission rate monoterpene [**ng/g needle dw/h]**: 1726 – 13,120	Monoterpenes with highest emissions: α-pinene 23% limonene 17% ß-pinene 15% ß-phellandrene 11% camphene 10% 3-carene 10% Elevated ozone concentrations -> increasing terpene emissions	Vol. 3CA, B.8.3.4/16 Kivimäenpää et al. (2013)
Investigation + quantification of photosynthesis and monoterpene emissions of beech tree (*Fagus sylvatica* L.)	Mixed temperate forest (Semi-urban Aelmoeseneie forest near Gent, Belgium) Late spring-early autumn	Leaves of beech tree (*Fagus sylvatica* L.)	Dynamic branch enclosure system (cuvettes) at 7, 14, 21 and 25 m in the canopy, photosynthesis measured by quantum sensors, infrared gas analyser, PTR-MS, GC-MS	Emission rate monoterpene [**µg/m²/s**]: 76 (25 m) – 144 (21 m)	Highest emissions on semi-shaded leaves (21 m), leaf physiology effect MT emissions; emitted total monoterpene composition varies with light intensity (sun, semi-shade)	Vol. 3CA, B.8.3.4/10 Simpraga et al. (2013)

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Investigation of potential change of monoterpene emissions depending on temperature and light	Deciduous forest in urban area (Research Center Jülich, Germany) Summer	Leaves of canopy top of European beech (*Fagus sylvatica* L.)	Open dynamic (flow-through) branch enclosure system (cuvettes), infrared gas analyser, GC-FID, GC-MS; storage at -30°C before refocusing of VOC species	Emission rate monoterpene [**µg/g/h**]: 4.1 – 12.9 (G97 function) 4.5 – 14.1 (S97 function)	Sabinene dominant monoterpene, emission is function of light and temperature; decomposing of sabinene during storage to p-cymene, α-phellandrene, ß-phellandrene, α-terpinene, g-terpinene, terpinolene and α-thujene, following saturation trend, yielding 55% of initial sabinene after 7-day storage	Vol. 3CA, B.8.3.4/11 Dindorf et al. (2006)
Measurements of mixing ratios and fluxes in and above fir canopy	Douglas fir (*Pseudotsuga menziesii*) forest in Speulderbos, the Netherlands Summer	Douglas fir	Canopy scale, PTR-MS coupled with virtual disjunct eddy covariance, PTR-MS instrument fitted with turbopump connected to the detection chamber, GC-MS	Emission rates [**µg/g_{dw}/h**] Monoterpene 0.8 Isoprene 0.09 – 0.16	α-pinene dominant monoterpene, emissions temperature-dependent,	Vol. 3CA, B.8.3.4/12 Copeland et al. (2014)
Speciation of emitted compounds and relationship with presence of *Phyllaphis fagi* L.	Field measurements: Aelmoeseneie forest near Gent, Belgium Field: Summer, early autumn (May-October)	*Fagus sylvatica* L. branches at 9, 16, 22 and 24 m canopy height; branches from middle of *F. sylvatica* trees (3-4 ears) in growth chambers	Dynamic enclosure system (cuvettes), GC-MS	Emission rate [**µg/m^2/h**]: Total terpenoid: 54 (non-infected conditions) 127 (upon infection) Sabinene (without aphids): 44 α-farnesene (aphid infection): 26 Linalool (aphid infection): 30	18 compounds in BVOCs. 11 monoterpene (MT), 1 oxygenated MT, 1 homoterpene (C14H18), 3 sesquiterpene (SQT), isoprene and methyl salicylate; shift from MT to linalool, α-farnesene, (E)-ß-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) due to aphid infection; sabinene most prevalent compound	Vol. 3CA, B.8.3.4/17 Joó et al. (2010)
Measurement of α-pinene in *Pinus halepensis* and *Quercus ilex* simultaneous with leaf environmental conditions	Mediterranean conditions, potted plants in open plastic tunnel at campus of Universitat Auto-noma of Barcelona, Catalonia, Spain Summer	Leaves of Mediterranean conifer *Pinus halepensis*, *Quercus ilex*	Cuvette system, ADC gas exchange system	Emission rate [**µg/g/h**] α-pinene: *P. halepensis*: 1.3 – 19.8 *Q. ilex*: 0.11 – 14.7	Significant exponential correlation between α-pinene emission rates with leaf temperature and rates of photosynthetic electron transport; correlation between temperature of previous days at *P. halepensis* (monoterpene storing species)	Vol. 3CA, B.8.3.4/13 Blanch et al. (2011)

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Results of initial investigation into monoterpene emissions from soil, focusing on source strength and monoterpene profiles	Sitka spruce (*Picea sitchensis*) forest in the Forestry Commission Grizedale Forest Plantation, Cumbria, UK Summer	3 soil layers and leaves of *Picea sitchensis* forest	Dynamic soil enclosure and dynamic branch enclosure, GC, GC-FID	Emission rates: **Soil** [**µg/m²/h**]: Mean: 13.0 – 199 α-pinene: 3.52 – 62.7 ß-pinene: 1.18 – 17.1 Myrcene: 1.23 – 9.66 Limonene: 5.08 – 68.9 **Foliage** [**ng/g_{dw}/h**]: Mean: 625 α-pinene: 91.7 ß-pinene: 66.3 Myrcene: 104 Limonene: 274	Monoterpene composition and emission rates depend on soil layers Limonene > α-pinene > camphene > myrcene > ß-pinene > α-phellandrene > 3-carene > α-terpinene > cymene ≈ cineole	Vol. 3CA, B.8.3.4/18 Hayward et al. (2001)
Investigation of rates of sesquiterpene emission in lab	Laboratory experiment	Secondary fungal metabolites	Aseptic flow-through apparatus (solid phase microextraction), SPME, GC-MS	Rates of sesquiterpene emissions [**ng/g/h**] 1 – 62 (depending on fungal species)	Number of detected sesquiterpenes (12-49) and emission rates depend on fungal species	Vol. 3CA, B.8.3.4/19 Horváth et al. (2011)
Quantification of changes of VOCs of soil after clear-cut and burning of slash	100-year-old-Norway spruce (*Picea abies* L.) forest Summer	Soil (glacial till)	Manual steady-state chamber system, GC-MS	VOC emission rate [**ng/m²/min**] <u>Monoterpene</u>: before clear-cutting: ~500 after cutting: ~ 400-800 after burning: ~900 <u>Sesquiterpene</u>: before clear-cutting: 2 – 4 after cutting~ 6 – 14 after burning: ~ 16	Soil VOCs 100-fold after clear-cutting, but decreased after burning	Vol. 3CA, B.8.3.4/03 Kulmala et al. (2014)

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Screening of 18 species for emissions of isoprene, monoterpene and other VOCs	Forest site in Castelporziano nature reserve Summer, autumn	18 tree and shrub species	Bag-enclosure system, GC-FID, GC-MS	Estimated VOCs emissions [**g/8 h**], depending on species and season **Dunes:** α-pinene: 0.2 – 1151.0 ß-pinene: 0.1 – 501.0 Myrcene: 0.4 – 154.0 α-phellandrene: 0.1-11.0 α-terpinene: 38.0 *p*-cymene: 1.9 Limonene: 0.1 – 110.0 γ-terpinene: 0.1 – 6.4 α-terpineol: 0.1 – 1.0 **Macchia:** α-pinene: 2.3 – 18418.0 ß-pinene: 0.7 – 8017 Myrcene: 4.3 – 2471 α-phellandrene: 0.9 – 170.0 α-terpinene: 608.0 *p*-cymene: 2.0 Limonene: 1.3 – 1763 γ-terpinene: 2.3 – 65.0 α-terpineol:1.9 – 19.0 **Monoterpene emission rates** [**µg/g_{dw}/h**] 0.002 – 58.5	30 emitted compounds identified, isoprene far exceeding other compounds in forest scrubs, in canopy vegetation mainly α-pinene, sabinene, ß-pinene, linalool. Temperature sensitivity of monoterpene emissions varied between species, isoprene emissions strong temperature depended. VOC composition	Vol. 3CA, B.8.3.4/28 Owen et al. (1997)
Distinguish boreal peatland BVOCs, effects of water table drawdown in hollows	Ombrotrophic peatland in Turvesuo peatland, Suonenjoki, Central Finland Autumn (sampling), then stored at 1-2 °C in the dark, growth chamber conditions corresp. to summer	Vascular plants, mosses and peat in hummocks (dry microsites) and hollows (wet microsites) of boreal peatland	Boreal peatland microcosm maintained in growth chambers, conventional chamber method, collection on adsorbent, GC-MS	Emission rate [**µg/m²/h**]: Hummock: Monoterpene: ~ 0.25 – 2.7 Sesquiterpene: ~ 0.02 – 0.35 Hollow: Monoterpenes: ~0.1-2.4 Sesquiterpenes: ~0.1-1.8	Hummock: 44 compounds (4 mono-, 2 sesquiterpenes, 36 other reactive VOCs, 2 other VOCs) Hollow: 47 compounds (4 mono-, 3 sesquiterpenes, 38 ORVOCs, 2 other VOCs) Monoterpene emissions with table water drawdown	Vol. 3CA, B.8.3.4/29 Faubert et al. (2010).

Species / Experimental aim / setup	Ecosystem/ Season	VOC source	Method	Substances/ Emission rates	Further details	Reference
Determination of normalized species-specific BVOC emission rates, investigation of BVOC´s composition	14 different forest areas in Turkey Summer-autumn	Branches of 31 tree species	Dynamic enclosure system (Nalofan bag), GC-MS	Normalized emission rates [**µg/m²/h**] Isoprene: 1.17 – 2310 Monoterpene: 0.45 – 3754 Sesquiterpene: 0.11 – 60.2 Oxygenated sesquiterpene: <LOD – 2.3 Oxygenated compounds: 0.72 – 828 Total BVOC: 2.89 –4366	65 BVOCs in 5 major groups: isoprene, monoterpene, sesquiterpene, oxygenated sesquiterpene, other oxygenated compounds), emission rates and compositions depending on species	Vol. 3CA, B.8.3.4/04 Aydin et al. (2014)

2.8.3.1 Hazardous to the ozone layer

Not considered in CLH report (July 2018).

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Not considered in CLH report (July 2018).

2.8.3.1.2 Comparison with the CLP criteria

Not considered in CLH report (July 2018).

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not considered in CLH report (July 2018).

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No monitoring data were provided or required due to natural occurrence of all TTO constituents and their emission to the environment.

2.8.5 Definition of the residues in the environment requiring further assessment

TTO and its constituents are included in definition of the residues in the environment requiring further assessment.

2.8.6 Summary of exposure calculations and product assessment

Soil exposure

The soil exposure was calculated for the worst case EU GAP comprising of 4 applications at 445 g a.s./ha with 7 days interval, covering also uses at lower application rate (4 x 334 g a.s./ha), longer interval between applications (10 days) and lower number of applications (3). Performed calculations covered also glasshouse uses of BM 608 to tomatoes.
Summary of the input parameters for selected TTO constituents is provided in table below.

Table 2.8.6-1: Summary of input parameters for selected TTO compounds considered in the soil exposure calculation following application of BM 608

Parameter	Terpinene-4-ol	γ-Terpinene	p-Cymene	1,8-Cineole	(+)-Aroma-dendrene	Globulol/ Viridiflorol
Molecular weight [g/mol]	154.3	138.3	134.2	154.3	204.4	222.4
max actual DT_{50} in soil [d]	0.42 [1)]	0.15	0.53	0.68	7.75	**10.9**
max actual DT_{50} in soil [hours]	10 [1)]	3.56	12.8	16.3	186	262
Mean occurrence in TTO [%] [2)]	44.25	18.61	1.51	4.74	0.23	0.03

[1)] Data for 1 soil only
[2)] According to the 5-batch analysis
Value in **bold** has been selected for calculation of the short- and long-term soil exposure as representing worst case

Calculations were performed using ESCAPE ver. 2 program, which for calculation of PEC_{soil} for the active compound without metabolites directly implements the FOCUS recommendations. The soil density of 1.5 g/cm^3 and the soil depth was set to 5 cm. Due to soil DT_{90} <365 days for all compounds, no accumulation is expected and for this reason plateau concentration and resulting $PEC_{soil, accu}$ were not calculated as not necessary.

Results obtained for assumptions provided above are presented in the following table.

Table 2.8.6-2: Predicted soil exposure following application of TTO in BM 608 according to the worst case use pattern

$PEC_{(s)}$ [mg/kg]		**Single application Actual**	**Single application Time weighted average**	**Multiple application Actual**	**Multiple application Time weighted average**
Initial		0.2967		0.6866	
Short term	24h	0.2784	0.2875	0.6443	0.6654
	2d	0.2612	0.2787	0.6046	0.6449
	4d	0.2300	0.2620	0.5324	0.6067
Long term	7d	0.1901	0.2395	0.4399	0.5681
	28d	0.0500	0.1386	0.1157	0.4610
	50d	0.0123	0.0895	0.0286	0.3481
	100d	0.0005	0.0466	0.0012	0.1904
Plateau concentration		Not relevant, no accumulation expected			

Since acceptable risk for soil organisms could be concluded for the exposure calculated above, no further calculations are deemed necessary.

In addition to the calculated above soil exposure, also information on natural background concentrations of some TTO constituents is available from the literature data. Summary of available information is provided below.

Table 2.8.6-3: Literature data on background level of terpene components in soil

Substance	**Study/endpoint type**	**Results**	**Reference**
Soil			
Monoterpenes	Concentration in upper soil horizons under monocultures of *Picea abies*, *Picea sitchensis* and *Pinus sylvestris* in UK	9.1 – 67.6 µg/g dw	Vol. 3CA, B.8.1.1.5/10 Ludley et al. (2009)
	Distribution in soil horizons around *Pinus halepensis* (California)	Range over mineral and organic horizons from 3 replicates (pentane extracts): 0 m from tree: 1.4 – 5740 µg/g ww 3 m from tree: 0.5 – 4666 µg/g ww 10 m from tree: 0.0 – 1043 µg/g ww	Vol. 3CA, B.8.1.5/02 Asensio et al. (2008)
α-Pinene	Concentration in different soil horizons under Norway Spruce	Organic horizons: 0.01 – 1.1 µmol/g dw Mineral horizons: < 0.01 µmol/g dw	Vol. 3CA, B.8.1.5/01 Maurer et al. (2008)
	Distribution in soil horizons around *Pinus halepensis* (California)	Range over mineral and organic horizons from 3 replicates (pentane extracts): 0 m from tree: 1.4 – 1094 µg/g ww 3 m from tree: 0.1 – 2845 µg/g ww 10 m from tree: 0.0 – 61 µg/g ww	Vol. 3CA, B.8.1.5/02 Asensio et al. (2008)
Limonene	Concentration in different soil horizons under Norway Spruce	Organic horizons: < 0.01 – 0.7 µmol/g dw Mineral horizons: < 0.01 µmol/g dw	Vol. 3CA, B.8.1.5/01 Maurer et al. (2008)
Sabinene	Distribution in soil horizons around *Pinus halepensis* (California)	Range over mineral and organic horizons from 3 replicates (pentane extracts): 0 m from tree: 0.0 – 224 µg/g ww 3 m from tree: 0.0 – 160 µg/g ww 10 m from tree: 0.0 – 24.8 µg/g ww	Vol. 3CA, B.8.1.5/02 Asensio et al. (2008)

Based on the available literature data it may be expected that the natural concentrations of TTO constituents in soil will exceed concentrations predicted after application of BM 608 according to the EU GAP.

Groundwater exposure

The groundwater modelling has been performed using FOCUS PEARL 4.4.4, FOCUS PELMO 5.5.3 and FOCUS MACRO 5.5.4 with consideration of the worst case GAP for each crop (i.e. 4 applications to tomatoes and vineyards at 445 g a.s./ha with 7 days interval) as covering also uses at lower application rate (4 x 334 g a.s./ha), longer interval between applications (10 days) and lower number of applications (3).
Although input parameters were available for selected main compounds of TTO, the RMS decided to perform the groundwater modelling for the single "virtual compound" constructed with assumption of the worst case input parameters derived for particular compounds and the application rate of the whole active substance attributed to this "virtual compound". In case PEC_{gw} values for these assumptions are <0.1 µg/L, no further calculations are deemed necessary and the groundwater exposure is considered to be covered for all compounds.

Summary of the selected worst case input parameters for the "virtual compound" is presented in table below.

Table 2.8.6-4: Summary of the worst case input parameters selected for the virtual compound, covering all TTO constituents

Parameter	TTO [1)]	Remarks
Molecular weight [g/mol]	222.4	Maximum molecular weight agreed for Globulol
Water solubility [mg/L]	3280 (20°C)	Maximum molecular weight agreed for Terpinene-4-ol
Saturated vapour pressure [Pa]	0.00495 (25°C)	Lowest vapour pressure agreed for globulol
K_{OC} [mL/g]	59 112201	Lowest Koc agreed for 1,8-Cineole, used in PEARL and PELMO Highest Koc agreed for Aromadendrene, used in MACRO
K_{OM} (K_{OC}/1.724) [mL/g]	34 65082	Calculated from K_{OC}
Freundlich sorption exponent (1/n)	1	Default
Soil DT_{50} [days]	7.75	Longest geometric mean normalised to 20ºC and pF 2 from lab studies agreed for Globulol
Mean occurrence in TTO [%]	100%	Worst case assumption

[1)] Virtual active substance based on worst case input parameters derived for particular compounds

Summary of the FOCUS default input parameters relevant for groundwater modelling is provided in table below.

Table 2.8.6-5: Summary of default FOCUS input parameters relevant for groundwater modelling

Parameter	Value	Remarks
Molar enthalpy of dissolution [kJ mol^{-1}]	27	FOCUS recommendation
Molar enthalpy of vaporization [kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	4.3 x 10^{-5} m^2 d^{-1} (20°C) (Pearl) 5.0 x 10^{-10} m^2 s^{-1} (20°C) (Macro)	FOCUS recommendation
Diffusion coefficient in gas [m^2 d^{-1}]	0.43 (20°C)	FOCUS recommendation
Temperature correction function Reference temperature [°C] MACRO: [K^{-1}] PRZM: Q_{10}	 20 0.095 2.58	 FOCUS recommendation EFSA recommendation EFSA recommendation
Moisture correction function Reference moisture PRZM / MACRO: moisture exponent Reference concentration in liquid phase [mg L^{-1}]	 pF 2 0.7 65.4	 FOCUS recommendation
Plant uptake	0	FOCUS default for Tier 1

Results obtained for assumptions provided above are presented in the following table.

Table 2.8.6-6: Predicted groundwater exposure following application of BM 608 according to the EU GAP (worst case application pattern for each crop)

FOCUS scenario	PECgw (µg/L)	
	Tomatoes (4 x 445 g a.s./ha, 7 d interval)	Vineyards (4 x 445 g a.s./ha, 7 d interval)
	TTO	TTO
FOCUS PEARL 4.4.4		
Châteaudun	0.0088	0.0126
Hamburg	-	0.0271
Jokioinen	-	-
Kremsmünster	-	0.0248
Okehampton	-	-
Piacenza	0.0024	0.0298
Porto	0.0006	0.0480
Sevilla	0.0001	0.0012
Thiva	0.0002	0.0004
FOCUS PELMO 5.5.3		
Châteaudun	0.004	0.019
Hamburg	-	0.027
Jokioinen	-	-
Kremsmünster	-	0.032
Okehampton	-	-
Piacenza	0.008	0.035
Porto	0.001	0.007
Sevilla	<0.001	0.001
Thiva	<0.001	0.001
FOCUS MACRO 5.5.4		
Châteaudun	<0.001	<0.001

"-" scenario not defined for the given crop

PEC_{gw} calculated using all modelling programs for the worst case "virtual compound" are below the threshold concentration of 0.1 µg/L in all relevant scenarios for both crops. Taking this into account no unacceptable leaching of TTO constituents following application of BM 608 in line with the EU GAP is expected and no further calculations for particular compounds are deemed necessary.

In opinion of the RMS, groundwater modelling performed for these worst case assumptions and field applications of BM 608 covers also intended glasshouse uses to tomatoes.

Surface water exposure

The surface water exposure has been calculated by the Applicant in Anonymous (2018c, Doc. No STK-2018-03) at Steps 1-4 for the intended uses of BM 608 presented in table below.

Table 2.8.6-7: Uses of BM 608 considered in surface water modelling

Crop	Growth stage	Application method	Number of applications	Application interval [days]	Application rate per treatment [g a.s./ha]
Tomato (field)	BBCH 10-99	Overall foliar spraying	3	7	445
Vineyard	BBCH 10-99	Overall foliar spraying	4	7	445
Tomato (glasshouse)	BBCH 10-99	Overall foliar spraying	4	7	445

The maximum application rate assumed in surface water modelling covers also application at lower rate (3 x 334 g a.s./ha) intended in all considered crops.

For the calculation of the PEC_{SW} and PEC_{SED} the component Terpinene-4-ol was chosen as lead component and used as surrogate for all components of the active substance, Tea Tree oil. The maximum use rate was assigned to

this lead component and the PEC calculations according to FOCUS were performed with the input parameters summarized in the tables below.

Table 2.8.6-8: Endpoints for the lead component of Tea Tree Oil, Terpinene-4-ol

Parameter		Value	Remarks
Molecular Mass	[g/mol]	154.25	As agreed in phys-chem section
Water solubility	[mg/L]	3280 (20°C)	As agreed in phys-chem section
Saturated vapour pressure	[Pa]	14.9 (20°C)	As agreed in phys-chem section
K_{OC}	[mL/g]	89.1	Determined using HPLC method
$K_{OM:}$		51.7	[K_{OC}/1.724]
Freundlich sorption exponent (1/n)		1	Default value
DT_{50} soil	[days]	0.3	Worst case lab studies (n = 1)
DT_{50} water (used for Step 3 + 4)	[days]	4.92	FOCUS recommendation, ascribe the whole system DT_{50} to the water phase for compounds with a K_{OC} < 100 mL/g
DT_{50} sediment (used for Step 3 + 4)	[days]	1000	FOCUS recommendation, default value
DT_{50} w/s system	[days]	4.92	Geometric mean from two systems (river, pond)
DT_{50} crop	[days]	10	FOCUS recommendation
% in TTO		44.25	Mean from 5-batch study
Plant uptake		0	Worst case default

Table 2.8.6-9: Summary of default input parameters for PEC_{SW} and PEC_{SED} calculations according to FOCUS

Parameter		Value	Remarks
Entry routes into surface water		Spray drift Volatilisation Runoff Drainage	-
Degradation in soil			
Temperature correction function Reference temperature MACRO: PRZM: Q_{10}	 [°C] [1/K] [-]	 20 0.0948 2.58	 FOCUS recommendation FOCUS recommendation FOCUS recommendation
Moisture correction function Reference moisture PRZM/MACRO moisture exponent	 [-] [-]	 pF 2 0.7	 FOCUS recommendation
Degradation in aquatic systems			
Temperature correction function Reference temperature TOXSWA: activation energy	 [°C] [J/mol]	 20 65 400	 FOCUS recommendation EFSA recommendation
Wash off coefficient PRZM: MACRO:	 [1/cm] [1/mm]	 0.5 0.05	 FOCUS recommendation

Simulations were performed using following FOCUS models: FOCUS Step 1&2 ver. 2, SWASH ver. 3.2, MACRO ver. 5.5.4, PRZM ver. 4.3.1, TOXWA ver. 4.4.3 and SWAN ver. 4.0.1.

Table 2.8.6-10: Application settings for FOCUS Step 3

Application on vines	
Application method:	Air blast
Chemical application method:	CAM 2
Incorporation depth [cm]:	4
Application on tomatoes	
Application method:	Ground spray
Chemical application method:	CAM 2
Incorporation depth [cm]:	4

Since in both crops TTO is intended to be applied during the whole season (BBCH 10-99), 3 application scenarios has been assumed:

1. Vineyards: early application 20 days after emergence, early application 90 days after emergence, late application 40 days before harvest.
2. Tomatoes (field): application 20, 50 and 80 days after emergence.

In order to account for volatilisation of Terpinene-4-ol, deposition rates were calculated with EVA 3 tool and implemented into the modelling. Obtained results are provided below.

Table 2.8.6-11: Step 1 and Step 2 surface water exposure (vineyards, 4x445 g a.s./ha, 7 days interval)

FOCUS STEP 1	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
Scenario	**Actual**	**Actual**
Early applications	546.34	472.52
Late applications	577.96	472.52
FOCUS STEP 2	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
Scenario	**Actual**	**Actual**
Southern EU (early)	5.85 (4.00)	3.26 (2.12)
Southern EU (late)	15.52 (11.91)	8.65 (6.32)

Values in **brackets** indicate exposure after single application

Table 2.8.6-12: Step 3 surface water exposure (vineyards, 4x445 g a.s./ha, 7 days interval)

Timing of application	**FOCUS STEP 3 Scenario**	**Waterbody**	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
			Actual	**Actual**
Single applications				
Early, 20 days after emergence	D6	ditch	3.963	1.016
	R1	pond	0.4368	0.1579
	R1	stream	2.614	0.3341
	R2	stream	3.177	0.1725
	R3	stream	3.537	0.4124
	R4	stream	2.429	0.1537
Early, 90 days after emergence	D6	ditch	3.851	1.071
	R1	pond	0.4330	0.1511
	R1	stream	2.630	0.2177
	R2	stream	3.204	0.1784
	R3	stream	3.392	0.3903
	R4	stream	2.575	0.2117
Late, 40 days before harvest	D6	ditch	8.178	3.918
	R1	pond	0.6267	0.4952
	R1	stream	6.346	0.2362
	R2	stream	8.238	0.1599
	R3	stream	8.517	0.5706
	R4	stream	6.211	0.4941
Multiple applications				
Early, 20 days after emergence	D6	ditch	3.928	1.421
	R1	pond	0.5237	0.4444
	R1	stream	2.418	0.2913
	R2	stream	2.897	0.1954
	R3	stream	3.324	0.4767
	R4	stream	2.225	0.2876
Early, 20 days after emergence	D6	ditch	3.897	2.311
	R1	pond	0.5320	0.3864
	R1	stream	2.439	0.2523
	R2	stream	2.923	0.2186
	R3	stream	3.160	0.5624
	R4	stream	2.394	0.2969
Early, 20 days after emergence	D6	ditch	7.536	4.389
	R1	pond	1.101	0.8653
	R1	stream	5.370	0.5661
	R2	stream	6.905	0.4445
	R3	stream	7.218	1.254
	R4	stream	5.262	0.5857

Table 2.8.6-13: Step 1 and Step 2 surface water exposure (tomatoes, 3x445 g a.s./ha, 7 days interval)

FOCUS STEP 1	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
Scenario	**Actual**	**Actual**
Early applications	410.03	354.39
Late applications	410.03	354.09
FOCUS STEP 2	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
Scenario	**Actual**	**Actual**
Southern EU (early)	4.56 (4.09)	2.53 (2.17)
Southern EU (late)	4.56 (4.09)	2.53 (2.17)

Values in **brackets** indicate exposure after single application

Table 2.8.6-14: Step 3 surface water exposure (tomatoes, 3x445 g a.s./ha, 7 days interval)

Timing of application	**FOCUS STEP 3 Scenario**	**Water body**	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg)**
			Actual	**Actual**
Single applications				
Early, 20 days after emergence	D6	ditch	2.787	0.3425
	R2	stream	2.832	0.1653
	R3	stream	2.818	0.3178
	R4	stream	2.707	0.5335
Early, 90 days after emergence	D6	ditch	2.796	0.3734
	R2	stream	2.833	0.1513
	R3	stream	2.818	0.3178
	R4	stream	2.157	0.1756
Late, 40 days before harvest	D6	ditch	2.804	0.3983
	R2	stream	2.830	0.1502
	R3	stream	2.883	0.3267
	R4	stream	2.171	0.1772
Multiple applications				
Early, 20 days after emergence	D6	ditch	2.058	0.4380
	R2	stream	2.016	0.2166
	R3	stream	2.130	0.3487
	R4	stream	1.937	0.5208
Early, 20 days after emergence	D6	ditch	1.993	0.3648
	R2	stream	2.021	0.7532
	R3	stream	2.078	0.3578
	R4	stream	2.354	0.4668
Early, 20 days after emergence	D6	ditch	2.027	0.3852
	R2	stream	2.011	0.1548
	R3	stream	2.110	0.4943
	R4	stream	1.581	0.1771

It is, however, noted that RMS has concerns regarding soil DT_{50} for Terpinene-4-ol since the rate of degradation of this compound was determined in only one soil while according to the data requirements as set in Commission Regulation (EU) No 283/2013, the rate of degradation for the active substance must be investigated in at least 4 soils. This requirement would be applicable also for Terpinene-4-ol, considered to be the lead component of TTO, especially for other components present in TTO at lower concentrations (γ-Terpinene, p-Cymene, 1,8-Cineole, Aromadendrene and Globulol) soil degradation was investigated in the sufficient number of soils.

It is further noted that the DT_{50} values of other tested compounds were clearly shorter in LUFA 2.4 soil comparing to other soils (LUFA 2.1, LUFA 2.2 and LUFA 6S). Therefore it may be expected that DT_{50} of Terpinene-4-ol in these other soils would be also longer than this derived in LUFA 2.4 soil.

Due to indicated above concerns, more detailed discussion with Member States and EFSA during the peer-review is necessary in order to decide on the most relevant input parameters and approach to be taken in estimation of the surface water exposure following application of BM 608 before new simulations are performed.

For the surface water risk assessment for greenhouse uses, some drift from the greenhouse towards a static water body has to be taken into account. Following the "Dutch Model", the maximum instantaneous PEC_{sw} value was calculated from entry through spray drift that occurred immediately after the last application, considering a drift of 0.5 % of the application rate from the glasshouse. With the depth of the static water body assumed as 30 cm and the single application rates of TTO of 445 g a.s./ha, the resulting maximum instantaneous **PEC_{sw}** value is **0.0074 µg a.s./L**.

In addition to the calculated above surface water exposure, also information on natural background concentrations of some TTO constituents is available from the literature data. Summary of available information is provided below.

Table 2.8.6-15: Literature data on background level of terpene components in soil, water and sediment

Substance	Study/endpoint type	Results	Reference
Water			
p-Cymene	Residues in water	6.4 % after 2 d 0.01 % after 7 d Test concentration: 0.1 mg/ml	Vol. 3CA, B.8.2.4/03 Park et al (2011)
	Concentration in estuarine water (UK)	35-45 ng/L	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)
Limonene	Residues in water	12.0 % after 2 d 0.14 % after 7 d Test concentration: 0.1 mg/ml	Vol. 3CA, B.8.2.4/03 Park et al (2011)
	Concentration in sea water (Alaska)	0.47 – 84 ng/L Large difference explained by input from terrestrial runoff after heavy rainfall events.	Vol. 3CA, B.8.2.4/02 Button & Jüttner (1989)
	Concentration in estuarine water (UK)	25-633 ng/L	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)
γ-Terpinene	Residues in water	2.37 % after 2 d Test concentration: 0.1 mg/ml	Vol. 3CA, B.8.2.4/03 Park et al (2011)
α-Terpinene	Residues in water	3.48 % after 2 d 0.10 % after 7 d Test concentration: 0.1 mg/ml	Vol. 3CA, B.8.2.4/03 Park et al (2011)
α-Pinene	Concentration in sea water (Alaska)	0.71 – 8840 ng/L Large difference explained by input from terrestrial runoff after heavy rainfall events.	Vol. 3CA, B.8.2.4/02 Button & Jüttner (1989)
	Concentration in estuarine water (UK)	25 - 412 ng/L	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)
Sabinene	Concentration in sea water (Alaska)	0.08 – 704 ng/L Large difference explained by input from terrestrial runoff after heavy rainfall events.	Vol. 3CA, B.8.2.4/02 Button & Jüttner (1989)
1,8-Cineole	Concentration in sea water (Alaska)	3.4 ng/L Large difference explained by input from terrestrial runoff after heavy rainfall events.	Vol. 3CA, B.8.2.4/02 Button & Jüttner (1989)
Sediment			
Monoterpenes	Marine sediments	0.5 mg/kg dw	Vol. 3CA, B.8.2.4/05 Sporstøl et al. (1983)
Sesquiterpenes	Marine sediments	0.3 mg/kg dw	Vol. 3CA, B.8.2.4/05 Sporstøl et al. (1983)
p-Cymene	Marine sediments	1.4 mg/kg dw	Vol. 3CA, B.8.2.4/05 Sporstøl et al. (1983)
	Concentration in estuarine sediment	200 – 350 ng/kg dw	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)
α-Pinene	Concentration in estuarine sediment	150 – 506 ng/kg dw	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)
	Concentration in wetland sediment	2.46 – 2.64 ng/g dw	Vol. 3CA, B.8.2.4/01 Palma-Fleming (2013)
Limonene	Concentration in estuarine sediment	105 – 807 ng/kg dw	Vol. 3CA, B.8.2.4/04 Bianchi et al (1991)

It is not possible to compare natural concentrations of TTO constituents provided in table above with the exposure expected after application of BM 608 according to the EU GAP, since FOCUS modelling was performed for Terpinene-4-ol, the main constituent of TTO and not for each compound separately. Nevertheless it may be concluded that available literature data indicate that TTO constituents are present in natural water bodies at considerable concentrations.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Summary of toxicity data for birds and mammals is presented in tables below.

Table 2.9.1-1: Toxicity of TTO to birds

Study type	Test substance	Species	Endpoint		Reference
Acute oral toxicity	BM 608 (Timorex Gold)	Japanese quail *(Coturnix coturnix japonica)*	LD_{50} (female)	>2000 mg product/kg bw (>472 mg a.s./kg bw)	Vol. 3CP, B.9.1.1/01 [redacted] (2008) Rep. No 22875
			Extrapolated	3228 mg product/kg bw/d (761.8 mg a.s./kg bw)	
	Timorex 66% EC	Japanese quail *(Coturnix coturnix japonica)*	LD_{50} (male)	>2000 mg product/kg bw (>1320 mg a.s./kg bw)	Vol. 3CP, B.9.1.1/02 [redacted] (2005a) Rep. No BI-6159/05
			Extrapolated	**3776 mg product/kg bw/d (2492 mg a.s./kg bw)**	
Long-term toxicity and reproduction	TTO	Japanese quail *(Coturnix coturnix japonica)*	**NOEL**	**100.0 mg a.s./kg bw/d**	Vol. CA, B.9.1.1.3/01 [redacted] Rep. No 34280

[1)] Extrapolation factor of 1.614 applied as 5 birds were tested with no mortality observed
[2)] Extrapolation factor of 1.888 applied as 10 birds were tested with no mortality observed
Values in **bold** were used in the risk assessment

Table 2.9.1-2: Toxicity of TTO to mammals

Study type	Test substance	Species	Endpoint		Reference
Acute oral toxicity	TTO	Rat	**LD_{50}**	**1049 mg a.s./kg bw**	Vol. 3CA, B.6.2.1/01 [redacted] (2015) Rep. No G9945
	TTO	Rat	LD_{50}	1682-1721 mg a.s./kg bw	Vol. 3CA, B.6.2.1/05 [redacted] (1989) Rep. No 400050
	BM 608 (Timorex Gold)	Rat	LD_{50}	>2000 mg product/kg bw (>476 mg a.s./kg bw)	Vol. 3CP, B.6.1.1/01 [redacted] (2007) Rep. No BIM/016/AOT
28-days, gavage	TTO	Rat	NOAEL	62.5 mg a.s./kg be/d	Vol. CA, B.6.3.1/01 [redacted] (2010) Rep. No N896
90-days, feeding	TTO	Rat	NOAEL (males)	30 mg a.s./kg bw/d	Vol. CA, B.6.3.2/01 [redacted] (2011) Rep. No G7153
			NOAEL (females)	60 mg a.s./kg bw/d	
2-generation reproduction study	TTO	Rat	**NOAEL (reproduction)**	**25 mg a.s./kg bw/d**	Vol. 3CA, B.6.6.1/01 [redacted] (2017) Rep. No G11090
			NOAEL (offspring)	25 mg a.s./kg bw/d	
Developmental toxicity	TTO	Rat	NOAEL (maternal)	30 mg a.s./kg bw/d	Vol. 3CA, B.6.6.2/01 [redacted] (2012) Rep. No G8373
			NOAEL (foetal)	60 mg a.s./kg bw/d	
Developmental toxicity	TTO	Rabbit	NOAEL (maternal)	75 mg a.s./kg bw/d	Vol. 3CA, B.6.6.2/02 [redacted] (2018) Rep. No G12442
			NOAEL (foetal)	30 mg a.s./kg bw/d	
			NOAEL (teratogenicity)	75 mg a.s./kg bw/d	

Values in **bold** were used in the risk assessment

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

Summary of toxicity data for aquatic organisms is presented in table below.

Table 2.9.2-1: Toxicity of TTO and BM 608 to aquatic organisms

Group	Test substance	Time-scale (Test type)	Endpoint	Toxicity [1)]	Reference
Laboratory tests					
Fish					
Oncorhynchus mykiss	TTO	Acute 96 hr semi-static	Mortality, LC_{50}	>2.85 mg a.s./L (mm)	Vol. 3CA, B.9.2.1//01 [redacted] (2015) Rep. No FAR16154
Oncorhynchus mykiss	BM 608	Acute 96 hr semi-static	Mortality, LC_{50}	2.66 mg product/L (mm) **(0.63 mg a.s./L)**	Vol. 3CP, B.9.3.1.1/02 [redacted] (2009a) Rep. No 080218BB
Pimephales promelas	TTO	28 day ELS flow-through	NOEC (Fry survival, swim-up and morphological / behavioural effects)	**0.244 mg a.s./L (mm)**	Vol. 3CA, B.9.2.2/01 [redacted] (2017a) Rep. No FSF16943
Aquatic invertebrates					
Daphnia magna	TTO	Acute 48 hr static	Immobilisation, EC_{50}	0.591 mg a.s./L (nom)	Vol. 3CA, B.9.2.4/01 Noack (2011) Rep. No DAI13977
Daphnia magna	BM 608	Acute 48 hr static	Immobilisation, EC_{50}	0.68 mg product/L (mm) **(0.16 mg a.s./L)**	Vol. 3CP, B.9.3.1.2/01 Scheerbaum (2009b) Rep. No 080218BB
Daphnia magna	TTO	21 day semi-static	NOEC (mortality, reproduction) EC_{10} (reproduction rate)	**0.303 mg a.s./L (mm)** Not reliable	Vol. 3CA, B.9.2.5/01 Scheerbaum (2017b) Rep. No DRE16943
Sediment-dwelling organisms					
Chironomus riparius	TTO	28 d static spiked water	NOEC (development) NOEC (emergence)	**4.36 mg a.s./L (imm)** 9.56 mg a.s./L (imm)	Vol. 3CA, B.9.2.5/02 Scheerbaum (2017c) Rep. No IZW16943

Group	Test substance	Time-scale (Test type)	Endpoint	Toxicity [1)]	Reference
Algae					
Raphidocelis subcapitata (formerly *Pseudokirchneriella subcapitata*)	TTO	72 hr static	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	2.17 mg a.s./L (mm) 1.96 mg a.s./L (mm) 1.83 mg a.s./L (mm) 1.26 mg a.s./L (mm) 1.76 mg a.s./L (mm) 1.54 mg a.s./L (mm) <0.91 mg a.s./L (mm)2 0.912 mg a.s./L (mm)	Vol. 3CA, B.9.2.6/01 Scheerbaum (2017d) Rep. No SPO16943
Desmodesmus subspicatus	BM 608	72 hr static	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	3.27 mg product/L (mm) **(0.77 mg a.s./L)** 2.89 mg product/L (mm) (0.68 mg a.s./L) 2.71 mg product/L (mm) (0.64 mg a.s./L) 1.07 mg product/L (mm) (0.25 mg a.s./L) 2.40 mg product/L (mm) (0.57 mg a.s./L) 2.06 mg product/L (mm) (0.49 mg a.s./L) 1.91 mg product/L (mm) (0.45 mg a.s./L) 1.07 mg product/L (mm) (0.25 mg a.s./L)	Vol. 3CP, B.9.3.1.3/01 Scheerbaum (2009c) Rep. No 080218BB

Group	Test substance	Time-scale (Test type)	Endpoint	Toxicity [1)]	Reference
Higher plant					
Lemna gibba	TTO	7 day semi-static	<u>Frond number</u> E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC <u>Dry weight</u> E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	 **10.3 mg a.s./L (mm)** 9.38 mg a.s./L (mm) 8.88 mg a.s./L (mm) 4.63 mg a.s./L (mm) 10.0 mg a.s./L (mm) 8.65 mg a.s./L (mm) 7.87 mg a.s./L (mm) 4.63 mg a.s./L (mm) 10.3 mg a.s./L (mm) 9.09 mg a.s./L (mm) 8.34 mg a.s./L (mm) 1.91 mg a.s./L (mm) 10.6 mg a.s./L (mm) 5.81 mg a.s./L (mm) 3.49 mg a.s./L (mm) 1.91 mg a.s./L (mm)	Vol. 3CA, B.9.2.7/01 Scheerbaum (2017e) Rep. No SLG16943

nom: nominal; mm: mean measured; imm: initial mean measured
Values in **bold** were used in the risk assessment

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

<u>The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.</u>

Table 2.9.2.1: Summary of relevant information on bioaccumulation

Method	Results	Reference
Measured	Terpinen-4-ol Log P_{OW} = 2.643 at 23.5°C and pH of 5.85.	Parsons, A.H. 2007
Experimental and estimated values	See nested table below	Li, J. Perdure, E.M. et al., 1998 Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. 1980 Griffin, S.; Wyllie, S.G. & Markham, J., 1999

Components	BCF	Log Pow
Terpinen-4-ol	66**	2.80[3] ;
α-Pinene	395**	4.83[1]; 4.48[3]
Limonene	360**	4.57[1]; 4.38[3]
γ-Terpinene	433**	4.50[1]; 4.36[3]
Terpinolene	296**	4.47[1]; 4.24[3]
α-Terpineol	68**	2.98[1]; 3.28[3]
p-Cymene	236**	6.34[2]; 4.10*
α-Terpinene	433**	4.25[3]
1,8-Cineole	30**	2.74[3]

Method	Results	Reference
	Aromadendrene 5129** 6.13** δ-Cadinene 6838** 6.32** Sabinene 577** 4.69** Globulol 529** 4.63** Viridiflorol 529** 4.63** Ledene 5543** 6.18** [1] Experimental value from Li & Perdue (1998) [2] Experimental value from Banerjee *et. al* 1980 [3] Experimental value from Griffin *et al.* 1999 *From Episuite v4.11, experimental database match ** From Episuite v4.11, estimated	

2.9.2.1.1 Estimated bioaccumulation

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Estimated BCF is < 500 for all monoterpene components, which account on average for > 95% of Tea Tree Oil. For the sesquiterpenes, BCF > 500 has been estimated, however, for the majority of these below 600, i.e. close to the trigger of 500. The sesquiterpene content of Tea tree oil is traces to max. 3.5% (individually), and cumulatively usually < 5%. Cumulative content of components with BCF > 600 (Cadinene, Aromadendrene and Ledene, BCF range 5000-7000) usually is below 2%.
Overall, Tea Tree Oil is considered to be not bioaccumulative.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

The experimental $logP_{OW}$ of Terpinen-4-ol, the main and representative component of Tea Tree Oil amounts to 2.643 at 23.5° and pH 5.85 and thus does not exceed the trigger value of 4 ($logP_{OW}$ < 4).
There are no bioaccumulation test data available.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Table 2.9.2.2-1: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results [mg as/L]	Remarks	Reliability score	Reference
Acute toxicity to fish OECD 203, GLP Semi static, 96 h	*Oncorhynchus mykiss*	Tea Tree Oil	LC_{50} = 7.45	measured	1	Anonymous (35) (2015)
Acute toxicity to fish OECD 203 96 h semi-static	*Brachydanio rerio*	Tea Tree Oil	LC_{50} > 100 NOEC = 100	nominal	3	Anonymous (36) (1999)
Literature study 96 h static Fish toxicity test	*Oncorhynchus mykiss*	α-Terpineol	LC_{50} = 6.6	measured	3	Stroh, J. et al (1998)
	Oncorhynchus kisutch	α-Terpineol	LC_{50} = 6.3	measured	3	Stroh, J. et al (1998)
Literature study US EPA 660/3-75-009 (1975) guideline	*Cyprinodon variegatus*	p-Cymene	LC_{50} = 48 NOEC = 10	measured	3	Heitmuller, P.T. et al (1981)

96 h static						
Acute toxicity to daphnia OECD 202, GLP Semi-static, 48 h	*Daphnia magna*	Tea Tree Oil	EC_{50} = 0.591 NOEC = 0.106	measured	1	Noack, M. (2011)
Literature study Acute toxicity to daphnia US EPA 660/3-75-009 guideline Static, 48 h	*Daphnia magna*	p-Cymene	EC_{50} = 6.5 NOEC < 4.6	nominal	3	LeBlanc, G.A. (1980)
	Daphnia magna	α-Pinene	LC_{50} = 41 NOEC = 8.8	nominal	3	LeBlanc, G.A. (1980)
Algae growth inhibition test OECD 201, GLP	*Pseudokirchneriella subcapitata*	Static, 72 h Tea Tree Oil	E_yC_{50} = 1.76 E_rC_{50} = 2.17	measured	1	Scheerbaum, D. (2017)
Aquatic plant toxicity test OECD 221, GLP	*Lemna gibba*	Semi-static, 7 d Tea Tree Oil	E_rC_{50} = 10.3 E_yC_{50} = 10.0	measured	1	Scheerbaum, D. (2017)

2.9.2.2.1 Acute (short-term) toxicity to fish

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Two acute toxicity studies on Tea Tree Oil showed short-term (96 hour) acute toxicity to fish in *Oncorhynchus mykiss* and *Brachydanio rerio*. 96 hour LC_{50} values were 7.45 mg as/L in *Oncorhynchus mykiss* and > 100 mg as/L in *Brachydanio rerio*.
Two studies on the component of Tea Tree Oil α-Terpineol on *Oncorhynchus mykiss* and *Oncorhynchus kisutch* lead to 96 hour LC_{50} values of 6.6 and 6.3 mg as/L, respectively.
One study on *Cyprinodon variegatus* with the component of Tea Tree Oil p-Cymene lead to a 96h LC_{50} value of 48 mg as/L.

Study 1: Anonymous 2015 (35), Extract from Tea Tree (Tee Tree Oil). Fish (Rainbow trout), acute toxicity test, semi-static, 96 h, OECD 203, GLP

Reliability statement: The study was performed in line with recommendation of OECD 203 with no major deviations. The validity criteria regarding oxygen concentration, environmental conditions and mortality in control groups were fulfilled and the study is considered acceptable (reliability score: 1).
The acute toxicity of Tea Tree Oil to Rainbow trout was determined in fresh water at 14°C using a semi-static 96 h test system at nominal concentrations of 1.71, 3.76, 8.26, 18.2 and 40.0 mg TTO/L.
Records of mortality and sublethal effects of exposure were made at 24, 48, 72 and 96 hours after the start of the exposure.
No mortality occurred at the three lowest test concentrations during the whole period of exposure of 96 h. A 100 % mortality was recorded at the highest tested concentration of 40 mg TTO/L.
The 96-hour LC_{50} for the rainbow trout was 7.45 mg TTO/L (95 % confidence limits: 3.85 to 7.64 mg/L), based on geometric mean measured concentrations.

Nominal concentration of Tea Tree Oil [mg/L]	**Cumulative Mortality [%]**				
	2 h	**24 h**	**48 h**	**72 h**	**96 h**
40	0	100	100	100	100
18.2	0	14	57	71	71
8.26	0	0	0	0	0
3.76	0	0	0	0	0
1.71	0	0	0	0	0
Control	0	0	0	0	0

Study 2: Anonymous 1999 (36), Main Camp Tea Tree Oil pharmaceutical grade: fish, acute toxicity test, OECD 203, pre-GLP

Reliability statement: Very limited information is presented in the study report, which seems to be rather summary than actual test report. No information regarding fish size is available. No information regarding purity of the test item is given (e.g. concentration of the "lead components"). Sub-lethal effects are not reported, but from the available information it cannot be deduced if they have not occurred or were not investigated.
The most significant deficiency of the study is lack of the verification of the test item concentrations in any of the freshly prepared or old test solutions. For this reason it cannot be confirmed if the nominal concentrations were maintained at ±20% of nominal, but it seems to be highly unlikely given the way of preparation of test solutions - due to stirring of the stock solution for 24 hours it may be expected that at least part of the components of the extract volatilised. Furthermore, the undissolved phase was removed and only the dissolved water phase was used in the study, so fish were exposed only to part of components of the tea tree extract.
Overall, taking into account deficiencies mentioned above, the study is considered unacceptable (reliability score: 3).

The acute toxicity of Tea Tree Oil to zebra fish (*Brachydanio rerio*) has been conducted in a 96-hour semi-static design according to OECD Guideline 203. Ten fish were exposed to the concentrations of 5, 10, 25, 50 and 100 mg Tea Tree Oil/L.
No mortality occurred throughout the exposure period of 96 h.
The 96-hour LC_{50} and NOEC were determined to be > 100 mg Tea Tree Oil/L, based on the nominal concentrations.

Study 3: Stroh, J. et al., 1998; Literature study (Evaluation of the acute toxicity to juvenile pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier; Bull. Environ. Contam. Toxicol. 60:923-930)

Reliability statement: This public literature study was performed in line with protocol outlined in Wan et al. (1990, 1991) and Environment Canada (1990a, 1990b). Description of the test methods shows that the test design partially followed recommendations of OECD 203.
However, important information is missing in the paper, e.g.:

- size of the fish at test initiation,
- exact test concentrations,
- more detailed data regarding test conditions (especially oxygen concentration, which is a test validity criterion),
- preparation of test solutions,
- numerical data on mortality in each test group including controls.

In addition to that, results of chemical analyses are reported only for eugenol with no information regarding measured concentrations of α-terpineol. Based on obtained results, the study authors concluded that the total loss of the test item from test solutions could be 90% of nominal. However, results of the test are based on nominal concentrations.
Overall, as the measured concentrations of α-terpineol were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OECD 203 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).
The acute toxicity of α-terpineol to juvenile (2-3 months old) rainbow trout (*Oncorhynchus mykiss*), and pacific coho salmon (*Oncorhynchus kisutch*) was determined in a 96-hour static test. Glass aquaria of 20 L volume served as test vessels, resulting in an average loading rate of 0.4 g fish/L. Fish were exposed to a series of 5 test concentrations below 100 mg α-terpineol/L. Each test concentration was tested in triplicate with ten fish per test vessel. Test media were slightly aerated throughout the test. Fresh ground water with a mean hardness of 95 mg/L $CaCO_3$ was used for dilution water. Testing was carried out at 15 ± 1 °C. Conductivity, pH, dissolved oxygen concentration, and temperature were measured frequently throughout the test.
Test substance concentrations during test: to evaluate chemical loss in the test materials, water samples were taken from control and treatment vessels containing 18 ppm (nominal) blended product (thyme oil: alpha-Terpineol: Eugenol (1:1:1)). Not measured for test substance α-terpineol vessels separately, just for blended product.
No data of these measurements given, but stated that there was a chemical loss in all tested vessels with and without fish and that it was more than 90% of the nominal concentration. This loss of test chemicals was explained by volatilization during the initial aeration process and glass adsorption. However, it was unclear if this statement referred only to the substance eugenol or also to alpha-terpineol. No fish tissue analyses were conducted after the test.

LC_{50} values of 6.6 and 6.3 mg alpha-Terpineol/L were determined for the rainbow trout (*Oncorhynchus mykiss*) and the pacific coho salmon (*Oncorhynchus kisutch*), respectively. Please refer also to the table below for more details on the results.

Test species	**LC_{50} [mg α-terpineol/L]**			
	24 h	**48 h**	**72 h**	**96 h**
O. mykiss	6.7	6.7	6.6	6.6
O. kisutch	6.8	6.5	6.5	6.3

Study 4: Heitmuller, P.T. et al (1981); Literature study (Acute toxicity of 54 industrial chemicals to sheepshead minnow (Cyprinodon variegatus). Bull. Environ. Contam. Toxicol. 27, 596-604)

Reliability statement: This public literature study was performed in line with US EPA guideline. Description of the test methods shows that the test design partially followed recommendations of OCSPP 850.1075 of 2016.
However, important information is missing in the paper, e.g.:

- exact test concentrations,
- whether fish were fed or not (it may be deduced that fish were not fed, but this is not explicitly indicated),
- more detailed data regarding test conditions (especially oxygen concentration, which is a test validity criterion),
- numerical data on mortality in each test group including controls.

Furthermore, in the publication it is stated that:
Many of the chemicals were insoluble in seawater and either floated upon the water surface or formed globules on the bottoms of the test containers.
It is, however, not indicated, which chemicals were insoluble.
In addition to that, chemical analyses were not performed and for this reason it is not known if the test concentrations were maintained at required ±20% of nominal.
Overall, as the measured concentrations of p-cymene were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OCSPP 850.1075 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).

The acute toxicity of p-Cymene to unfed sheepshead minnow (*Cyprinodon variegatus*) 14 to 28 days old, post hatch, length of 8 to 15 mm) was determined in a static, 96- hour test according to the US EPA 660/3-75-009 (1975) guidelines.
Chemicals tested were analytical grade with a minimum purity of 80%. There was no aeration. Filtered natural seawater was used as dilution water. In the separate tests, a stock solution was prepared using a solvent (triethylene or acetone) or the substance was added directly to dilution water.
Treatments consisted of a series of concentrations (actual values not indicated), a dilution water control and/or a solvent control when necessary. 10 fish were used per treatment level. Tests were conducted in either 4-L glass jars containing 3 L of test medium or 19-L glass jars containing 15 L of test medium.
Test substance concentrations were not determined in the test media. Water quality parameters dissolved oxygen and pH were determined during testing; actual values are not indicated in the published report.
It has been stated that results were not considered acceptable if control mortality exceeded 10%.
The LC_{50} value of 48 mg p-Cymene/L was determined for sheepshead minnow (*Cyprinodon variegatus*). Please refer also to the table below for more details on the results.

LC_{50} (95 % confidence interval) **[mg p-Cymene/L]**				**NOEC**
24 h	**48 h**	**72 h**	**96 h**	**[mg p-Cymene/L]**
56 (32 – 100)	50 (38 – 68)	48 (36 – 64)	48 (36 – 64)	10

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

One acute toxicity study with Tea Tree Oil showed short-term (48 hour) acute toxicity to *daphnia* with an EC_{50} value of 0.591 mg as/L.

Two acute toxicity studies on the components of Tea Tree Oil p-Cymene and α-Pinene revealed short-term (48 hour) acute toxicity to *daphnia* with EC_{50} values of 6.5 and 41 mg as/L, respectively.

Study 1: Noack, M. 2011, Tea Tree Oil: Acute immobilisation test to Daphnia magna, semi-static, 48 hours, OECD 202, GLP

Reliability statement: The study was performed in line with recommendations of OECD 202 with no major deviation. All validity criteria were met and the study is considered acceptable (reliability score: 1).
In order to investigate the acute toxicity of Tea Tree Oil in aquatic invertebrates, the daphnid *Daphnia magna* was exposed over a period of 48 h under semi-static conditions to 6 concentrations of the test item ranging from 0.250 to 8.00 mg/L set up in a geometric series with a dilution factor of 2. Twenty (20) test organisms were exposed to each concentration and control.
The 48-hour EC_{50} value for *Daphnia magna* for Tea Tree Oil was determined to be 0.591 mg/L (95% confidence limits: 0.499 – 0.700 mg/L) based on measured concentrations. The no observed effect concentration (NOEC) was 0.106 mg/L based on measured concentrations.

Geometric mean measured Concentrations of test item [mg/L]	**Number of *Daphnia magna***	**Mean Immobilization [%] Time**	
		24 h	**48 h**
2.65	20	75	100
1.60	20	45	95
0.750	20	30	60
0.374	20	0	25
0.169	20	5	15
0.106	20	0	0
Control	20	0	0

Study 2: LeBlanc, G.A. 1980, Literature study (Acute toxicity of priority pollutants to water flea (Daphnia magna); Bull. Environ. Contam. Toxicol. 27, 684-691) LeBlanc, G.A., 1980

Reliability statement: This public literature study was performed in line with US EPA guideline. Description of the test methods shows that the test design partially followed recommendations of OCSPP 850.1010 of 2016.
However, important information is missing in the paper, e.g.:

- exact test concentrations,
- solvent used to dissolve p-cymene and α-pinene in water (if any),
- numerical data on immobilisation in each test group including controls.

Furthermore, 15 daphnids per concentration were used, while according to the guideline minimum 20 daphnids should be used per test group.
In addition to that, chemical analyses were not performed and for this reason it is not known if the test concentrations were maintained at required ±20% of nominal.
Overall, as the measured concentrations of p-cymene and α-pinene were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OCSPP 850.1010 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).

The acute toxicity of p-Cymene and α-Pinene to unfed *Daphnia magna* (< 24 hours old) was determined separately in static, 48-hour tests according to the US EPA 660/3-75-009 guidelines. Reconstituted water was used as dilution water. Depending upon solubility, either a stock solution was prepared with or without using a solvent (triethylene, glycol, ethanol, acetone, or dimethylformamide) or the substance was added directly to dilution water. Using the corresponding method, the test substance was added to 500 mL of dilution water in a 2 L jar to prepare each test solution. Depending upon solubility and volatility, the test solution was either divided in three 150 mL aliquots to provide three replicates or retained as it was to omit substance losses due to handling.
Treatments consisted of a series of 5 to 8 concentrations (actual values not indicated), a dilution water control and a solvent control when necessary. 15 daphnids were used per treatment level. For non-soluble substances that may be lost through volatilisation, one closed test vessel with 15 daphnids per treatment level was used. Tests were conducted at 22 ± 1°C. Test substance concentrations were not determined in the test media.
Control mortality was below 10% during this study.
The 48-hour EC_{50} for *Daphnia magna* was determined to be 6.5 mg/L for p-Cymene and 41 mg/L for alpha-Pinene, based on nominal concentrations.

Test substance	LC_{50} (95 % confidence interval) [mg a.i./L] 24 h	48 h	NOEC [mg a.i./L]
p-Cymene	9.4 (7.9 -11)	6.5 (4.3 - 10)	< 4.6
α-Pinene	68 (24 – 190)	41 (27 – 62)	8.8

In the table below, data from the open scientific literature is presented as an overview. These data are regarded solely as supplemental information.

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
α-Pinene	*Aedes aegypti* larvae	48 h Mortality [%]: 40.0 ± 8.1 at 100 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: > 50 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 15.87 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
		24h LC_{50}: 15.4 mg/L	No	3	Lucia et al. (2007)
	Aedes albopictus larvae	24h LC_{50} (+): 68.68 mg/L 24h LC_{50} (-): 72.30 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: > 50 mg/L	Yes	3	Cheng et al. (2009)
		24h LC_{50}: > 100 mg/L	Yes	3	Cheng et al. (2013)
		24h LC_{50}: 34.09 mg/L	No	3	Govindarajan et al. (2016)
	Anopheles suspictus larvae	24h LC_{50}: 32.09 mg/L	No	3	Govindarajan et al. (2016)
	Culex quinquefasciatus larvae	24h LC_{50}: 95 mg/L	No	3	Pavela (2015)
α-Terpinene	*Daphnia magna*	48h LC_{50}, 8.45 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 25.0 mg/L 5.0 ± 2.8 at 12.5 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 28.1 mg/L	No	3	Cheng et al. (2009)
	Aedes albopictus larvae	24h LC_{50}: 22.4 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
	Culex tritaeniorhynchus larvae	24h LC_{50}: 36.75 mg/L	No	3	Govindarajan et al. (2016)
p-Cymene	*Daphnia magna*	48h LC_{50}, 3.54 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 50 mg/L 5.0 ± 5.0 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 43.3 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 12.49 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: 69.4 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 34.9 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 68.3 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: 21 mg/L	No	3	Pavela (2015)
		24 h LC_{50}: 20.6 µl/L**	No	3	Pavela et al. (2017)
(-)-Limonene	*Daphnia magna*	48h LC_{50}, 7.22 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 100 mg/L 97.5 ± 2.5 at 50 mg/L 32.5 ± 10.3 at 25 mg/L	Yes	2	Park et al. (2011)
	Aedes albopictus larvae	24h LC_{50}: 34.89 mg/L	No	3	Giatropoulos et al (2012)

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
(+)-Limonene	*Daphnia magna*	48h LC_{50}, 7.85 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 100 mg/L 50.0 ± 9.1 at 50 mg/L 10.0 ± 4.0 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 19.4 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 39.7 mg/L	No	3	Chung et al. (2010)***
		24h LC_{50}: 71.9 mg/L	No	3	Cheng et al. (2013)
		24h LC_{50}: 29.1 mg/L	No	3	Tabanca et al. (2015)
	Aedes albopictus larvae	24h LC_{50} (+): 35.99 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: 15.0 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 19.84 mg/L	No	3	Liu et al. (2013)
		24h LC_{50}: 41.75 mg/L	No	3	Liu et al. (2015)
		24h LC_{50}: 41.2 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: 40 mg/L	No	3	Pavela (2015)
γ-Terpinene	*Daphnia magna*	48h LC_{50}, 3.45 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 50 mg/L 7.5 ± 2.5 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 26.8 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 0.4 mg/L	No	3	Lucia et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 20.21 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: 22.8 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: 26 mg/L	No	3	Pavela (2015)
Terpinolene	*Aedes aegypti* larvae	48 h Mortality [%]: 100 at 100 mg/L 97.5 ± 2.5 at 50 mg/L 32.5 ± 10.3 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 32.1 mg/L	No	3	Cheng et al. (2009)
	Aedes albopictus larvae	24h LC_{50}: 21.3 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: 21 mg/L	No	3	Pavela (2015)
		24 h LC_{50}: 25.7 µl/L**	Yes	2	Pavela et al. (2017)
Terpinene-4-ol	*Aedes aegypti* larvae	48 h Mortality [%]: 0 at 100 mg/L, lower concentrations not tested	Yes	2	Park et al. (2011)
		24h LC_{50}: 38.77 mg/L	No	3	Lucia et al. (2013)
α-Terpineol	*Aedes aegypti* larvae	48 h Mortality [%]: 2.5 ± 2.5 at 100 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 76.68 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: 331.7 mg/L	No	3	Pandey et al. (2013)
		24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
1,8-Cineole	*Aedes aegypti* larvae	24h LC_{50}: 53.63 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: > 200 mg/L	No	3	Chung et al. (2010)***

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
		24h LC_{50}: 57.2 mg/L	Yes	2	Lucia et al. (2007)
	Culex pipiens	48h LC_{50}: > 200 mg/L	No	3	Koliopoulos et al (2010)
		24h LC_{50}: 105.6 mg/L	No	3	Kimbaris et al (2012)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
Sabinene	*Culex quinquefasciatus* larvae	24h LC_{50}: 25.01 mg/L	No	3	Govindarajan (2010)
		24 h LC_{50}: 57.7 µl/L**	No	3	Pavela et al. (2017)
	Aedes aegypti larvae	24h LC_{50}: 21.20 mg/L	No	3	Govindarajan (2010)
		24h LC_{50}: 74.1 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 39.5 mg/L	No	3	Cheng et al. (2013)
	Anopheles stephensi larvae	24h LC_{50}: 19.67 mg/L	No	3	Govindarajan (2010)

* as usual for scientific literature, the testing was not conducted to fulfil regulatory requirements. Where reliability score 2 was assigned, analytical determination was conducted in any way, mostly to determine the presence of the test substance in the mixture, but not for verification of the test item concentration. This data is considered as supportive information. Where reliability score 3 was assigned, no analytical validation and measurement was conducted. These publications can only be considered as additional information.
** concentration in mg/L cannot be concluded from information given in the publication.
*** publication has been retracted by the author subsequently, since deemed not reliable

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

An acute algae growth inhibition test with Tea Tree Oil with *Pseudokirchneriella subcapitata* is available. It lead to an E_yC_{50} of 1.76 mg as/L, an E_rC_{50} of 2.17 mg as/L with a NOEC of 0.912 mg as/L.
The semi-static 7d aquatic plant toxicity test with *Lemna gibba* resulted to a E_rC_{50} of 10.3 mg as/L, an E_yC_{50} of 10.0 mg as/L and a NOEC of 1.91 mg as/L.

Study 1: Scheerbaum, D. 2017 Tea Tree Oil (TTO): Aquatic plant toxicity test, Lemna gibba, semi-static, 7 days, OECD 221, GLP

Reliability statement: The study was performed in line with OECD 211 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1)
The effects of Tea Tree Oil on the growth of the monocotyledonous aquatic plant species *Lemna gibba* applied at 5 dose rates 6.25, 12.5, 25.0, 50.0 and 100 mg Tea Tree Oil/L (nominal) was observed over a period for 7 days in a semi-static test system. Three replicates at each test concentration and six replicates for the control were included in the test. 2 additional replicates were treated at the highest concentration level and the control, respectively, and incubated and were used for the test on the recovery of the test system.
Frond numbers were assessed on days 0, 2, 5 and 7.
The 7-day E_yC_{50} value for Tea Tree Oil was determined to be 10.0 mg Tea Tree Oil/L and 10.6 mg Tea Tree Oil/L, based on fond number and dry weight, respectively. The 7-day E_rC_{50} value was estimated to be 10.3 mg Tea Tree Oil/L, based on fond number and dry weight. The NOEC was 4.63 mg Tea Tree Oil/L based on geometric mean measured concentrations.

Frond number		**Dry weight**	
Growth rate inhibition [mg/L]			
NOEC	4.63	**NOEC**	1.91
LOEC	9.06	**LOEC**	4.63
EC_{r50} (95 % confidence limits)	10.3 (9.17 – 14.4)	**EC_{rdw50}** (95 % confidence limits)	10.3 (9.35 – 14.1)
Inhibition of yield [mg/L]			
NOEC	4.63	**NOEC**	1.91
LOEC	9.06	**LOEC**	4.63
EC_{y50} (95 % confidence limits)	10.0 (9.31 – 13.7)	**EC_{ydw50}** (95 % confidence limits)	10.6 (9.42 – 11.7)

Study 2: Scheerbaum, D. 2017, Tea Tree Oil (TTO): Alga, growth inhibition test with Pseudokirchneriella subcapitata, 72 h, OECD 201, GLP

Reliability statement: The study was performed in line with OECD 201 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1).
The acute toxicity of Tea Tree Oil upon the growth of freshwater green algae *(Pseudokirchneriella subcapitata*) applied at 5 dose rates (2.56, 6.40, 16.0, 40.0 and 100 mg Tea Tree Oil/L) was observed over a period of 72 hours in a static test system. The study was carried out in closed bottles without headspace to avoid losses of the test item. Three replicate flasks at each test concentration and six control replicate flasks (Algal Growth Medium alone) were included in the test.
The 72-hour E_yC_{50} value for Tea Tree Oil was determined to be 1.76 mg Tea Tree Oil/L and the 72-hours E_rC_{50} value was estimated to be 2.17 mg Tea Tree Oil/L). The NOEC was 0.912 mg Tea Tree Oil/L based on geometric mean measured concentrations. Potential of recovery after exposure was observed at the geometric mean measured concentration of 3.15 mg Tea Tree Oil/L (algistatic effect) and an algicidal effect at the concentration levels of 7.45 and 19.8 mg Tea Tree Oil/L.

Geometric mean measured Concentration of Tea Tree Oil [mg/L]	**Mean Cell Concentration [cells/mL] time [h]**			
	0	**24**	**48**	**72**
19.8	6305	n.a.	n.a	2407
7.45	6305	3996	n.a.	2913
3.15	6305	11166	7671	6203
1.26	6305	35797	203152	634166
0.912	6305	37246	209688	637905
Control	6305	40966	252889	716307

n.a. = data not applicable

Geometric mean measured Concentration of TTO [mg/L]	**Mean Growth Rate [day^{-1}]**	**Inhibition of Growth Rate [%]**	**Yield [cells/mL]**	**Inhibition of Yield [%]**
19.8	(+) -0.322	100	(+) -3898	100
7.45	(+) -0.260	100	(+) -3392	100
3.15	(+) - 0.006	99	(+) -102	100
1.26	(-) 1.54	3	(+) 627861	12
0.912	(-) 1.54	3	(-) 631600	11
Control	1.58	-	710002	-

(-) = statistically non-significant differences compared to the control values.
(+) = statistically significant differences compared to the control values.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No further relevant aquatic effects data were available for purposes of CLH report.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Table 2.9.2.3-1: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results [mg as/L]	Remarks	Relia-bility score	Reference
Early life stage test with fish OECD 210 GLP Flow-through, ELS	*Pimephales promelas*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.244	measured	1	Anonymous (37) (2017)
Reproductive and developmental toxicity to *Daphnia* OECD 211 GLP Semi-static, 21d	*Daphnia magna*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.303 EC_{10} = 0.411	measured	1	Scheerbaum, D. (2017)
Development and emergence in *Chironomus* OECD 219 GLP Water-sediment, 28d	*Chironomus riparius*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 4.36 NOEC = 13.32 mg as/kg sediment EC_{50} = 28.3 EC_{50} = 86.47 mg as/kg sediment	measured	1	Scheerbaum, D. (2017)
Algae growth inhibition test OECD 201, GLP Static, 72 h	*Pseudo-kirchneriella subcapitata*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.912	measured	1	Scheerbaum, D. (2017)
Aquatic plant toxicity test OECD 221, GLP	*Lemna gibba*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol (in compliance with ISO specification)	NOEC = 1.91	measured	1	Scheerbaum, D. (2017)

2.9.2.3.1 Chronic toxicity to fish

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

One chronic toxicity study on Tea Tree Oil (early life stage test with fish) to *Pimephales promelas* leads to a NOEC of 0.244 mg as/L.

Study 1: Anonymous(37) 2017, Tea Tree Oil (TTO): Early-Life Stage Toxicity Test with Fathead Minnow (Pimephales promelas) under Flow-Through Conditions, OECD 210, GLP

Reliability statement: The study was performed according to OECD 210 with no major deviations. All validity criteria were met and the study is considered acceptable (reliability score: 1).
The effects of the test item Tea Tree Oil (TTO) to the early-life stage of fish (*Pimephales promelas* / Fathead minnow) were determined according to OECD Guideline 210.
A test was conducted under flow-through conditions with the nominal test item concentrations of 0.640, 1.60, 4.00, 10.0 and 25.0 mg/L, corresponding to arithmetic mean measured concentrations of 0.244, 0.373, 0.885, 1.40 and 3.15 mg/L. Due to the low solubility of the test item in water, methanol was used as solvent with a loading of 0.10 mL per L dilution water.
The test was started by placing fertilized eggs into the test vessels and lasted 34 days (28 days post-hatch). 80 eggs of *Pimephales promelas* were exposed to each test concentration, the solvent control and the control (4 replicates with 20 eggs each).
The water quality parameters pH-value, oxygen concentration, temperature and total hardness were within the acceptable limits.
On study day 6, 85% of the control and 86% of the solvent control larvae had hatched. Therefore, study day 6 was defined as post hatch day 0 (= PHD 0).
Different toxicological endpoints were determined: hatch, time to hatch, fry growth (expressed as length and fresh weight), morphological and behavioural effects and post-hatch survival.
Specific analysis of various concentrations of TTO in the test media and the control groups was carried out via SPME-GC-MS.
The test media were sampled and analysed prior to exposure on day -1 and during the exposure on study days 0, 6, 14, 21, 23, 28 and 33. The measured concentrations of the test media during the exposure were in the range of 5% to 49% of the nominal values.
All effect values are given based on arithmetic mean measured concentrations of the test item Tea Tree Oil.
The results of the parameters hatch, time to hatch, fry growth (expressed as weight and length) and post-hatch survival were checked for statistically significant differences. The effect values NOEC and LOEC were determined based on the statistical results. The results are presented in the table below:

Parameter	**NOEC**	**LOEC**	**LC_{50} (95% C.I.)**
Hatch	0.373	0.885	
Fry Growth expressed as: Length Weight	 ≥ 3.15 ≥ 3.15	 > 3.15 > 3.15	
Post hatch survival	≥ 3.15	> 3.15	
Cumulative survival	0.244	0.373	0.818 (0.500 - 0.884)

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

One chronic toxicity study on Tea Tree Oil to *Daphnia magna* is available and leads to a NOEC of 0.303 mg as/L and a corresponding EC_{10} of 0.411 mg as/L.

> *Study 1: Scheerbaum, D. 2017, Tea Tree Oil (TTO): Daphnia magna reproduction test, semi- static, 21 days, in a closed system without headspace, OECD 211, GLP*

Reliability statement: The study was performed in line with OECD 211 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1).
A *Daphnia magna* reproduction test (semi-static, 21 d) with Tea Tree Oil (TTO) was conducted according to OECD 211 (2012).
Ten daphnids (2 to 24 hours old) held individually, were used per concentration level and control.
The study was carried out in a closed system without headspace (sealed glass flasks filled up to the top with the test solutions) under semi-static conditions with a daily renewal of the test solutions. The aim of the test was to assess the effects on the reproduction capacity and other sub-lethal effects.
Five concentration levels of the test item TTO were tested, prepared in a geometric series with a separation factor of 2.5: 0.640, 1.60, 4.00, 10.0, 25.0 mg/L.
The concentrations of TTO were analytically verified via GC-MS in fresh media on days 0, 7, 14 (0 hours) and in old media on days 1, 8, 15 (24 hours) in all concentration levels with surviving parental daphnids and the control.

The measured concentrations of the test item in the fresh media (0 hours) were in the range of 17 to 25% of the nominal concentrations. At the end of the respective exposure intervals (24 hours), the measured concentrations in the old media were in the range of 13 to 34% of the nominal values.
The geometric mean measured concentrations of the test item were: 0.132 - 0.303 - 0.747 – 2.02 - 5.75 mg/L.
The effect-concentrations ($EC_{10\ /\ 50}$, $LC_{20\ /50\ /\ 100}$, LOEC and NOEC) were based on the geometric mean measured concentrations of the test item.
The test item induced statistically significant adult mortality of 40% in the concentration level 0.747 mg/L and 100% in the concentration levels of 2.02 and 5.75 mg/L. In the two lowest concentration levels of 0.132 and 0.303 mg/L and in the control all parental daphnids survived until the end of the test (21 days).
A statistically significant reduction of the reproductive output in comparison to the reproductive output in the control was determined at the three highest concentration levels of 0.747 to 5.75 mg/L. At the two lowest concentration levels of 0.132 and 0.303 mg/L, the reproductive output was comparable to the reproductive output of the control.
A summary of all endpoints based on the geometric mean measured concentrations of the test item Tea Tree Oil (TTO) is given in the following table.

Effect values	Tea Tree Oil (TTO)
EC_{10} Reproduction (with 95% confidence limits)	**0.411** (0.0838 2.02)
EC_{50} Reproduction (with 95% confidence limits)	0.531 (0.0998 - 2.95)
LOEC Reproduction	0.747
NOEC Reproduction	**0.303**
LC_{20} Adult mortality after 21 days (with 95% confidence limits)	0.677 (Not applicable)
LC_{50} Adult mortality after 21 days (with 95% confidence limits)	0.779 (Not applicable)
LC_{100} Adult mortality after 21 days	2.02
LOEC Adult mortality after 21 days	0.747
NOEC Adult mortality after 21 days	0.303

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Please refer to previous point 2.9.2.2.3 where the toxicity tests with the substance on algae are included

2.9.2.3.4 Chronic toxicity to other aquatic organisms

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

One chronic toxicity study on the effects of Tea Tree Oil to *Chironomus riparius* is available and lead to a NOEC of 4.36 mg as/L and a corresponding EC_{50} of 28.3 mg as/L.

> *Study 1: Scheerbaum, D. 2017,* Tea Tree Oil (TTO): Sediment-water chironomid toxicity test using spiked water

Reliability statement: The study was performed in line with OECD 219 with no major deviations. All validity criteria were fulfilled and the test is considered acceptable (reliability score: 1).
The effects of the test item Tea Tree Oil (TTO) on the development of the common non-biting midge *Chironomus riparius* in a water-sediment system were determined. The study was carried out according to the principles of OECD Guideline 219.
A dose response test was conducted by spiking the water layer. Five test item concentrations (nominal concentrations in the aqueous layer) of 10.2, 25.6, 64, 160, 400 mg/L (factor of 2.5) corresponding to initial measured concentrations of 1.91, 4.36, 9.56, 27.8, 73.4 mg/L were tested. 100 first instar larvae were exposed to each test concentration and to the control (5 replicates with 20 larvae each, 4 for the biological part and one for the analytical part on day 7).
Water quality parameters such as temperature, pH-value and O_2-content were determined regularly throughout the study. Also, ammonium and total hardness were analysed at the day of application (day 0) and at test end (day 28) from the control and the highest test item group.

The concentrations of the test item were analytically verified via GC-MS on days 0, 7 and 28. The test item was analysed in the sediment layer after extraction and liquid injection. Analysis of pore water and overlying water phase were carried out via SPME injection.

In the control, emergence of the midges started 13 days after larvae insertion and was finished at day 20 after larvae insertion with an emergence ratio of 94%. In the test item concentrations, emergence of the midges started 13 days after larvae insertion and was finished at day 23 after larvae insertion and the emergence ratio ranged from 0 to 95%.
Tea Tree Oil did affect the emergence rate and development rate of *Chironomus riparius* at the test item concentrations of 27.8 mg/L and higher compared to the control. At 73.4 mg/L no mean emergence rate and development rate was determinable.
The results indicate a NOEC for the emergence rate at 9.56 mg/L and the development rate at 4.36 mg/L. The EC_{50} for mortality is determined 28.3 mg/L. A summary of all endpoints based on the measured concentrations of the test item TTO is given in the following table.

Effect values [mg/L]	**Tea Tree Oil (TTO)**
EC_{50} (Emergence)	28.3
LOEC (Emergence Rate) Lowest tested concentration with an observed effect on the emergence rate after 28 d	27.8
NOEC (Emergence Rate) Highest tested concentration without any observed effect on the emergence rate after 28 d	9.56
LOEC (Development Rate) Lowest tested concentration with an observed effect on the development rate after 28 d	9.56
NOEC (Development Rate) Highest tested concentration without any observed effect on the development rate after 28 d	4.36

Although in the current study the "No Observed Effect Concentration' (NOEC) for the emergence rate (9.56 mg/L) and the development rate (4.36 mg/L) have been only expressed in mg/L, based on the water overlying the sediment (i.e. 550 ml per test vessel), the corresponding NOEC values, based on the sediment, could be calculated by taking into account the test sediment amount of 180 g dry weight per test vessel. Therefore, the NOEC for the emergence rate and the development rate, based on the sediment, could be calculated as 29.21 mg/kg sediment dry weight and 13.32 mg/kg sediment dry weight, respectively.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Aquatic acute toxicity data on Tea Tree Oil are available for fish, invertebrates, algae and higher aquatic plants. Daphnids are the most acutely sensitive trophic group with EC_{50} values ≤ 1.0 mg/L. The lowest value is 0.591 mg/L for *Daphnia magna*. On this basis, Tea Tree Oil meets the criteria from the CLP regulation (Annex I, section 4.1, table 4.1.0) for classification in Category Acute 1.

As the lowest acute toxicity endpoint is $0.1 < L(E)C_{50} \leq 1$ mg/L the corresponding Acute M-factor is 1.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Degradation

As summarised in section 10.1., Tea Tree Oil is considered rapidly degradable according to CLP criteria.

Bioaccumulation

Estimated BCF is < 500 for all monoterpene components, which account on average for > 95% of Tea Tree Oil. For the sesquiterpenes, BCF > 500 has been estimated, however, for the majority of these below 600, i.e. close to the trigger of 500. The sesquiterpene content of Tea tree oil is traces to max. 3.5% (individually), and cumulatively usually < 5%. Cumulative content of components with BCF > 600 (Cadinene, Aromadendrene and Ledene, BCF range 5000-7000) usually is below 2%.
Overall, Tea Tree Oil is considered to be not bioaccumulative. On this basis, the substance does not meet CLP criteria as a bioaccumulative substance.

Chronic toxicity

As discussed in sections above, there are reliable chronic toxicity endpoints for fish, aquatic invertebrates, algae, aquatic plants and sediment dweller. The lowest chronic endpoint is for fish, i.e. the fish early life stage NOEC endpoint of 0.244 mg as/L. As this endpoint is > 0.1 to <1 mg/L and as Tea Tree Oil is considered rapidly degradable, the corresponding chronic classification is Chronic Category 3. No relevant Chronic M-factor is appointed to this category.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

The following information was copied directly from the CLH Report for Tea Tree Oil (July 2018) with no amendments made by the RMS.

Classification

Based on toxicity data, Tea Tree Oil should be classified as Category Acute 1 (Acute M Factor 1), Category Chronic 3 (Chronic M Factor: none).

Labelling

Based on classification of acute 1 the appropriate labelling is as follows:

GHS09 Pictogram

Signal word 'Warning'
Hazard statement H410 Very toxic to aquatic life.

2.9.3 Summary of effects on arthropods

Summary of toxicity data for non-target arthropods is presented in tables below.

Table 2.9.3-1: Toxicity of TTO and representative formulation (BM 608) to bees and other pollinators

Substance	Species	Time scale	Developmental stage	Endpoint	Value	Reference
BM 608	*Apis mellifera*	acute	adult	oral LD_{50}	**>95.8 µg a.s./bee**	Vol. 3CP, B.9.5.1.1/01 Bruhnke (2009) Rep. No. 080218BB/IBA12306
				contact LD_{50}	**136.0 µg a.s./bee (lower limit LD_{50} due to wide CI)**	
BM 608	*Bombus terrestris*	acute	adult	oral LD_{50}	>105.5 µg a.s./bumblebee	Vol. 3CP, B.9.5.1.4/01 Kling (2009) Rep. No. S08-02674 Supportive information [1)]
				contact LD_{50}	>100.0 µg a.s./bumblebee	
BM 608	*Apis mellifera*	chronic	adult	oral LDD_{50}	Study not valid (data gap)	Vol. 3CP, B.9.5.1.2/01 Rathke (2016a) Rep. No. 141103SB/IBC16153
BM 608	*Apis mellifera*	acute	larvae	$NOED_{larvae}$	Study not valid (data gap)	Vol. 3CP, B.9.5.1.3/01 Rathke (2016b) Rep. No. 141103SB/IBL16153

Values in **bold** were used in the risk assessment

[1)] The test item concentrations in the test diet and test solutions were not confirmed in the chemical analyses since in absence of the respective test guideline in 2009, the study was performed in line with OECD 213 and 214, according to which the chemical analyses are not required. Taking this into account the RMS is of the opinion that lack of chemical analyses is a deviation from the current test guidelines for bumblebees, but the performed study may be used as additional information indicating that the oral and contact toxicity of BM 608 to bumblebees is expected to be low

Table 2.9.3-2: Toxicity of BM 608 (representative formulation of TTO) to non-target arthropods other than bees

Test substance	Species	Life stage	Substrate	Tested rates [mL prod./ha]	Corrected mortality [%] [1)]	Sub-lethal effects [%] [1)]	LR_{50} ER_{50} [mL prod./ha]	Reference
Laboratory studies								
BM 608	*Typhlodromus pyri* [2)]	Protonymphs	glass plates (2D)	270 540 1080 2160 4320	-2.6 0.0 10.3 6.5 17.9	2.4 15.3 41.2 51.8 77.6	>4320 (961.2 g a.s./ha) 1780 (396.1 g a.s./ha)	Vol. 3CP, B.9.5.2.1/01 Adelberger (2009a) Rep. No. S08-02646
	Aphidius rhopalosiphi	Adults	glass plates (2D)	270 540 1080 2160 4320	7.5 2.5 20.0 95.0 77.5	27.0 12.6 49.5 - -	>1080 [3)] (240.3 g a.s./ha) >1080 (240.3 g a.s./ha)	Vol. 3CP, B.9.5.2.1/02 Adelberger (2009b) Rep. No. S08-02645
Extended laboratory studies								
BM 608	*Typhlodromus pyri*	Protonymphs	bean leaves (2D)	579 1331 3062 7043 16200	-7.9 0.0 3.9 17.6 33.3 [4)]	9.4 17.1 8.5 20.5 15.4	>16200 (3558.7 g a.s./ha) >16200 (3558.7 g a.s./ha)	Vol. 3CP, B.9.5.2.2/01 Höhn (2010a) Rep. No. S10-00184
BM 608	*Aphidius rhopalosiphi*	Adults	barley seedlings (3D)	1013 2025 4050 8100 16200	4.0 4.0 0.0 0.0 0.0	-11.0 22.7 8.8 -5.4 12.5	>16200 (3558.7 g a.s./ha) >16200 (3558.7 g a.s./ha)	Vol. 3CP, B.9.5.2.2/02 Höhn (2010b) Rep. No. S10-00183
BM 608	*Orius laevigatus*	Nymphs	bean leaves (2D)	415 1037 2592 6480 16200	8.0 16.0 5.3 14.6 8.0	Reprod. 14.3 -6.5 20.8 11.7 10.4 Hatching 1.5 -8.6 5.2 10.2 -9.4	>16200 (3558.7 g a.s./ha) >16200 (3558.7 g a.s./ha)	Vol. 3CP, B.9.5.2.2/03 Klug (2010) Rep. No. S10-00185

[1)] Negative value indicates stimulation comparing to control

[2)] Delayed development with 11.3% protonymphs not developed to adults at 1080 mL/ha and with >30% protonymphs not developed to adults at 2160 and 4320 mL/ha was observed; effects >30% are, however, covered by the ER_{50} derived for reproductive effects

[3)] Exact LR_{50} could not be calculated due to no clear dose-response and oily droplets avoided by wasps seen in the plates of two highest treatment groups; the LR_{50} was thus estimated to be above the rate at which residues completely dried and mortality <50% were observed

[4)] High escape rate in 3 highest test item groups was observed (up to 40%), so the actual mortality due to exposure to the test item was lower than may be concluded from the mortality data

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Summary of toxicity data for soil macro- and meso-fauna is presented in table below.

Table 2.9.4-1: Toxicity of TTO representative formulation (BM 608) to soil meso- and macro-fauna

Test substance	Test/duration	Endpoint	Value [mg/kg dw soil]	Reference
Earthworms				
BM 608	Chronic, 56 days	Mortality, reproduction NOEC $NOEC_{corr}$ EC_{10}	 ≥13.5 mg a.s./kg dw soil **≥6.75 mg a.s./kg dw soil** Could not be calculated	Vol. 3CP, B.9.7.1.2/01 Winkelmann (2015a) Rep. No. RBR16153
Collembola				
BM 608	Chronic, 28 days	Reproduction NOEC $NOEC_{corr}$ Mortality: NOEC $NOEC_{corr}$	 28.43 mg a.s./kg dw soil **14.22 mg a.s./kg dw soil** ≥227.4 mg a.s./kg dw soil ≥113.7 mg a.s./kg dw soil	Vol. 3CP, B.9.7.2/01 Rathke (2015) Rep. No. ICR16153
Soil mite				
BM 608	Chronic, 14 days	Mortality, reproduction NOEC $NOEC_{corr}$ EC_{10}	 ≥240.0 mg a.s./kg dw soil **≥120.0 mg a.s./kg dw soil** Not relevant for limit test	Vol. 3CP, B.9.7.2/02 Rathke (2017) Rep. No. IHL17173

Values in **bold** were used in the risk assessment

2.9.5 Summary of effects on soil nitrogen transformation

Summary of effects of TTO on soil microbial activity is presented in table below.

Table 2.9.5-1: Effects of BM 608 on soil nitrogen transformation

Compound	Parameter	Tested concentration [mg a.s./kg dw soil]	Effect at test end [1)]	Reference
BM 608	nitrate formation rate (day 28)	0.564 2.82	-2.0% -9.0%	Vol. 3CP, B.9.9/01 Winkelmann (2015b) Rep. No. TBN16153

[1)] Negative values indicate stimulation comparing to control

2.9.6 Summary of effects on terrestrial non-target higher plants

Summary of toxicity data for non-target terrestrial plants is presented in tables below.

Table 2.9.6-1: Effects of BM 608 on non-target terrestrial plants

Test substance	Type of the test	Species	ER_{50} [1)] [L product/ha]	Reference
BM 608	vegetative vigour	*Avena sativa* (oats) *Allium cepa* (onion) *Beta vulgaris* (sugar beet) *Brassica napus* (oilseed rape) *Lactuca sativa* (lettuce) *Glycine max* (soybean)	>2.0 (>423 g a.s./ha)	Vol. 3CP, B.9.11.2/01 Fiebig (2015) Rep. No. TNW16153
	seedling emergence	*Avena sativa* (oats) *Allium cepa* (onion) *Beta vulgaris* (sugar beet) *Brassica napus* (oilseed rape) *Daucus carota* (carrot) *Glycine max* (soybean)	>2.0 (>446.4 g a.s./ha)	Vol. 3CP, B.9.11.1/02 Fiebig (2017) Rep. No. TNW17173

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No studies on other terrestrial organisms were performed or required.

2.9.8 Summary of effects on biological methods for sewage treatment

No valid study has been provided (data gap).

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Risk assessment for birds

The risk assessment for birds has been performed in line with recommendations of EFSA (2009) with consideration of the EU GAP. For indoor uses the assumptions as for field uses were taken as representing worst case. Only active compound was considered, as no relevant TTO metabolites were found in soil and aquatic systems metabolism studies.

Results of the screening step evaluation are provided in table below.

Table 2.9.9.1-1: Screening risk assessment for avian indicator species

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Acute risk									
Tomatoes (field) 3x0.334 kg a.s.ha, 7 days interval	Small omnivorous bird	2492	0.334	158.8	1.6	not relevant	84.9	29.4	≥10
Tomatoes (field) 3x0.445 kg a.s./ha, 7 days interval	Small omnivorous bird	2492	0.445	158.8	1.6		113.1	22.0	
Tomatoes (indoors) 4x0.334 kg a.s./ha, 7 days interval	Small omnivorous bird	2492	0.334	158.8	1.8		95.5	26.1	
Tomatoes (indoors) 4x0.445 kg a.s./ha, 7 days interval	Small omnivorous bird	2492	0.445	158.8	1.8		127.2	19.6	
Vineyards 4x0.334 kg a.s./ha, 7 days interval	Small omnivorous bird	2492	0.334	95.3	1.8		57.3	43.5	
Vineyards 4x0.445 kg a.s./ha, 10 days interval	Small omnivorous bird	2492	0.445	95.3	1.5		63.6	39.2	
Long-term risk									
Tomatoes (field) 3x0.334 kg a.s./ha, 7 days interval	Small omnivorous bird	100.0	0.334	64.8	2.0	0.53	22.9	**4.4**	≥5
Tomatoes (field) 3x0.445 kg a.s./ha, 7 days interval	Small omnivorous bird	100.0	0.445	64.8	2.0	0.53	30.6	**3.3**	
Tomatoes (indoors) 4x0.334 kg a.s./ha, 7 days interval	Small omnivorous bird	100.0	0.334	64.8	2.2	0.53	25.2	**4.0**	
Tomatoes (indoors) 4x0.445 kg a.s./ha, 7 days interval	Small omnivorous bird	100.0	0.445	64.8	2.2	0.53	33.6	**3.0**	
Vineyards 4x0.334 kg a.s./ha, 7 days interval	Small omnivorous bird	100.0	0.334	38.9	2.2	0.53	15.1	6.6	
Vineyards 4x0.445 kg a.s./ha, 10 days interval	Small omnivorous bird	100.0	0.445	38.9	1.9	0.53	17.4	5.7	

Values in **bold** indicate unacceptable risk

Acute TER values calculated at the screening step are all above the trigger of 10 demonstrating acceptable acute risk to birds following application of BM 608 according to the EU GAP.
The long-term TER values for intended uses in vineyards are above the respective trigger indicating acceptable long-term risk to birds following this intended use.
The long-term screening step TER values for uses in tomatoes are below the trigger and for this reason Tier 1 evaluation is performed below.

Table 2.9.9.1-2: Tier 1 long-term risk assessment for avian generic focal species

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Long-term risk									
Tomatoes 3x0.334 kg a.s./ha 7 days interval Field	Frugivorous bird "crow" BBCH 71-89	100.0	0.334	32.0	2.0	0.53	11.3	8.8	≥5
	Small granivorous bird "finch", BBCH 10-49	100.0	0.334	11.4	2.0	0.53	4.0	25.0	
	Small granivorous bird "finch", BBCH ≥50	100.0	0.334	3.4	2.0	0.53	1.2	83.3	
	Small omnivorous bird "lark", BBCH 10-49	100.0	0.334	10.9	2.0	0.53	3.9	25.6	
	Small omnivorous bird "lark", BBCH ≥50	100.0	0.334	3.3	2.0	0.53	1.2	83.3	
	Frugivorous bird "starling", Fruit stage, BBCH 71-89	100.0	0.334	20.7	2.0	0.53	7.3	13.7	
	Small insectivorous bird "wagtail", BBCH 10-19	100.0	0.334	11.3	2.0	0.53	4.0	25.0	
	Small insectivorous bird "wagtail", BBCH ≥20	100.0	0.334	9.7	2.0	0.53	3.4	29.4	
Tomatoes 3x0.445 kg a.s./ha 7 days interval Field	Frugivorous bird "crow" BBCH 71-89	100.0	0.445	32.0	2.0	0.53	15.1	6.6	≥5
	Small granivorous bird "finch", BBCH 10-49	100.0	0.445	11.4	2.0	0.53	5.4	18.5	
	Small granivorous bird "finch", BBCH ≥50	100.0	0.445	3.4	2.0	0.53	1.6	62.5	
	Small omnivorous bird "lark", BBCH 10-49	100.0	0.445	10.9	2.0	0.53	5.1	19.5	
	Small omnivorous bird "lark", BBCH ≥50	100.0	0.445	3.3	2.0	0.53	1.6	62.5	
	Frugivorous bird "starling", Fruit stage, BBCH 71-89	100.0	0.445	20.7	2.0	0.53	9.8	10.2	
	Small insectivorous bird "wagtail", BBCH 10-19	100.0	0.445	11.3	2.0	0.53	5.3	18.9	
	Small insectivorous bird "wagtail", BBCH ≥20	100.0	0.445	9.7	2.0	0.53	4.6	21.7	
Tomatoes 4x0.334 kg a.s./ha 7 days interval Indoors	Frugivorous bird "crow" BBCH 71-89	100.0	0.445	32.0	2.2	0.53	12.5	8.0	≥5
	Small granivorous bird "finch", BBCH 10-49	100.0	0.445	11.4	2.2	0.53	4.4	22.7	
	Small granivorous bird "finch", BBCH ≥50	100.0	0.445	3.4	2.2	0.53	1.3	76.9	
	Small omnivorous bird "lark", BBCH 10-49	100.0	0.445	10.9	2.2	0.53	4.2	23.8	
	Small omnivorous bird "lark", BBCH ≥50	100.0	0.445	3.3	2.2	0.53	1.3	76.9	
	Frugivorous bird "starling", Fruit stage, BBCH 71-89	100.0	0.445	20.7	2.2	0.53	8.1	12.3	
	Small insectivorous bird "wagtail", BBCH 10-19	100.0	0.445	11.3	2.2	0.53	4.4	22.7	
	Small insectivorous bird "wagtail", BBCH ≥20	100.0	0.445	9.7	2.2	0.53	3.8	26.3	

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Tomatoes 4 x 0.445 kg a.s./ha 7 days interval Indoors	Frugivorous bird "crow" BBCH 71-89	100.0	0.445	32.0	2.2	0.53	16.6	6.0	≥5
	Small granivorous bird "finch", BBCH 10-49	100.0	0.445	11.4	2.2	0.53	5.9	16.9	
	Small granivorous bird "finch", BBCH ≥50	100.0	0.445	3.4	2.2	0.53	1.8	55.6	
	Small omnivorous bird "lark", BBCH 10-49	100.0	0.445	10.9	2.2	0.53	5.7	17.5	
	Small omnivorous bird "lark", BBCH ≥50	100.0	0.445	3.3	2.2	0.53	1.7	58.8	
	Frugivorous bird "starling", Fruit stage, BBCH 71-89	100.0	0.445	20.7	2.2	0.53	10.7	9.3	
	Small insectivorous bird "wagtail", BBCH 10-19	100.0	0.445	11.3	2.2	0.53	5.9	16.9	
	Small insectivorous bird "wagtail", BBCH ≥20	100.0	0.445	9.7	2.2	0.53	5.0	20.0	

Long-term Tier 1 TER values calculated for all intended uses in tomatoes are above the trigger of 5 demonstrating that application of BM 608 to tomatoes will not pose unacceptable risk to birds.

The risk resulting from exposure via drinking water was also considered and assessed to be low.

Evaluation of the risk of secondary poisoning was deemed not necessary as accumulation potential of particular TTO constituents is assessed as low based on available data.

2.9.9.2 Risk assessment for mammals

The risk assessment for mammals has been performed in line with recommendations of EFSA (2009) with consideration of the EU GAP. For indoor uses the assumptions as for field uses were taken as representing worst case. Only active compound was considered, as no relevant TTO metabolites were found in soil and aquatic systems metabolism studies.

Results of the screening step evaluation are provided in table below.

Table 2.9.9.2-1: Screening risk assessment for mammalian indicator species

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Acute risk									
Tomatoes (field) 3x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	1049	0.334	136.4	1.6	not relevant	72.9	14.4	≥10
Tomatoes (field) 3x0.445 kg a.s./ha, 7 days interval	Small herbivorous mammal	1049	0.445	136.4	1.6		97.1	10.8	
Tomatoes (indoors) 4x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	1049	0.334	136.4	1.8		82.0	12.8	
Tomatoes (indoors) 4x0.445 kg a.s./ha, 7 days interval	Small herbivorous mammal	1049	0.445	136.4	1.8		109.3	**9.6**	
Vineyards 4x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	1049	0.334	136.4	1.8		82.0	12.8	

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Vineyards 4x0.445 kg a.s./ha, 10 days interval	Small herbivorous mammal	1049	0.445	136.4	1.5		91.0	11.5	
Long-term risk									
Tomatoes (field) 3x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	25.0	0.334	72.3	2.0	0.53	25.6	**0.98**	≥5
Tomatoes (field) 3x0.445 kg a.s./ha, 7 days interval	Small herbivorous mammal	25.0	0.445	72.3	2.0	0.53	34.1	**0.73**	
Tomatoes (indoors) 4x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	25.0	0.334	72.3	2.2	0.53	28.2	**0.89**	
Tomatoes (indoors) 4x0.445 kg a.s./ha, 7 days interval	Small herbivorous mammal	25.0	0.445	72.3	2.2	0.53	37.5	**0.67**	
Vineyards 4x0.334 kg a.s./ha, 7 days interval	Small herbivorous mammal	25.0	0.334	72.3	2.2	0.53	28.2	**0.89**	
Vineyards 4x0.445 kg a.s./ha, 10 days interval	Small herbivorous mammal	25.0	0.445	72.3	1.9	0.53	32.4	**0.77**	

[1)] MAF_{90} and MAF_m relevant for 4 applications with 7 days interval, covering all intended uses in tomatoes and vineyards (including indoor uses in tomatoes)

Calculations performed for field application of BM 608 in tomatoes demonstrated acceptable acute risk for all indicator species. In case of indoor uses, acceptable acute risk could be concluded for lower application rate (4x0.334 kg a.s./ha), while potentially unacceptable risk was indicated for higher application rate (4x0.445 kg a.s./ha). Tier 1 risk assessment is presented below. For application in vineyards acceptable risk could be concluded based on screening step.

With regard to long-term risk, TER values were below the respective trigger for all intended uses and for this reason Tier 1 calculations was performed and is presented below.

Table 2.9.9.2-2: Tier 1 acute risk assessment for mammalian generic focal species following indoor application to tomatoes at 4x0.445 kg a.s./ha

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF	f_{TWA}	DDD		
Acute risk									
Tomatoes 4 x 0.445 kg a.s./ha 7 days interval	Frugivorous mammal “rat”, BBCH 71-89	1049	0.445	45.2	1.8	not relevant	36.2	29.0	≥10
	Small insectivorous mammal “shrew”, BBCH 10-19	1049	0.445	7.6	1.8		6.1	172.0	
	Small insectivorous mammal “shrew”, BBCH ≥20	1049	0.445	5.4	1.8		4.3	244.0	
	Small herbivorous mammal “vole”, BBCH 10-49	1049	0.445	136.4	1.8		109.3	**9.6**	
	Small herbivorous mammal “vole”, BBCH ≥50	1049	0.445	40.9	1.8		32.8	32.0	
	Small omnivorous mammal “mouse”, BBCH 10-49	1049	0.445	17.2	1.8		13.8	76.0	
	Small omnivorous mammal “mouse”, BBCH ≥50	1049	0.445	5.2	1.8		4.2	249.8	

Values in **bold** indicate unacceptable risk

Tier 1 calculations performed for indoor application of BM 608 in tomatoes at 4x0.445 kg a.s./ha demonstrated acceptable acute risk for all generic focal species with exception of small herbivores exposed following uses at BBCH 10-49. However, in opinion of the RMS the risk from this use may be considered as sufficiently addressed as the TER of 9.6 is only slightly below the trigger, none of the parameters was refined (calculations were based on worst case defaults, while taking into account properties of TTO constituents rapid degradation may be expected on plants, so the MAF value is considered to be extremely unrealistic) and this use is intended to be performed indoors, which will considerably limit the exposure.

Tier 1 long-term risk assessment for field uses is provided below.

Table 2.9.9.2-3: Tier 1 long-term risk assessment for mammalian generic focal species following uses in tomatoes

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF [1)]	f_{TWA}	DDD		
Long-term risk									
Tomatoes 3x0.334 kg a.s./ha 7 days interval Field	Frugivorous mammal "rat", BBCH 71-89	25.0	0.334	25.2	2.0	0.53	8.9	**2.8**	≥5
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.334	4.2	2.0	0.53	1.5	16.7	
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.334	1.9	2.0	0.53	0.7	35.7	
	Small herbivorous mammal "vole", BBCH 10-49	25.0	0.334	72.3	2.0	0.53	25.6	**0.98**	
	Small herbivorous mammal "vole", BBCH ≥50	25.0	0.334	21.7	2.0	0.53	7.7	**3.2**	
	Small omnivorous mammal "mouse", BBCH 10-49	25.0	0.334	7.8	2.0	0.53	2.8	8.9	
	Small omnivorous mammal "mouse", BBCH ≥50	25.0	0.334	2.3	2.0	0.53	0.8	31.3	
Tomatoes 3x0.445 kg a.s./ha 7 days interval Field	Frugivorous mammal "rat", BBCH 71-89	25.0	0.445	25.2	2.0	0.53	11.9	**2.1**	≥5
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.445	4.2	2.0	0.53	2.0	12.5	
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.445	1.9	2.0	0.53	0.9	27.8	
	Small herbivorous mammal "vole", BBCH 10-49	25.0	0.445	72.3	2.0	0.53	34.1	**0.73**	
	Small herbivorous mammal "vole", BBCH ≥50	25.0	0.445	21.7	2.0	0.53	10.2	**2.5**	
	Small omnivorous mammal "mouse", BBCH 10-49	25.0	0.445	7.8	2.0	0.53	3.7	6.8	
	Small omnivorous mammal "mouse", BBCH ≥50	25.0	0.445	2.3	2.0	0.53	1.1	22.7	
Tomatoes 4x0.334 kg a.s./ha 7 days interval Indoors	Frugivorous mammal "rat", BBCH 71-89	25.0	0.334	25.2	2.2	0.53	9.8	**2.6**	≥5
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.334	4.2	2.2	0.53	1.6	15.6	
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.334	1.9	2.2	0.53	0.7	35.7	
	Small herbivorous mammal "vole", BBCH 10-49	25.0	0.334	72.3	2.2	0.53	28.2	**0.89**	
	Small herbivorous mammal "vole", BBCH ≥50	25.0	0.334	21.7	2.2	0.53	8.5	**2.9**	
	Small omnivorous mammal "mouse", BBCH 10-49	25.0	0.334	7.8	2.2	0.53	3.0	8.3	

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF [1)]	f_{TWA}	DDD		
	Small omnivorous mammal "mouse", BBCH ≥50	25.0	0.334	2.3	2.2	0.53	0.9	27.8	
Tomatoes 4 x 0.445 kg a.s./ha 7 days interval Indoors	Frugivorous mammal "rat", BBCH 71-89	25.0	0.445	25.2	2.2	0.53	13.1	**1.9**	≥5
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.445	4.2	2.2	0.53	2.2	11.4	
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.445	1.9	2.2	0.53	1.0	25.0	
	Small herbivorous mammal "vole", BBCH 10-49	25.0	0.445	72.3	2.2	0.53	37.5	**0.67**	
	Small herbivorous mammal "vole", BBCH ≥50	25.0	0.445	21.7	2.2	0.53	11.3	**2.2**	
	Small omnivorous mammal "mouse", BBCH 10-49	25.0	0.445	7.8	2.2	0.53	4.0	6.3	
	Small omnivorous mammal "mouse", BBCH ≥50	25.0	0.445	2.3	2.2	0.53	1.2	20.8	

Table 2.9.9.2-4: Tier 1 long-term risk assessment for mammalian generic focal species following uses in vineyards

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF [1)]	f_{TWA}	DDD		
Long-term risk									
Vineyards 4 x 0.334 kg a.s./ha 7 days interval	Large herbivorous mammal "lagomorph", BBCH 10-19	25.0	0.334	6.7	2.2	0.53	2.6	9.6	≥5
	Large herbivorous mammal "lagomorph", BBCH 20-39	25.0	0.334	5.5	2.2	0.53	2.1	11.9	
	Large herbivorous mammal "lagomorph", BBCH ≥40	25.0	0.334	3.3	2.2	0.53	1.3	19.2	
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.334	4.2	2.2	0.53	1.6	15.6	
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.334	1.9	2.2	0.53	0.7	35.7	
	Small herbivorous mammal "vole", BBCH 10-19	25.0	0.334	43.4	2.2	0.53	16.9	**1.5**	
	Small herbivorous mammal "vole", BBCH 20-39	25.0	0.334	36.1	2.2	0.53	14.1	**1.8**	
	Small herbivorous mammal "vole", BBCH ≥40	25.0	0.334	21.7	2.2	0.53	8.5	**2.9**	
	Small omnivorous mammal "mouse", BBCH 10-19	25.0	0.334	4.7	2.2	0.53	1.8	13.9	
	Small omnivorous mammal "mouse", BBCH 20-39	25.0	0.334	3.9	2.2	0.53	1.5	16.7	
	Small omnivorous mammal "mouse", BBCH ≥40	25.0	0.334	2.3	2.2	0.53	0.9	27.8	
Vineyards 4 x 0.445 kg a.s./ha 10 days interval	Large herbivorous mammal "lagomorph", BBCH 10-19	25.0	0.445	6.7	1.9	0.53	3.0	8.3	≥5
	Large herbivorous mammal "lagomorph", BBCH 20-39	25.0	0.445	5.5	1.9	0.53	2.5	10.0	
	Large herbivorous mammal "lagomorph", BBCH ≥40	25.0	0.445	3.3	1.9	0.53	1.5	16.7	
	Small insectivorous mammal "shrew", BBCH 10-19	25.0	0.445	4.2	1.9	0.53	1.9	13.2	

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF [1)]	f_{TWA}	DDD		
	Small insectivorous mammal "shrew", BBCH ≥20	25.0	0.445	1.9	1.9	0.53	0.9	27.8	
	Small herbivorous mammal "vole", BBCH 10-19	25.0	0.445	43.4	1.9	0.53	19.4	**1.3**	
	Small herbivorous mammal "vole", BBCH 20-39	25.0	0.445	36.1	1.9	0.53	16.2	**1.5**	
	Small herbivorous mammal "vole", BBCH ≥40	25.0	0.445	21.7	1.9	0.53	9.7	**2.6**	
	Small omnivorous mammal "mouse", BBCH 10-19	25.0	0.445	4.7	1.9	0.53	2.1	11.9	
	Small omnivorous mammal "mouse", BBCH 20-39	25.0	0.445	3.9	1.9	0.53	1.7	14.7	
	Small omnivorous mammal "mouse", BBCH ≥40	25.0	0.445	2.3	1.9	0.53	1.0	25.0	

Values in **bold** indicate unacceptable risk

Calculations performed for application of BM 608 in tomatoes demonstrated acceptable long-term risk for most of generic focal species with exception of small herbivores exposed following uses at all BBCH stages and frugivores exposed at BBCH 71-89.
In case of application of BM 608 in vineyards, acceptable long-term risk for most of generic focal species could be concluded with exception of small herbivores exposed following uses at all BBCH stages.
The risk to frugivorous mammals was refined with consideration of the residue trials performed in tomatoes used to refine MAF and fTWA. Respective calculations are presented in table below.

Table 2.9.9.2-5: Long-term risk assessment for mammalian generic focal species of concern based on detailed GAP

Crop	Indicator species	Toxicity [mg a.s./kg bw]	Exposure					TER	Trigger
			Appl. rate [kg/ha]	SV	MAF [1)]	f_{TWA} [1)]	DDD		
Long-term risk									
Tomatoes 3x0.334 kg a.s./ha 7 days interval Field	Frugivorous mammal "rat" BBCH 71-89	25.0	0.334	25.2	1.0	0.069	0.6	41.7	≥5
Tomatoes 3x0.445 kg a.s./ha 7 days interval Field	Frugivorous mammal "rat" BBCH 71-89	25.0	0.445	25.2	1.0	0.069	0.8	31.3	
Tomatoes 4x0.334 kg a.s./ha 7 days interval Indoors	Frugivorous mammal "rat" BBCH 71-89	25.0	0.334	25.2	1.0	0.069	0.6	41.7	
Tomatoes 4x0.445 kg a.s./ha 7 days interval Indoors	Frugivorous mammal "rat" BBCH 71-89	25.0	0.445	25.2	1.0	0.069	0.8	31.3	

[1)] MAF and f_{TWA} refined according to recommendations of EFSA (2009) with consideration of conservative DT_{50} of 1 day in fruits

Refined TER values for frugivorous mammals are all below the trigger of 5 demonstrating acceptable risk following application of BM 608 to tomatoes.

No relevant risk refinement options were available for purposes of refinement of the risk to small herbivorous mammals following application of BM 608 to tomatoes and vineyards and potentially unacceptable risk cannot be ruled out.

However, in opinion of the RMS, following indoor uses in tomatoes the exposure of mammals to TTO may be considered as minimal, provided that the application is performed in permanent structures. In such case acceptable risk could be concluded.

The risk resulting from exposure via drinking water was also considered and assessed to be low.

Evaluation of the risk of secondary poisoning was deemed not necessary as accumulation potential of particular TTO constituents is assessed as low based on available data.

2.9.9.3 Risk assessment for aquatic organisms

The risk assessment for aquatic organisms was performed according to recommendations of EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290.
At Tier 1, the RAC_{sw} values for acute and chronic exposure were obtained dividing the relevant endpoints by assessment factors (100 for acute and 10 for chronic). Resulting RAC values were compared with exposure estimated in area of environmental fate and behaviour. In case RAC_{sw} is greater than max PEC_{sw}, the risk was concluded to be low.

Please note that due to issues indicated in area of exposure assessment, the presented below risk assessment is only illustrative and may be changed, once relevant approach in surface water modelling for TTO is agreed among the experts and new surface water exposure calculated.

For greenhouse uses the risk assessment was performed with consideration of emission from glasshouse at 0.5%.

Respective calculations are presented in tables below.

Table 2.9.9.3-1: FOCUS$_{sw}$ step 1-3 - PEC/RACs for TTO – vineyards at 4 x 445 g a.s./ha with 7 days interval (covering also 10 days interval)

Scenario	PEC global max (µg/L) [1)]		Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Sed. dweller prolonged water-spiked	Algae	Higher plant
		Species	*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*C. riparius*	*D. subspicatus*	*L. gibba*
		Endpoint	LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
		µg/L	630	244	160	303	4360	770	10300
		AF	100	10	100	10	10	10	10
		RAC µg/L	6.3	24.4	1.6	30.3	436	77	1030
FOCUS Step 1	577.96		**91.7**	**23.7**	**361.2**	**19.1**	**1.33**	**7.5**	0.56
FOCUS Step 2									
North Europe	not calculated [2)]		-	-	-	-	-	-	-
South Europe	15.52		**2.46**	0.64	**9.7**	0.51	0.04	0.20	-
FOCUS Step 3									
D6 / ditch	8.178		**1.30**	-	**5.11**	-	-	-	-
R1 / pond	0.6267		0.10	-	0.39	-	-	-	-
R1 / stream	6.346		**1.007**	-	**3.97**	-	-	-	-
R2 / stream	8.238		**1.31**	-	**5.15**	-	-	-	-
R3 / stream	8.517		**1.35**	-	**5.32**	-	-	-	-
R4 / stream	6.211		0.99	-	**3.88**	-	-	-	-
Trigger			1	1	1	1	1	1	1

[1)] Maximum of single/multiple and early/late applications ; values include volatilisation

[2)] Uses in vineyards are intended in Southern Europe only

Values in **bold** indicate unacceptable risk

Table 2.9.9.3-2: FOCUS$_{sw}$ step 1-3 - PEC/RACs for TTO – tomatoes at 3 x 445 g a.s./ha with 7 days interval (field use)

Scenario	PEC global max (µg/L) [1]		Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Sed. dweller prolonged water-spiked	Algae	Higher plant
		Species	*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*C. riparius*	*D. subspicatus*	*L. gibba*
		Endpoint	LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
		µg/L	630	244	160	303	4360	770	10300
		AF	100	10	100	10	10	10	10
		RAC µg/L	6.3	24.4	1.6	30.3	436	77	1030
FOCUS Step 1	410.03		**65.1**	**16.8**	**256.3**	**13.5**	0.94	**5.33**	0.40
FOCUS Step 2									
North Europe	not calculated [2]		-	-	-	-	-	-	-
South Europe	4.56		0.72	0.19	**2.85**	0.15	-	0.06	-
FOCUS Step 3									
D6 / ditch	2.804		-	-	**1.78**	-	-	-	-
R2 / stream	2.830		-	-	**1.77**	-	-	-	-
R3 / stream	2.883		-	-	**1.80**	-	-	-	-
R4 / stream	2.707		-	-	**1.69**	-	-	-	-
Trigger			1	1	1	1	1	1	1

[1] Maximum of single/multiple and early/late applications ; values include volatilisation
[2] Field uses in tomatoes are intended in Southern Europe only
Values in **bold** indicate unacceptable risk

Table 2.9.9.3-3: PEC/RACs for TTO – tomatoes at 4 x 445 g a.s./ha with 7 days interval (greenhouse use)

Scenario	PEC (µg/L) [1]		Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Sed. dweller prolonged water-spiked	Algae	Higher plant
		Species	*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*C. riparius*	*D. subspicatus*	*L. gibba*
		Endpoint	LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
		µg/L	630	244	160	303	4360	770	10300
		AF	100	10	100	10	10	10	10
		RAC µg/L	6.3	24.4	1.6	30.3	436	77	1030
Greenhouse	0.0074		0.001	0.0003	0.005	<0.001	<0.001	<0.001	<0.001
Trigger			1	1	1	1	1	1	1

[1] Calculated assuming emission from greenhouse at 0.5%

Performed calculations demonstrated acceptable risk to all aquatic organisms from greenhouse uses of TTO to tomatoes at 4 x 445 g a.s./ha with 7 days interval.

The illustrative risk assessment based on FOCUS Step 1-3 PEC_{sw} demonstrated acceptable chronic risk from field uses of BM 608 to vineyards and tomatoes for fish, *Daphnia magna*, *Chironomus riparius*, algae and aquatic macrophytes with no need for further assessment. The acute risk to fish following application to tomatoes was also acceptable. The performed calculations demonstrated potentially unacceptable acute risk to fish from uses in vineyards and *Daphnia magna* from the both intended field uses.

Risk assessment based on Step 4 PEC_{sw} values was not presented by the RMS, since in the risk assessment the Applicant considered either mean measured active substance or nominal formulation endpoints, which were higher than values agreed by the RMS. For this reason no Step 4 simulations were performed by the Applicant for tomatoes and for vineyards only some scenarios were considered at Step 4.

Nevertheless, as already indicated by the RMS above, risk assessment presented in tables above is only illustrative, since further discussion on approach and input parameters for surface water modelling is deemed necessary before final calculations agreed by all the MS experts are performed.

It should be also noted that all TTO constituents are characterized by a high vapour pressure and it may be expected that they will volatilise from soil and plant surfaces shortly after the application. Hence, it is highly unlikely that they would be a subject to drainage and run-off and spray drift seems to be the main route of migration of these compounds to surface water.

In addition to that, the available literature data presented in area of residue and efate section show that majority of TTO components occurs naturally in multiple plant species, including crops such as tomatoes and vines. Therefore the emission from natural and agricultural sources may potentially exceed exposure after application of BM 608. Although available literature studies report emissions from gram or number of the investigated plant species, there are some data showing emissions from the surface unit (m^2 or ha). Examples are listed below:

- emission of terpenes estimated from forests: 480 mio tons/year,
- emission of terpenes from pastures: 5.72-12.81 kg/ha/year,
- emission of monoterpenes from leaves of beech tree : 76-144 $\mu g/m^2/s$,
- emission of total terpenoid from *Fagus sylvatica* branches : 54 $\mu g/m^2/h$,
- emission of monoterpenes (Limonene, α-Pinene, camphene, myrcene, β-Pinene, α-phellandrene, 3-carene, α-Terpinene and cymene) from soil in *Picea sitchensis* forest : mean 13-199 $\mu g/m^2/h$.

It is especially easy to compare emission from pastures at 5.72-12.81 kg/ha/year with the cumulative TTO application rate (1.78 kg/ha/year), which shows that the natural emission from plants is much higher than this after application of BM 608. Therefore it may be questioned if the estimation of exposure using FOCUS models is actually necessary since aquatic organisms will be naturally exposed to terpenoids emitted from various sources and application of BM 608 will have only marginal impact on the natural exposure.

2.9.9.4 Risk assessment for bees

The risk assessment for bees has been performed in line with recommendations of EFSA Guidance on risk assessment on bees (EFSA Journal 2013;11(7):3295). However, in absence of valid chronic and larvae endpoints only acute risk assessment could be performed. Exposure calculations and risk assessment are presented below.

Table 2.9.9.4-1: Screening step exposure of bees following application of BM 608

Crop	Route of exposure	Source of exposure	Developmental stage	SV value	Single application rate	Exposure
Tomato, vineyard	Contact	Overspray	Adult	not relevant	445 g a.s./ha	445 g a.s./ha
Tomato	Oral	Nectar and pollen	Adult	7.6 (downward spraying)	445 g a.s./ha	3382 g a.s./ha
			Larvae	4.4 (downward spraying)	445 g a.s./ha	1958 g a.s./ha
Vineyard	Oral	Nectar and pollen	Adult	10.6 (upward spraying)	445 g a.s./ha	4717 g a.s./ha
			Larvae	6.1 (upward spraying)	445 g a.s./ha	2715 g a.s./ha

Table 2.9.9.4-2: Screening risk assessment for bees exposed to TTO in BM 608

Risk quotient	Exposure [1)]	Endpoint	Value	Result	Trigger
$HQ_{contact}$	445 g a.s./ha	$LD_{50contact}$	136 µg a.s./bee	3.3	<42
$ETR_{acute\ adult\ oral}$	4.717 kg a.s./ha	LD_{50oral}	>95.8 µg a.s./bee	<0.05	<0.2

[1)] maximum exposure calculated for application to vineyards at 445 g a.s./ha as representing worst case and covering all intended uses of BM 608

Based on presented above calculations acceptable acute risk to adult bees may be concluded following application of BM 608 according to the intended EU pattern.

The chronic and larvae risk assessment could not be performed and a data gap for valid toxicity studies is identified.

In evaluation of the risk from guttation water the water solubility of the active substance is considered. However, TTO is a mixture of multiple compounds and for this reason it is not possible to determine its solubility in water. The individual values for particular constituents are not sufficient to perform the respective risk assessment since toxicity data are available for the whole mixture and not for particular components. Taking this into account it is not possible to perform the risk via guttation water in line with EFSA Journal 2013;11(7):3295.
Nevertheless, it should be noted that all TTO constituents occur naturally in various plants and the main constituents of TTO (Terpinene-4-ol, α-Terpinene, α-Terpineol, α-Pinene, p-Cymene, 1,8-Cineole and Limonene) were found in both intended crops (grapes and tomatoes) as well as in multiple other crops (e.g. *Thymus vulgaris*, pine tree, spearmint, raspberry, apple, carrot, grapefruit, lemon, mandarin, pepper). For this reason it may be expected that bees would be naturally exposed to these compounds and their metabolites in the guttation fluid.

The risk resulting from exposure via surface water was assesses as low.

2.9.9.5 Risk assessment for non-target arthropods

The risk assessment for non-target arthropods has been performed in line with recommendations of ESCORT 2 guidance document with consideration of the in- and off-field exposure calculated for the worst case use pattern, covering all intended uses of BM 608.

Summary of exposure estimates is given in tables below.

Table 2.9.9.5-1: In-field PER values for applications of BM 608 to intended crops

Crop	Application rate [g a.s./ha]	MAF		$PER_{in\text{-}field}$ [g a.s./ha]	
		Foliar	Soil	Foliar	Soil
Vineyards	4 x 445	2.7	3.4	1201.5	1513
	4 x 334	2.7	3.4	901.8	1135.6
Tomatoes	4 x 445	2.7	3.4	1201.5	1513
	4 x 334	2.7	3.4	901.8	1135.6

Table 2.9.9.5-2: Tier I off-field PER values for applications of BM 608 to intended crops

Crop	Application rate [g a.s./ha]	MAF	Drift [1)]	Vegetation distribution factor	Correction factor	$PER_{off\text{-}field}$ [g a.s./ha]
Vineyards	4 x 445	2.7	6.71 [1)]	10	10	80.6
	4 x 334	2.7	6.71 [1)]	10	10	60.5
Tomatoes (field)	4 x 445	2.7	1.85 [2)]	10	10	22.2
	4 x 334	2.7	1.85 [2)]	10	10	16.7

[1)] relevant for 4 applications to "grapevine, late", covering also early applications in vineyards
[1)] relevant for 4 applications to "field crops"

In-field risk assessment

The resulting hazard quotients are presented in table below. Please note that at Tier I endpoints based on mortality data were considered, in line with indications of ESCORT 2.

Table 2.9.9.5-3: Tier I in-field risk assessment for non-target arthropods exposed to BM 608 following application to intended crops

Crop	Application rate [g a.s./ha]	Species	$PER_{in\text{-}field}$ [g a.s./ha]		LR_{50} [g a.s./ha]	$HQ_{in\text{-}field}$		Trigger
			Foliar	Soil		Foliar	Soil	
Vineyards Tomatoes (field)	4 x 445	*T. pyri*	1201.5	1513	>961.2	<1.3	<1.6	≤2
		A. rhopalosiphi			>240.3	**<5.0**	**<6.3**	
Vineyards Tomatoes (field)	4 x 334	*T. pyri*	901.8	1135.6	>961.2	<0.9	<1.2	
		A. rhopalosiphi			>240.3	**<3.8**	**<4.7**	

Based on performed calculations acceptable in-field risk may be concluded for *Typhlodromus pyri* for all intended uses. The Tier I HQ values for *Aphidius rhopalosiphi* are above the trigger indicating potentially unacceptable risk. For this reason further assessment has been performed using Tier II toxicity data generated for both standard indicator species and one additional species (*Orius laevigatus*), in line with ESCORT 2 recommendations.

For purposes of the Tier II risk assessment rates that caused <50% mortality and sub-lethal effects in extended laboratory studies were compared with predicted in- and off-field exposure. The risk assessment is presented in table below.

Table 2.9.9.5-4: Tier II in-field risk assessment for non-target arthropods exposed to BM 608 following application to intended crops

Crop	Application rate [g a.s./ha]	Species	Substrate	LR_{50} ER_{50} [g a.s./ha]	PER in-field [g a.s./ha]		Risk acceptable?	Trigger
					Foliar	Soil		
Vineyards Tomatoes (field)	4 x 445	*T. pyri*	bean leaves	>3558.7 >3558.7	1201.5	1513	Yes Yes	<50%
		A. rhopalosiphi	barley seedlings	>3558.7 >3558.7	1201.5	1513	Yes Yes	
		O. laevigatus	bean leaves	>3558.7 >3558.7	1201.5	1513	Yes Yes	
Vineyards Tomatoes (field)	4 x 334	*T. pyri*	bean leaves	>3558.7 >3558.7	901.8	1135.6	Yes Yes	
		A. rhopalosiphi	barley seedlings	>3558.7 >3558.7	901.8	1135.6	Yes Yes	
		O. laevigatus	bean leaves	>3558.7 >3558.7	901.8	1135.6	Yes Yes	

In all extended laboratory studies effects on mortality and reproduction of tested species were clearly below 50% up to application rate more than 2 times higher than the maximum in-field exposure. Based on that acceptable in-field risk may be concluded for non-target arthropods from all intended uses of BM 608.

Off-field risk assessment

The resulting hazard quotients are presented in table below. Please note that at Tier I endpoints based on mortality data were considered, in line with indications of ESCORT 2.

Table 2.9.9.5-5: Tier I off-field risk assessment for standard indicator species exposed to BM 608 following application to intended crops

Crop	Application rate [g a.s./ha]	Species	$PER_{off\text{-}field}$ [g a.s./ha]	LR_{50} [g a.s./ha]	$HQ_{off\text{-}field}$	Trigger
Vineyards	4 x 445	*T. pyri*	80.6	>961.2	<0.08	≤2
		A. rhopalosiphi		>240.3	<0.34	
	4 x 334	*T. pyri*	60.5	>961.2	<0.06	
		A. rhopalosiphi		>240.3	<0.25	
Tomatoes (field)	4 x 445	*T. pyri*	22.2	>961.2	<0.02	
		A. rhopalosiphi		>240.3	<0.09	
	4 x 334	*T. pyri*	16.7	>961.2	<0.02	
		A. rhopalosiphi		>240.3	<0.07	

Based on performed above calculation acceptable off-field risk from all intended uses of BM 608 may be concluded for non-target arthropods already at Tier I.

Overall, risk assessment performed on the basis of laboratory studies demonstrated acceptable risk to non-target arthropods from the intended uses of BM 608. However, literature data indicated TTO constituents have significant effects on arthropod stages that are not tested in the standard studies (eggs and larvae). Taking this into account the RMS is of the opinion that further studies on toxicity of BM 608 to non-target arthropods should be performed with exposure of the most sensitive stages (i.e. eggs and larvae) included in the exposure regime.

2.9.9.6 Risk assessment for earthworms and other soil meso- and macro-organisms

The risk assessment was performed in line with recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final). The potential long-term risk to earthworms, springtails and soil mites resulting from exposure to TTO was assessed by comparing the worst case $PEC_{soil,ini}$ values with respective NOEC values.

Respective risk assessment is presented in table below.

Table 2.9.9.6-1: TER_{LT} values for soil meso- and macro-fauna exposed to TTO following the intended uses

Compound	Use pattern	Species	$NOEC_{corr}$ [mg a.s./kg dw soil]	max PEC_{soil} [mg a.s./kg dw soil] [1)]	TER_{LT}	Trigger
TTO	Vineyards and tomatoes (field) 4 x 445 g a.s./ha 7 d interval	*Eisenia fetida*	≥6.75	0.6866	≥9.8	≥5
		Folsomia candida	14.22	0.6866	20.7	
		Hypoaspis aculeifer	≥120.0	0.6866	≥174.8	

[1)] since accumulation is not expected, maximum $PEC_{soil,ini}$ was considered in evaluation

Performed risk assessment demonstrated acceptable long-term risk to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* exposed to TTO following application of BM 608 to vineyards and tomatoes at 4 x 445 g a.s./ha with 7 days interval. Performed evaluation represents worst case and covers also application of the lower rate (4 x 334 g a.s./ha with 7 days interval) as well as longer interval between applications (10 days) and is also protective for greenhouse applications to tomatoes.

2.9.9.7 Risk assessment for soil micro-organisms

The risk assessment was performed in line with recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final). The risk assessment was performed by comparison of maximum PEC_{soil} values for particular compounds with concentrations of the test items at which effects were below 25%. Respective evaluation is presented in table below.

Table 2.9.9.7-1: Risk assessment for soil nitrogen transformation following exposure to TTO following the intended uses

Compound	Use pattern	max PEC_{soil} [mg a.s./kg dw soil] [1)]	Tested concentrations [mg a.s./kg dw soil]	Effect on nitrate formation rate	Trigger
TTO	Vineyards and tomatoes (field) 4 x 445 g a.s./ha 7 d interval	0.6866	0.564	-2.0%	<25%
			2.82	-9.0%	

[1)] since accumulation is not expected, maximum $PEC_{soil,ini}$ was considered in evaluation

TTO tested as BM 608 had no significant effects on soil nitrogen transformation at concentrations approximately 4 times higher than maximum PEC_{soil} values. On this basis it may be concluded that application of BM 608 according to recommendations will have no unacceptable effects on soil nitrogen transformation.

2.9.9.8 Risk assessment for non-target terrestrial plants

The risk assessment has been performed in line with Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev.2 final, 2002) by comparison of toxicity values to off-field exposure in order to obtain TER values. Results of performed calculations are presented in table below.

Table 2.9.9.8-1: Risk assessment for non-target terrestrial plants exposed to BM 608

Type of the test	ER_{50} [g a.s./ha]	Worst case $PER_{off-field}$ [g a.s./ha] [1)]	TER	Trigger
Vegetative vigour	>423	35.7	11.8	5
Seedling emergence	>446.4	35.7	12.5	

[1)] Calculated for late application to vineyards at 445 g a.s./ha with drift rate 8.02% and default distance 3 m

The risk assessment performed for the worst case off-field exposure resulted with TER values greater than the trigger of 5. On this basis acceptable risk to non-target plants following application of BM 608 according to proposed use pattern may be concluded.

2.10 ENDOCRINE DISRUPTING PROPERTIES

The assessment of endocrine disrupting properties of TTO was done according to the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

2.10.1 Human health

The adverse effects relevant for human health which could potentially be related to endocrine disrupting properties of TTO, which were identified in the standard toxicity assessment studies, were confined mainly to male reproductive system (DRAR, Vol. 3, B.6.8.3). TTO has been demonstrated in several repeated dose toxicity studies in male rats and dogs and in 2-generation study in rats to cause decrease in sperm count and motility, increase in sperm abnormalities, decrease in weight of testes and epididymides, histopathological changes in testes and epididymides, reduction of mean litter size and reduced pups survival by day 4 (P-Generation), reduced male and female mating and fertility indexes (P-Generation), reduction of no. of corpora lutea and no. of implantations at 50 mg/kg bw/d. The highest dose level at which these effects were not observed in 2-generation study was 25 mg/kg bw/d, which thus considered as No Observed Adverse Effect Level (NOAEL) for reproductive toxicity.

In literature search on potential endocrine disruption properties of TTO, performed according to the relevant guidance (EFSA, 2011), four *in vitro* studies were identified showing either no estrogenic response, or weak-estrogenic response, weak anti-androgen response, and no anti-estrogenic response. The reliability of the studies is low since they were not performed according to recognised guidelines, had poor reporting not allowing to assess key elements of design and results of studies. In some studies a cytotoxicity of TTO at concentration of 0.025% was observed.

To investigate potential endocrine disruption properties of TTO, noting that TTO have decreased fertility and affected sperm cells in rats and dogs the applicant has submitted the results of two studies: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties and the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists, which were done to clarify whether these effects could be mediated by interaction of TTO with androgen and estrogen receptor in germ and somatic cells.

In the Hershberger assay performed according to OECD 441 and in GLP conditions TTO at doses of 60-120 mg/kg, sufficient to affect the seminiferous epithelium in male rats and dogs, did not interact with androgen receptor in androgen sensitive tissues of male rats, therefore it was possible to conclude that it does not have the androgenic or antiandrogenic activity in this test system .

In the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists performed on the (h)ERα-HeLa-9903 cell line according to OECD TG 455 and in GLP conditions Tea Tree Oil tested in seven concentrations ranging from 100 pg/mL to 100 μg/mL was found to be nontoxic for (h)ERα-HeLa-9903 cells, and it did not induce a response specific for estrogen receptor agonist.

The results of both studies demonstrate that TTO does not have androgenic or anti-androgenic activity as well as estrogenic activity therefore these results do not provide evidence that the effect on testes and sperm cells are mediated by endocrine disrupting mechanism. This conclusion is further supported by lack of effect of TTO on histopathology or weight of prostate and seminal vesicles or male rat mammary glands, lack of effect of TTO on oestrus cycle of females exposed to this substance in 2-generation study at doses up to 50 mg/kg bw/d, lack of effect in non-reproductive endocrine organs such as pituitary or adrenals.

There were no histopathological changes in thyroid in any repeated dose toxicity study in rats and dogs, no increase in thyroid weight was observed in 2-generation reproduction toxicity study in rats , but increase in absolute thyroid weight was seen in rats exposed to TTO in the repeated dose 90-day oral toxicity study at 60 mg/kg bw/d after 4 and 16 weeks recovery period and in dogs in the repeated dose 90-day oral toxicity study at 120 mg/kg bw/d. These data do not provide clear evidence that TTO may affect HPT axis, since no histopathological changes were seen in hypothalamus, pituitary and thyroid in any study in rats, dogs, and rabbits and the effect on thyroid weight was seen only in some, but not in all studies. However, the potential endocrine effect on thyroid may need to be investigate further, since thyroid hormones were not measured in any study.

The applicant made a thorough analysis of the mode of action (MoA) providing a description of a series of potential biological events, i.e. key events (KEs) that may result in degeneration of spermatogenic epithelium in testis and alterations in epididymis, concluding that cytotoxic destruction of sperm cells may be responsible for all effects observed in male reproductive system of mammals. Tea Tree oil (TTO), and its major active terpene

component, terpinen-4-ol, have been shown to have significant anti-proliferative activity against two tumour cell lines by causing primary necrotic cell death and cell cycle arrest in MTT assay (Greay et al. 2010).

The known cytotoxicity of TTO could thus be responsible for all the effects found in the male reproductive system of mammals, without a need to initiate these effects by disruption of endocrine system, particularly of the HPG axis. In the opinion of RMS, based on the existing data reviewed in this report and based on a fact that no estrogenic and/or anti-androgenic properties of TTO were detected in reliable tests, it is plausible to assume that TTO does not have endocrine disrupting properties and it affects the male germ cells by direct cytotoxic mechanism. It seems to be important to note the effect of TTO on reproductive system has a clear threshold, since at a dose level of 25 mg/kg bw/d this effect is not observed, what does not seem probable for the substance with endocrine disrupting properties. It is however admitted that this conclusion is based on limited number of studies.

To remove the uncertainties of the above assessment of potential ED properties of TTO, if considered necessary, the recommendation on further testing of the OECD Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD 2018) can be applied.

In case of TTO the recommendations listed in scenario D, E, F, G, H and I of the OECD Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption for interpretation of two-generation study (OECD TG 416) may apply, since:

- TTO was positive in OECD TG 416 test and in repeated dose toxicity studies for effects in testis, but negative in in vitro Estrogen Receptor OECD TG 455 without added metabolising system (scenario D) or
- TTO was positive in OECD TG 416 and in repeated dose toxicity studies for effects in testis, but negative in Hershberger Bioassay in Rats (OECD TG 441) and in in vitro Estrogen Receptor OECD TG 455 (scenario E)

In these scenarios the advised steps which could be taken to strengthen weight of evidence is to perform in vitro thyroid hormone receptor (TR), steroidogenesis (S) assays with added metabolising system. *In vitro* androgen receptor (AR) and *in vitro* estrogen (ER) receptor assays does not seem to be needed since the negative results in Estrogen Receptor assay (OECD TG 455) and Hershberger Bioassay in Rats (OECD TG 441) were found. These recommendations are in principle compatible with tests proposed by the applicant:

- OECD TG 456 or OPPTS 890.1200 to sufficiently investigate endocrine activity on S modality,
- OECD TG 440 uterotrophic bioassay to sufficiently investigate endocrine activity on E modality,
- OPPTS 890.1500 male pubertal assay to asses androgen activity with an intact HPG axis and to investigate thyroid parameters, and/or
- OECD TG 408 to investigate hormone levels in vivo especially to conclude on thyroid activity.

Evaluation procedure

In order to determine whether a substance causes adverse effect(s) that can be plausibly linked to endocrine activity, ED-relevant information and supporting toxicity information on the substance needs to be collected and assessed in accordance with the endocrine GD. The starting point is therefore to gather scientific information which is available for the active substance. The standard information requirements for PPPs include a number of studies that were generated in accordance with internationally agreed study protocols (standard studies; see Table 6.8.3-1 Available toxicity studies for Tea Tree Oil) that are useful for the ED assessment. Additionally, non-guideline in vivo studies and in vitro mechanistic studies can often be found in open literature and should also be considered.
For the evaluation the following sources of information were considered: data base information (ToxCast), results of an open literature search and repeated dose toxicity studies in terrestrial and aquatic vertebrates. Due to the availability of a valid and up-to-date Level 5 two-generation reproduction toxicity study, it was decided not to consider further lower-tier Level 1 data since, according to the endocrine GD, the EAS-mediated adversity with regard to humans and mammals is referred to as sufficiently investigated as stated in Point 3.4.1 of the GD when an acceptable, two-generation reproductive toxicity study (OECD TG 416) is avaiable.

Since concerns for endocrine disruption in humans and the environment grew within the last decades, intensive research and development was done to establish validated study protocols especially for detecting endocrine activity or endocrine adversity in mammals and other vertebrates. Meanwhile, there were several OECD TGs available to aid the assessment of endocrine disrupting properties of active substances, and the protocols of most of the toxicological standard studies were reviewed and, if applicable, endocrine sensitive endpoints were added.

These test guidelines are compiled in the OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD GD 150, 2018), which includes the 'OECD Conceptual Framework (OECD CF) for Testing and Assessment of Endocrine Disrupters' introducing a grouping of the studies into five levels according to the kind of information provided.

The OECD GD 150 lists tests (test guidelines) and parameters that are considered relevant when investigating the ED properties of substances. In addition, this document provides guidance on how to interpret parameters relevant for identification of ED properties measured in the standardised test guidelines with respect to EATS[47] modalities. All the parameters listed by the OECD GD 150 (and measured in assays listed in the OECD CF) are grouped into four groups. The grouping reflects the fact that, based on OECD GD 150, some effects are considered to be strong indicators of effects being mediated by an EATS modality, while some others are considered to be potentially sensitive to, but not diagnostic of EATS modalities. Furthermore, some parameters are measured by in vitro- and others by in vivo-test methods.

In general, in vitro-effects provide information on the mechanism through which a substance may exert endocrine activity (e.g. by binding to and activating a receptor), whereas in vivo-test methods may inform on endocrine activity, adverse effects or both.
In the following, data for TTO were evaluated in order to allocate the endocrine relevant effects observed within the standard toxicity studies to the four groups suggested by the OECD GD 150. The same was done for the effects reported within the in vitro/in vivo mechanistic studies. Furthermore, an open literature search was performed according to the guidance document developed by EFSA (EFSA, 2011) with focus on endocrine mechanisms or endocrine adverse effects.

It is suggested that available information is reported in an Excel template provided with the EFSA/ECHA endocrine GD (please refer to Appendix A provided with this document). A data matrix is generated by "extracting" the parameter assessed from standard toxicity studies, mechanistic studies and open literature studies. Both, positive and negative results should be recorded. Furthermore, if available, data from the ToxCast database [48] were reported. Both data from the mammalian toxicology section and the ecotoxicology section has been tabulated in a single spreadsheet.

The excel template consists of several columns which capture different types of information related to the study design and the effects observed in relevant parameters (e.g. OECD TG used, animal species, doses administered, exposure duration, type of effect observed, etc.). In the template, each row reports the changes observed in a certain parameter within a specific study. In order to facilitate the evaluation of the data collected in the template, the data are re-organised into a data matrix which is built automatically. The advantage of the data matrix is that it shows within one row all the effects observed from one study (this allows summarising the information collected that was before spread over several rows in the template). Then, a Weight of evidence approach (WoE) shall be applied for the assessment of the available scientific data i.e. the evidence of adversity should be assessed, both for each individual study (e.g. dose-response, statistical significance etc.) and for all studies combined for a particular effect (e.g. in how many studies decreased testes weight is seen). This step is aided by following the procedure given by the endocrine GD and will end up in so called "lines of evidence summary tables" (see Annexes 1 – 4). Within each group suggested by the OECD GD 150, separate lines of evidence were drawn and assessed, integrated and finally a conclusion for the overall evidence (endocrine vs. not-endocrine) for the respective group was drawn. Originating from these lines of evidence a mode of action was postulated to explain the observed effects. This step also included an uncertainty assessment in line with the requirements according to the endocrine GD document.

2.10.1.1. Evaluation procedure integrated lines of evidence for endocrine activity

2.10.1.1.1 Data base information

It is required by the endocrine GD that information from data bases which can provide information on endocrine modes of action (moa) should also be considered for ed evaluation and included into the matrix.
The US EPA data base TOXCAST is highly recommended for estrogen moa, and gives also information on androgen and thyroidal moa. The TOXCAST data base however, does not contain any more information on tea tree oil.

[47] EATS = estrogen, androgen, thyroid, steroidogenesis
[48] https://comptox.epa.gov/dashboard.

2.10.1.1.2. Literature studies

Mechanistic *in vitro* data can potentially provide evidence for endocrine activity, which is considered a key information in the assessment. Only a limited number of mechanistic (*in vitro*) assays are currently available as oecd test guidelines. Those young aged mechanistic study protocols, especially designed for testing of endocrine activity were not part of the standard data package required for approval active substances used for plant protection and are therefore most often not available initially. However, the open literature can provide further valuable information on endocrine mechanisms, mainly generated from *in vitro* assays. Nevertheless, it has to be noted that those open literature studies were in most cases not performed according to validated and accepted test protocols. It is therefore indispensable to perform an adequate reliability assessment of the published studies which were considered relevant for the assessment of ed properties of an active substance.
For tto an open literature search was performed according to the guidance document developed by efsa (efsa, 2011) as required by regulation (ec) no 1107/2009. The search included the active substan ce tto and its major constituents terpinen-4-ol, γ-terpinene, 1,8-cineole (eucalyptol), and α-terpinene. The search strategy, the results and the relevance and reliability assessment were described in document m-ca, section 9.
An overwhelming number of references were found after searching the data bases for TTO, its single terpene components, its synonyms and Timorex as representative formulation. After an initial rapid assessment for relevance followed by a full text assessment, three studies were evaluated to be of appropriate reliability and are listed below in Table 2.10.1.1.2-1. The remaining references failed the check for relevance and/or reliability and were therefore not mentioned within this document but listed according to EFSA (2011) in M-CA, Section 9, where also the reasons for non-reliability/relevance were presented.

In these three studies, Tea Tree Oil was tested for estrogenic, anti-estrogenic, androgenic and anti-androgenic toxicity. Four different test systems were utilized:

i) An engineered E. coli biosensor ending up in bacterial growth after estrogenic stimulation (Gawrys et al., 2009; (B.6.8.3/05),

ii) A transactivation assay measuring luciferase activity after estrogenic stimulation or antagonism of transfected MCF-7 cells (Henley et al., 2007) (B.6.8.3/06),

iii) A transactivation assay measuring luciferase activity after androgenic stimulation or antagonism of transfected MDA-kb2 cells (Henley et al., 2007) (B.6.8.3/07), and

iv) A MCF-7 proliferation assay measuring the increase in cell number after estrogenic stimulation of proliferation (or decrease of cell number after antagonism of preceding stimulation with estradiol) (Nielsen, 2008) (B.6.8.3/08),.

The two assays using the MCF-7 cell line both showed weak estrogenicity of TTO (Henley et al., 2007, Nielsen 2008). No anti-estrogen or androgen effect was detected. Henley et al. (2007) showed weak anti-androgenic effects for TTO. For Terpinen-4-ol, α-Terpineol and Eucalyptol no influence on endocrine receptors could be detected. It is to be mentioned that in all three studies TTO was identified to be toxic to the test cells which is consistent with its natural antimicrobial properties. A concentration of 0.025 % (v/v) and above of TTO in ethanol was described as the threshold concentration for cytotoxicity (Henley et al., 2007, Nielsen, 2008). The study results were summarized in Table 2.10.1.2..1-1.

Overall, the open literature search revealed studies in which endocrine activity of TTO was reported. However, as indicated earlier, these studies do not follow any agreed guideline for detection of endocrine effects (see Table 2.10.1.2..1-1.). Furthermore, there were some uncertainties which restrict the reliability of these studies. As a consequence, the results of these studies should rather be interpreted as a possible indication of endocrine activity of TTO in mammalian cell systems. The uncertainty due to the missing validation of the in vitro test method is too profound to conclude with evidence that TTO exhibits endocrine activity. Furthermore, the tested concentrations were also of notable importance. As expected, the estrogenic activity expressed in the cell-culture assays was dose dependent. The response was negative at low levels and became positive at first at levels about a million times higher than the effective estradiol concentration, which was the positive control. Hence the estrogenic activity of TTO must be considered as very weak. The same is true for the anti-androgenic activity reported by Henley et al. (2007).

In terms of ecotoxicological relevance, no data on endocrine disrupting properties of TTO or its constituents on terrestrial or aquatic organisms (vertebrate and invertebrate) has been found.

Table 2.10.1.2..1-1 Results of the open literature search after evaluation of the found references for relevance and reliability (references can be provided upon request)

Endocrine mechanism tested	Test item	Test system	Tested concentrations	Outcome	Reasons for restriction of study reliability	Reference
Estrogen	Plantlife® TTO 100% pure oil	An engineered *E. coli* biosensor strain expressing the ligand-binding domain of the human estrogen receptor β (ERβ) as part of a larger allosteric reporter enzyme. The result is a bacterial growth assay, where estrogenic activity of a test compound is reflected by increased cell growth on a simple defined medium.	Tenfold serial dilutions of the pure oil in ethanol. Concentration range was not given	No estrogen induced effect could be observed for TTO; at the tested concentrations TTO was toxic to cells; consistent with its natural antimicrobial properties.	• the results for TTO were only described in words, no numerical or graphical data shown • Concentration range of serial dilutions was not given • concentrations at which cytotoxicity appeared were not given • described method is not a validated test method • the source of the test system was not given	Gawrys, 2009

Endocrine mechanism tested	Test item	Test system	Tested concentrations	Outcome	Reasons for restriction of study reliability	Reference
Estrogen Androgen Anti-androgen	pure TTO	<u>Estrogenicity:</u> MCF-7 cells transiently transfected with an estrogen-inducible luciferase reporter plasmid containing an estrogen-response element (3x-ERE-TATA-luciferase), measurement of luciferase activity Expression of estrogen-regulated endogenous genes <u>(Anti)-androgenicity:</u> MDA-kb2 cells stably transfected with an androgen-inducible and glucocorticoid-inducible mouse mammary-tumor virus (MMTV)-luciferase reporter plasmid, measurement of luciferase activity Expression of androgen-regulated endogenous genes	Dilutions of the pure oil in ethanol 0.001, 0.005, 0.01 and 0.025% (v/v)	<u>weak estrogen</u> (max. response at 0.025%) Cytotoxicity above 0.025% <u>weak anti-androgen</u> (max. response at 0.005%) <u>no androgenicity</u>	• method of endpoint determination not clearly described • measurement values for positive control missing • Number of cells not given • maximal concentration of solvent in exposed cells not given • information on test system properties, conditions of cultivation and maintenance only fragmentary reported	Henley, 2007 *

Endocrine mechanism tested	Test item	Test system	Tested concentrations	Outcome	Reasons for restriction of study reliability	Reference
Estrogen Anti-estrogen	TTO Terpinen-4-ol α-Terpineol Eucalyptol Eucalyptus oil	MCF-7 cell proliferation assay	Serial dilutions in ethanol. Concentration range between 0.00075 and 0.1% (v/v)	weak estrogenic response for TTO (max. response at 0.0125%) no anti-estrogenic response no hormonal response for other test items Cytotoxicity at 0.025% and above	• purity of TTO not stated • measurement values for positive control missing	Nielsen, 2008

*Please note: The publication by Henley *et al.* (2007) consists of an *in vitro* and an *in vivo* study part. The *in vitro* part is tested for relevance and reliability according to the criteria which were established within the literature search project required by EFSA (EFSA, 2011). The outcome of the study, a weak estrogenicity for TTO, is summarized in section B.6. Furthermore, an observation study in young boys developing potentially estrogen induced prepubertal gynecomastia after the usage of TTO containing home care products is reported. This study part was also evaluated according the criteria of relevance and reliability for *in vivo* studies, however failed the reliability check in several issues. Therefore the *in vivo* part of Henley *et al.* (2007) is not mentioned within this document but listed as prescribed by EFSA in Section M-CA 9, where also the reasons for non-reliability were presented.

The lines of evidence for mechanistic parameters based on results of these studies are presented in Annex 1 of this section.

2.10.1.1.3. In vitro and in vivo mechanistic information

To rule out the uncertainties which arose during literature search with respect to estrogenicity and androgenicity, and to be sure not to miss endocrine activity induced by Tea Tree Oil, the applicant decided to perform further tests according to internationally agreed OECD guidelines to test for possibly underlying mechanisms of endocrine disruptive action: A Hershberger Assay to test for (anti)androgenicity (OECD 441; in vivo mechanistic; OECD CF level 3) and an Estrogen Receptor Transcriptional Activation assay (OECD 455; in vitro mechanistic; OECD CF level 2) to test for estrogenicity (See Table 6.8.3-1). In both test systems, the response to TTO was clearly negative compared to the respective positive controls. Therefore, the concerns raised within the open literature for estrogenicity and androgenicity of TTO could be eliminated.

Concluding from the above presented information, further studies to complete the investigation on endocrine activity are proposed in accordance with the guidance document. As androgen modalities are quite advancedly investigated, a male pubertal assay (OPPTS 890.1500) is proposed to fully conclude on any effects regarding the HPG axis.

Furthermore, this test can be used to start an investigation on thyroid activity. Based on the available studies the T-modality of endocrine activity has not been sufficiently investigated. Therefore, to fully conclude the investigation on thyroid hormonal activity, in vivo studies are necessary according to the guidance document. To avoid unnecessary testing, a 90-day rat study according to OECD TG 408 including hormonal measurements should be conducted. This would either prove that no thyroidal activity is found or, if a hormonal imbalance is observed, further investigations would be required. As an additional benefit, a measurement of sexual hormones is also foreseen by the design of this study type. This information is useful to substantiate the proposed MoA.

For the lines of evidence for mechanistic parameters please refer to Annex 1

2.10.1.2. Integrated lines of evidence for adversity

2.10.1.2.1. EATS-mediated parameters

2.10.1.2.1.1 Evaluation of observed effects in male reproductive organs/tissues

To discuss the observed effects on the male reproductive organs the general function has to be taken into account.

As reviewed by Lanning et al. (2002), the most common morphological consequence of injury is a disturbance in spermatogenesis almost regardless of the cellular target of toxicity within the reproductive system. This is because spermatogenesis is sensitive to functional perturbations taking place in most other parts of the reproductive tract. Spermatogenic disruption may reflect a direct effect on the seminiferous epithelium, affecting either the Sertoli cell or any one of the germ cell populations, or it may occur as a secondary response to altered hormone levels, altered vascular supply, or altered fluid balance, either within the testis or within the epididymis.

The pattern of disturbance can be very specific and diagnostic of the mechanism of toxicity, but generally this is only seen during the early development of the lesion. With longer periods of dosing, the development of maturation depletion (whereby death of a specific precursor germ cell causes the progressive loss of its descendant generations) reduces the specificity of the pattern of spermatogenic disturbance as the tubules become depleted of more and more germ cells.

Lanning et al. (2002), Creasy et al. (1997), and Creasy et al. (2001) provide a detailed description of damages in the reproductive tissues, mainly testicular damage as well as epididymides impairments and their causes.

The sperm parameters measured in the studies available for TTO are briefly described below to give an idea of how changes in theses parameters could be interpreted in the context of other findings.

Studies have shown that there is a relationship between sperm motility and fertility, but there is no generally accepted standard of how much of a change in motility should be considered adverse. The specific protocol should be considered, as well as information from the other sperm parameters and histopathology. Since sperm motility is dependent on testicular and epididymal function, damage to the testis, as revealed by the histologic appearance, may lead to changes in sperm motility. However, motility can be altered by direct effects on the epididymides or the sperm themselves (OECD, 2008).

Sperm morphology is related to fertility, but there is no generally accepted standard of how much of a change in morphology should be considered as being adverse. Information on sperm motility and count, as well as histopathology should be considered in the interpretation of sperm morphology. Histological lesions of sufficient

magnitude can impact sperm morphology. However, normal sperm morphology is dependent on numerous factors including the correct assembly of protamines, so changes in sperm morphology should not be disregarded in the absence of histological lesions (OECD, 2008).

Studies have demonstrated a strong relationship between sperm counts and fertility in all species that have been examined. Again, information on the other sperm parameters and histopathology should be considered in the overall interpretation. Testicular lesions of sufficient magnitude will be reflected in the sperm counts, but changes in sperm counts should not be disregarded in the absence of histological lesions. Similarly, a reduction in sperm count may not result in reduced fertility, particularly in rodent studies. This is due to the fact that rats and mice have a tremendous excess of spermatozoa in their ejaculates, and as such sperm counts have to be reduced by as much as 90% to affect fertility. It is important to note that sperm concentrations in human males are highly variable and generally lower than in rodents. The distribution of counts is such that many men have sperm concentrations near or below WHO reference values for fertility. Therefore, even a small decrease in sperm concentration across a population would be expected to shift the fertility potential of the group and move some men into the infertile or subfertile range. For this reason, a statistically significant change in sperm count in a rodent study is considered to be indicative of a potential effect on fertility in humans (OECD, 2008).

Also, further reproductive organs which have not found to be impaired have to be considered when interpreting the results.

In the prostate and seminal vesicles, the major response would be increased or decreased secretory activity, which often becomes manifest as a weight change. Since the secretory activity of the accessory sex glands is extremely sensitive to androgen levels, weight change and altered secretory activity in the prostate and seminal vesicle can be used as a good, and relatively rapid, integrated indicator of altered circulating androgen levels.

The structure of the male rat mammary gland responds to hormonal stimulation, and can serve as a sensitive indicator for endocrine disruption. Disruption of circulating levels of estrogen, androgen, prolactin, growth hormone or their receptors has the potential to result in detectable changes in the male and/or female mammary gland. The main changes are atrophy, hyperplasia, or altered differentiation (feminization of male pattern or masculinization of female pattern). The precise change will depend on the hormones that have been disturbed. However, in most cases, disruption of one hormone generally causes secondary disruption of other hormones, so the picture can be complex (OECD, 2009a, b; part 4).

Lanning et al. (2002), Creasy et al. (1997), and Creasy et al. (2001) provide a detailed description of damages in the reproductive tissues, mainly testicular damage as well as epididymis impairments and their causes.

The observed effects for TTO are confined to the testis and epididymis which show degenerative changes and in correlation a reduction in organ weight.

The observed changes in sperm number motility and morphology indicate a cytotoxic mechanism which leads to a reduction in sperm number and motility as well as to a changed sperm morphology.

This mechanism is supported by the absence of findings in the prostate, seminal vesicles and mammary gland as well as by the fact that the fertility is impaired and the number of implantations is significantly lowered. Due to the long duration of the studies only late stages were observed. Additionally, also in ecotoxicological studies an impaired fertility was observed which might support a cytotoxic destruction of sperms throughout different species.

In case of an endocrine mechanism further reproductive organs would show changes in weight and histopathology (OECD, 2009a, b; Part 2).

In the large and small intestine histopathological changes like hyperplasia and hemorrhage were found. These tissues are also sensitive for cytotoxic mechanisms therefore the finding supports the postulated toxicological mechanism. (Delgado et al., 2016)

As TTO is highly volatile it probably has molecular characteristics regarding size and stability to pass the blood testis barrier to cause the observed cytotoxic effects, whereas more robust organs like liver or kidney only react at much higher concentrations.

Furthermore, the mechanistic in vitro studies show strong cytotoxicity already at low concentrations which supports the theory that especially the sperms which are sensitive to cytotoxic effects are reduced by TTO.

In addition, the observed effects show a clear dose dependency as they are only observed at mid or high dose levels and not at low doses tested. Taking into account the complete reversibility demonstrated in the 8- and 16-

weeks recovery period study to cover the full spermatogenesis cycle the observed effects rather show a cytotoxic pattern than an endocrine mode of action (Morthorst et al., 2010)

Two parameters known as sensitive to endocrine disruption show inconclusive results. The ano-genital distance is slightly decreased but well within the range of historical control data. This parameter is also correlated to the body weight. As shown by Gallavan et al. (1999), this correlation can explain the slight changes in this parameter within historical control ranges.

The age at balanopreputial separation also gave inconclusive results. According to Engelbregt et al. (2000) intra uterine growth retardation or undernutrition can also result in a delayed onset of puberty. Consequently, this effect is not necessarily evoked by endocrine disruption.

To assess the effect of TTO on the intact HPG axis, the male pubertal assay according to US EPA OPPTS 890.1500 can be a useful study as it is specifically designed to assess endocrine disrupting properties and includes more specific parameters than a normal repeated dose study and can accordingly be recommended in this case.

For summary of the lines of evidence for EATS mediated parameters based on the effects observed in male reproductive organs/tissues please refer to Annex 2.

2.10.1.2.1.2 Findings in female reproductive organs/tissues and in thyroid

Female reproductive tissues are also sensitive to hormonal imbalance and several patterns of disruption would become visible if those organs or tissues were exposed to hormonal acting substances. For a detailed scientific review of the female reproductive system, see (OECD, 2009a, b; Part 3, section 1 - 5).
Endocrine disrupting substances can affect the hypothalamic-pituitary-ovarian (HPO) axis in several ways, producing alterations in the female reproductive tract that may be detected histologically. Endocrine disruptors may cause overt histological changes in the vagina, uterus and ovary that are relatively straightforward to identify. Within the study, parameters were determined that would have to show a clear effect under endocrine influence, e.g. the uterus and mammary gland weight and distinct histopathological changes, particularly in view of the repeated exposure of 28 or 90 days.
There were no effects with significant toxicological relevance found within the TTO toxicity studies. If so, in the absence of histopathological abnormalities, these effects were considered as being not of toxicological relevance with respect to endocrine disruption.

The absence of any finding in the female reproductive organs throughout the studies conducted with TTO supports a cytotoxic mechanism as tissue sensitivity to cytotoxicity can be different and effects which are observed only in strictly confined tissues cannot be attributed to a general endocrine mechanism.

The thyroid weight changes were only seen during recovery period and are probably a secondary effect of liver toxicity since an induction in metabolic activity in the liver often leads to an increase in thyroid activity. This effect is a known mechanism of general toxicity (Brändli-Baiocco at al., 2018, Melching-Kollmuss presentation at Fresenius conference on endocrine Disruptors 2018)

For summary of the lines of evidence for EATS mediated parameters based on the effects observed in female reproductive organs/tissues please refer to Annex 2.

2.10.1.2.2 Parameter sensitive to, but not diagnostic of, EATS

2.10.1.2.2 .1 Further developmental parameters gathered from toxicological and ecotoxicological studies

Developmental parameters as gathered in developmental and two generation studies in toxicology and ecotoxicology often help to identify endocrine mechanisms as developmental and reproductive parameters can often be used to detect endocrine patterns. Though some parameters in the presented studies show effects, there is no conclusive pattern that can be attributed to an endocrine mode of action. This is supported by the fact, that there were no histopathological findings in the tissues of the female reproductive tract as described in section B.6.8.3.

Within the 2-generation toxicity study, several fertility and gestational parameters were affected at the highest tested dose in the F1 generation (see Table 6.8.3-1 and Annex 3).

These endpoints are not specifically indicative of an endocrine-mediated mechanism of toxicity per se - they may also be influenced by overall growth and health status of the animal, or by toxicants that influence homeostasis of gravidity through other mechanisms.
In case of TTO several of the reported effects either turned out to be statistically not significant or within the overall historical control data (HCD) deliverd by the test institute. Those effects have been reported in Appendix A but will not be further considered when evaluating the endocrine properties of TTO.

For TTO, an impairment of sperm integrity (number, morphology and motility) was observed in several studies. It is reported that reduced sperm quality may be accompanied by an overt effect on reproductive outcome (ECETOC, 2002; OECD, 2008). In studies where males were treated with a sperm toxicant (e.g. metronidazole, nicotine or tolnidamine) and were mated with untreated females, reduced fertility, increase in post- and pre-implantation loss, decreased litter size and decreased litter weights has been observed (Oyeyipo, 2011; Kumari, 2013; Singh, 2008; Sharp, 2012). The authors, however, were only speculating which mechanisms may be causative. Nevertheless, since the sperm damage induced by the applied test substances is comparable to those observed with TTO it is reasonable to assume, that the impairment of fertility and the higher frequency of pre-natal deaths could be attributed to the reduced sperm quality and should not necessarily be considered as an effect induced by disruption of the hormonal balance during gravidity.
In the 2-generation study, the litter/pup weight is considered to be decreased. This is most likely due to due to extensive exposure of the pups via breast milk, since a constant level of the test item in the diet leads to peaks of exposure to the neonate from the end of the first week of lactation when the offspring is potentially exposed to the test compound by the dietary route (and consumes a high amount of diet relative to its bodyweight) and via lactation (ECETOC, 2002).

In addition to those effects observed in the 2-generation study, there were also developmental effects observed in the prenatal developmental toxicity studies in rats (reduced fetal weight, increased number of resorptions and increased number of females showing resorptions) and rabbits (increased post implantation loss, increased number of resorptions). Since in this kind of study type no males, but only the pregnant females are dosed after implantation, the underlying mechanism must be different from the mechanism assumed for the 2-generation toxicity study. As mentioned above, embryonic and foetal development depends on the overall growth and health status of the dams. It is frequently reported that maternal toxicity might also be causative for developmental impairment (ECETOC, 2002; OECD, 2008). Within the developmental toxicity study with rats, an affected health status of the dams was observable (reduced food consumption and body weight gain) even at concentrations lower than the respective developmental effect concentrations. Therefore, it is reasonable to assume that the observed foetal effects were due to maternal toxicity rather than endocrine disruption.

It is further notable that mainly parameters concerning survival of the embryos or pups are affected whereas parameters concerning developmental effects do not show any deviation from control animals. The number of embryonic deaths, pre- and post-implantation loss are increased. The same can be seen for the ecotoxicology where the egg viability is decreased and less viable embryos and hatchlings are detected. For an endocrine pattern, however, also developmental parameters like genital abnormalities or sex ratio should be impaired as well, but these parameters do not show any deviation from the control animals. In addition, the more sensitive EAS parameter such as an estrus cyclicity is not affected.

This inconsistency of an endocrine pattern can be found at different points in the lines of evidence and thus is not conclusive for an endocrine pathway but rather for a general toxicity mechanism.

Taking the whole effect pattern into account, an endocrine mechanism of the effects on fertility, gestational and developmental parameters is highly unlikely and a general toxicological mechanism based on the strong cytotoxic effect of TTO is more in line with the findings presented. However, to provide further evidence that the underlying mechanisms are not EAS mediated, it is recommended to perform further mechanistic testing for endocrine activity as required by the ED GD (see section 3.4.2 and Table 3 of ED GD). For TTO there are already two mechanistic studies available, i.e. estrogen receptor agonism and androgen receptor (ant)agonism (see section B.6 of this document). Since the Level 2 test for estrogenicity gave negative results, it is necessary to go for Level 3 testing. The uterotrophic bioassay (OECD TG 440) is adequate in this case. For the androgen modality, Level 3 testing is already addressed since a Hershberger assay (OECD TG 441) is available. According to the ED GD it is also required to test for the steroidogenesis modality of endocrine activity. For TTO, no tests are available. Therefore, further testing is necessary, the steroidogenesis assay (OECD TG 456) or the aromatase assay (OPPTS 890.1200) are feasible.

If all Level 2 and Level 3 tests will provide negative results, it is evident to conclude that TTO does not exhibit endocrine activity via EAS mediated mechanisms and that it is highly unlikely that the observed effects on fertility and developmental parameters as described above have been induced by endocrine mechanisms - consequently supporting and strengthening the argument that it is more reasonable to assume that direct toxic effects or maternal effects were causative.

For summary of the lines of evidence for parameters sensitive to but not diagnostic of EATS refer to Annex 3.

2.10.1.2.2.2 Findings in male and female non-reproductive endocrine organs/tissues

In addition to hormone-sensitive reproductive organs, further organs are involved in hormonal regulation and homeostasis, with the pituitary gland involved in each of the hormonal axes. Those three axes are the hypothalamic-pituitary-gonadal- (HPG), the hypothalamic-pituitary-adrenal- (HPA) and the hypothalamic-pituitary-thyroid- (HPT) axis. The main organs involved are the adrenals and the pituitary. Depending on the mechanism of disruption, common findings are increase or decrease of organ weight and atrophy or hypertrophy of glandular tissue. The mechanisms of disruption are quite complex and thus the effect pattern might be very diverse.Adrenals and pituitary were weighed and histologically evaluated in most of the available TTO studies.

Neither in male nor in female animals any effects have been observed which were of toxicological relevance. Some findings are common background findings and can be considered to be incidental. The organ weight changes in the adrenals are not consistent and therefore cannot be regarded as conclusive for an endocrine mechanism.
The described hypertrophy of the zona fasciculata is known to be induced by stress and also to occur during the aging process of rats (Ulrich-Lai, 2006 and Laast, 2014). It was described as incidental by the study authors and no dose dependency could be established. As the rats in the described studies were not old enough for an aging lesion, the most plausible explanation is that the observed lesions were stress induced especially as rats are normally held in groups which always bears the possibility of stress due to inter-individual rivalries. This would also conclude for the lacking dose dependency and the fact that in most of the studies these lesions could not be reproduced or other follow up lesions like hyperplasia or tumors were observed.

With the exception of the thyroidal effects described under point 0, there were no further histopathological effects of toxicological relevance observed within the thyroids.

For summary of the lines of evidence for parameters sensitive to but not diagnostic of EATS observed in male and female non-reproductive endocrine organs/tissues please refer to Annex 3.

2.10.1.2.2.3 Evidence of general toxicity

In addition to the above results, effects have also been noted, which in particular indicate a general toxicity of TTO. As such, food consumption in rats during the gestation or lactation period shows a dose-dependent decrease already at a test concentration of 10 mg/kg/day. While these effects are treatment-related, they are not considered to have an adverse effect, as reduced food intake at this level has revealed no effect on body weight. This relationship, however, is immediately detectable at higher test concentrations. Here, the body weight shows a significant, dose-dependent decline starting at an effect concentration of 50 mg/kg/day. In the case of rabbits, such a decline has been observed as early as 30 mg/kg/day, which also corresponds to the concentration at which a decline in food consumption has been recorded for this species. As a non-mammalian representative, the same correlation between dose-dependent reduction in food intake and body weight reduction was also found in a tested bird species (Japanese quail), however, only at test concentrations of 300 mg/kg/day in males and or 1000/600 mg/kg/day in females, respectively.
As a further sign of general toxicity, an increased mortality of adult rats was observed occasionally at an effect concentration of 120 mg/kg/day and in adult Japanese quails at 1000/600 mg/kg/day, respectively, which in both cases corresponded to the highest test concentration. In addition, quails at this dose also showed clinical signs in the form of e.g. reduced motility, staggering gait and ruffled feathers. The increased slight salivation seen in the two-generation study with rats, however, is considered non-treatment related. Apart from these exceptions, the test organisms were without findings in terms of mortality and clinical signs. Also, no abnormalities were detected during macroscopic inspection.

For the most part, this also applies to the target organ toxicity, where no effects on kidney histopathology, kidney weight, spleen histopathology, spleen weight and thymus weight were found. In contrast, slight changes could

be measured in relation to some liver parameters. Here, rats showed vacuolation and an increase in liver weights at the highest test concentrations (60, 120 and 125 mg/kg/day, respectively). In this regard, the increased thyroid weight seen in two repeated-dose 90-day oral toxicity studies in rats during the subsequent recovery period may be considered a secondary effect as described above.
The histopathological findings in the small and large intestine in the dog in the form of lymphoid hyperplasia, autolysis and haemorrhage in the Peyers patches, which were recorded at an effect dose of 120 mg/kg/day, can be considered as further supporting evidence for a cytotoxic mechanism which, in addition, may be closely related to the effects observed in the male (mammalian) and female (avian) reproductive organs.
For summary of the lines of evidence coming from findings in general toxicity please refer to Annex 4.

According to the endocrine GD, also fish and invertebrate data have to be included in the assessment, in order to be able to conclude on the ecotoxicological relevance of potential endocrine disruptive properties of the active substance under consideration. For TTO, a fish early life stage toxicity test (FELS, OECD TG 210, CF level 4) with fathead minnow as test species is available. For this type of study, the endocrine GD states that it has not been reported in the example evaluation table (Table 15, pp.78) since it "includes only 'sensitive to, but not diagnostic of, EATS' parameters". Nevertheless, the study should be (and has been) evaluated for effects related to such parameters and any relevant results are to be reported. However, since - except for a slightly increased cumulative mortality at the highest test concentration (3.15 mg/L) - no statistically significant (and no non-significant) effects relevant for endocrine evaluation were found, no further data from this study has been included in the lines of evidence assembly procedure but is instead described here.

While, as mentioned above, information on invertebrate non-target organisms, if available, should be considered in the assessment applying the general principles of the endocrine GD, due to the scarce knowledge on the endocrinology of invertebrates the GD does not specifically cover these organisms and does not provide explicit assistance on how to adapt this information for evidence assembly. In terms of TTO, a *Daphnia magna* reproduction test (OECD TG 211) is available, covering test concentrations (measured) of 0.132 to 5.75 mg/L. The results of this test revealed a statistically significant, dose-dependent and treatment-related increase of adult mortality and decrease of reproduction (based on number of offspring) starting from 0.747 mg/L. For the two highest test concentrations (2.02 and 5.75 mg/L), adult mortality was 100 %. Compared to control levels, the mean numbers of offspring dropped from 106 to 4.4 at 0.747 mg/L, simultaneously accompanied by a marked increase of stillborn juveniles, aborted eggs and overall percentage of dead juveniles at the same treatment level. However, even if considering the drastic explicitness of these results, the nature of the findings points towards adversity by general toxicity rather than endocrine disruption, since it can be considered highly likely and assumable that a substance, that causes 100 % mortality at the next higher test concentration, may already cause severe reproductive impairment at a lower treatment level. All in all, these findings principally also comply with the effects on reproductive organs found in terrestrial vertebrates, however, it cannot be excluded that Daphnids are even more susceptible to the active substance due to their small body size and the additionally intensified contact via their respiratory surfaces.

2.10.1.2.2.4 Mode of action (MOA) analysis

To identify a link between the observed effects and observed activity, a mode of action analysis has been performed. The above presented lines of evidence have been assessed and the conclusions that have been presented were then combined to postulate a mode of action which explains the effects seen.
As the effect pattern does not support an endocrine mode of action, a non-endocrine mode of action based on general cytotoxicity has been developed.

	Brief description of key events (KE)	**Supporting evidence**
Molecular Initiation Event (MIE)	Cell cycle arrest and necrosis	Cell debris seen in histopathology
KE 1	Uptake in the intestine	Lesions in the large and small intestine indicating cytotoxic effects
KE 2	Passage of blood testis barrier	Only testis and epididymis are affected in all species
KE 3	Cytotoxic destruction of sperms	Reduced sperm numbers and motility, changed sperm morphology
Adverse effect (AE)	Degeneration in testis and epididymis	Histopathology

According to Greay et al. (2010) TTO causes cytotoxicity via cell cycle arrest and necrosis. This can be also observed in the intestine after oral application and also after passage of the blood testis barrier in the testis and epididymis. This leads to a reduction in sperm number and in the observed degenerative changes in the histopathology.
In the following table, the conclusion on the biological plausibility of the link between the adverse effects of TTO and the above postulated MoA for general toxicity is summarized.

Table 2.10.1.2.2-1: Summary of biological plausibility

	Key event relationships (KER)				
	MIE to KE 1		**KE 1 to KE 2**	**KE 2 to KE 3**	**KE 3 to AE**
Biological Plausibility for the KERs	Moderate – It is known that TTO has a cytotoxic effect but the exact mechanism based on cell cycle arrest and necrosis is still not completely understood.		Moderate – after the uptake from the intestine TTO is transported in the blood stream; However, passage of the blood-testis barrier is not fully investigated but the confinement of effects found indicates a passage.	Strong – in case of a passage through the blood-testis barrier the cytotoxic effect on the sperms is biologically plausible.	Strong – it is a known mechanism for TTO to induce cytotoxicity and cell death which leads to the observed degenerative changes.
Empirical support for the KERs	Moderate – Cytotoxicity is clearly stated in different *in vitro* studies, the lesions in the intestine were however only seen in one species (dog).		Moderate – the confined changes are observed in a dose dependent manner in more than one species.	Strong – in all studies where sperm parameters were measured changes were observed in a dose dependent manner. Full reversibility was shown.	Strong – the observed degenerative changes were comparative in all studies and showed reversibility in rat and dog.
	MIE	**KE 1**	**KE 2**	**KE 3**	**AE**
Essentiality of KEs	No data				
		Moderate- there are no stop studies but reversibility was assessed and after dosing was stopped the observed effects were completely reversible.			
			See KE 1		
				See KE 1	
					See KE 1
Consistency	The KEs have been observed consistently in five different studies with different duration. The pattern of effects is consistent between the studies, there are no conflicting observations. Consistency across species is given as the effect was seen in rats and dogs.				
Analogy	No information. But other essential oils are known to show cytotoxic effect as well. A MoA has not been developed.				
Specificity	The cytotoxic effect is well known and still only a very confined compartment is showing adverse effects indicating a specific reaction in these tissues.				

Identified uncertainties	**Comment**
Uncertainty 1 [Lack of a full understanding of the MIE]	Cytotoxicity can also be observed due to apoptosis.

Uncertainty 2 [KE 2 not proven]	The passage itself cannot be proven with the information available.
Uncertainty 3 [KE 3 only observed in one species]	It is known that interspecies differences may lead to such observations.
Overall conclusion on the Postulated MoA	
The postulated mode of action describes a cytotoxic process which is initiated by cell cycle arrest and necrosis. After passage of the intestine and absorption in the blood, the blood testis barrier is passed and sperm damage and degenerative changes in testis and epididymis are observed. The observed KEs show a moderate (MIE KE 1 and KE 3) to strong (KE 2, KE 4 and AE) biological plausibility and empirical support. The essentiality cannot be fully assessed but reversibility in different species has been demonstrated. Consistency between studies and species have been demonstrated. Regarding the confined effects found in a definite compartment and the known cytotoxic property of TTO, this mode of action is biologically plausible. The observed effect pattern does not support an endocrine mode of action.	

2.10.1.2.3. Summary and discussion of endocrine disrupting properties of tea tree oil

During an open literature search for endocrine activity and endocrine disruptive properties of TTO, three studies passed the assessment for relevance and reliability as described in Table B.6.8.3.1- Within two of those studies, TTO showed weak endocrine activity. One study reported estrogenic and anti-androgenic activity; the other study reported TTO showing estrogenic activity. As already mentioned, there were some uncertainties which restrict the reliability of these studies – specifically since these studies do not follow any agreed guideline for detection of endocrine activity. As a consequence, the results of these studies should rather be interpreted to give a possible indication of endocrine activity of TTO in mammalian cell systems. The uncertainty due to the missing validation of the applied in vitro test method is too profound to conclude with evidence that TTO exhibits endocrine activity. To confirm (or disprove) the literature findings, two further tests according to internationally agreed OECD guidelines were performed. A Hershberger Assay to test for (anti)androgenicity and an Estrogen Receptor Transcriptional Activation assay to test for estrogenicity. In both test systems, no test substance-related (anti)androgenic or estrogenic activity was observed. Therefore, the concerns raised within the open literature for estrogenicity and androgenicity of TTO could be eliminated.

In order to evaluate whether oral exposure to TTO might disrupt the hormonal system due to potentially endocrine activity, all available in vivo tests were inspected for apparent abnormalities within the test animals which are indicating hormonal disruption. For TTO there is no explicit evidence of endocrine disruption. The affected sperm parameters are most likely the consequence of a direct toxic impairment of the loci of spermatogenesis. This is shown by the outcome of the in vivo studies. All shown damages or observations seem to be the result of impaired spermatogenesis. Such a massive impairment of spermatogenesis already occurring after 28 days however might also indicate the reaction to a substance with low androgen potential, which in turn should exert a clear effect on the other hormone sensitive tissues since even low levels of androgen were sufficient to disrupt the regulatory mechanisms of the HPG axis with its consequences on testicular function and secretory function of the prostate and seminal vesicles (OECD, 2009a, b; Creasy, 2001). Within the studies with TTO, such organs, which are extremely sensitive for hormonal influences, do not show any abnormalities. Neither weight nor histopathology has changed when compared to the control animals. This applies for male as well as for female individuals. In both sexes estrogens as well as androgens should show influences on the hormone sensitive tissues as they are able to affect the hypothalamic- pituitary axes already in small quantities. This also applies for substances that reduce the supply and effect of testosterone and estrogen. Anti-androgen effects cannot be reliably depicted in females (Kunimatsu et al., 2004). Such clearly confined effects exclusively affecting testis and epididymides do not correspond to any typical endocrine pattern which would be expected after exposure to an endocrine disruptive substance.
A comparable situation arises with regard to the reproductive, developmental and fertility parameters. Increased embryonic death in rats, rabbits and quails, reduced litter and egg viability could principally be ascribed to a cytotoxic mode of action, since, in case of an underlying endocrine MoA, disruptive effects on estrus cyclicity or sex ratio should also have occurred. However, these parameters remained unchanged in the studies.

In both sexes, TTO generally has no harmful impact on the non-reproductive endocrine organs. There is no histopathological evidence of the HPA or HPT axis being impaired. Spleen and liver are additional target organs not playing a pivotal role in terms of endocrinology. Moreover, there is no convincing evidence for a disruption of the HPG axis, since as mentioned above, the spermatogenesis is indeed clearly impaired, but none of the other hormone sensitive tissues (e.g. the weight and histopathology of the uterus, vaginal histopathology, mammary glands and prostate) displays any harmful effect.

Another point which has to be considered, is the presence of a distinct dose dependency of the effects, which becomes clearer in the 28-day study since there was a further higher dose tested showing a clear increase in the number of affected animals and severity of effects in highest dosed animals. This certainly does not rule out any endocrine action. However, especially for testicular damage, the peculiarity of an inverse dose dependency can be observed in a way that exposure to higher concentrations of androgens does not necessarily lead to more severe testicular damage. Indeed, exposure to low doses of exogenous androgens impair the hormonal axis leading to testicular damage and degeneration accompanied by disrupted spermatogenesis, whereas exposure to higher concentrations mask the impairment of the hormonal axis by local effectiveness in testis tissue, restoring the hormonal function.

Antimicrobial and cytotoxic properties were described for TTO (Shapiro et al. 1994; Soylu et al., 2006; Gawrys et al., 2009; Henley et al., 2007; Nielsen, 2008). It is quite plausible that these properties could also be causative for testicular damage observed in the rats. It is not known whether TTO is distributed into testicular tissues since adequate studies were missing, leaving the mechanism of toxicity partly unresolved. Nevertheless, it is conceivable that TTO is able to overcome the blood-testes barrier and due to its cytotoxic properties damages cells (Leydig or Sertoli cells) within the fragile system of spermatogenesis without showing signs of general toxicity in more robust organs like e.g. the liver.

In terms of ecotoxicological relevance, according to guidance reference should be made to the results of the mammalian evaluation, while additionally all available information as to other vertebrates and invertebrates should be considered. For TTO, an avian reproductive study along with a fish early life stage study are available for vertebrate assessment, while only one study investigating chronic toxicity on Daphnia magna is available to assess effects on invertebrates. The results of the avian study have already been discussed within the context of the mammalian evaluation. The FELS-study yielded no effects assignable to endocrine activity nor adversity. In the aquatic invertebrate study, severe reproductive effects were detected at low exposure levels. However, since mortality reached 100 % already at the next higher dose levels, these effects could most likely be explained by a state of declining health, resulting in impaired reproductive capacity.

To ensure a better overview of the above described effects table 2.10.1.2.3-12 and 2.10.1.2.3-2 summarize all effects and also parameters that have not been measured in the available studies separated for the T modality and for the EAS modalities.
These tables should also be helpful to conclude on studies needed for further investigations as especially endocrine activity has not been sufficiently investigated to conclude on this point. Furthermore these tables summarize quite well the effects with regard to an endocrine pattern as has been described above in this document.
Especially for the T modality the table shows that essential hormone measurements have not been conducted therefore these data gaps will be addressed in the next chapter. Furthermore the available evidence could be interpreted as a consequence of general toxic effects which should be clarified by further investigations.

Table 2.10.1.2.3-1: Summary of Effects of T-mediated adversity for TTO

Endpoints for T-mediated adversity as listed in ED GD	**Endpoints for T-mediated adversity available for TTO**	**Species**
HDL/LDL ratio	not measured	rat, dog
Liver Weight	no effect	dog
	increase	rat
Thyroid histopathology	no effect	rat, dog
Thyroid weight	increase	dog, rat
T3/T4 level	not measured	dog, rat
TSH level	not measured	rat, dog

For the EAS modalities the main parameters have been evaluated in the available tox studies and the detailed explanations have been given in the above sections regarding possible toxic mechanisms. It becomes quite clear

that there is a lack of effects in the female reproductive system which is not in accordance with an antiandrogenic mode of action which could be supposed by the efects recorded in the testis and epididymides. Aigain it has to be emphasized that still some findings are inconclusive and have to be further investigated with special regard to endocrine activity .

Table 2.10.1.2.3-13 - 2: Summary of Effects of EAS mediated Adversity for TTO

Endpoints for EAS-mediated adversity as listed in ED GD	Endpoints for EAS-mediated adversity available for TTO	Species
Accessory sex organs histopathology	not measured	dog, rat
Age at first oestrus	not measured	rat
Age at balanopreputial separation	Increase	rat
Age at vaginal opening	no effect	rat
Anogenital distance	Increase	rat
Cervix histopathology	no effect	rat
	not measured	dog
Coagulating gland histopathology	no effect	rat
	not measured	dog
Coagulating gland weight	no effect	rat
	not measured	dog
Cowper's gland weight	not measured	rat, dog
Epididymis histopathology	various sperm effects	rat, dog
Epididymis weight	Decrease	rat, dog
Oestrus cyclicity	no effect	rat
Glans penis weight	not measured	rat
Genital abnormalities	no effect	rat, rabbit
LABC weight	not measured	rat
Mammary gland histopathology (male)	no effect	rat, dog
Mammary gland histopathology (female)	no effect	rat, dog
Nipple Development (A)	not measured	rat
Ovary histopathology	no effect	dog, rat
Ovary weight	no effect	dog
	Increase (only in recovery)	rat
Oviduct histopathology	not measured	dog
	no effect	rat
Prostate histopathology (with seminal vesicles and coagulating glands)	reduced in size	dog
	no effect	rat
Prostate weight	no effect	dog, rat
Seminal vesicles histopathology	no effect	rat, dog
Seminal vesicles weight	no effect	rat, dog
Sperm morphology	increase in damaged sperms	dog, rat
Sperm motility	decreased	dog, rat
Sperm numbers	decreased	dog, rat
Testis histopathology	degenerative changes	dog, rat

Testis weight	decreased	dog, rat
Uterus histopathology (with cervix)	no effect	dog, rat, rabbit
Uterus weight (with cervix)	no effect	dog, rat, rabbit
Vagina histopathology	no effect	dog, rat, rabbit
Vaginal smears	not measured	dog, rat

2.10.1.2.4. Additional testing recommendations for sufficient investigation and data-gap closing

Mammalian species

For mammalian species, further toxicological studies are proposed to finally conclude on open points. Detailed reasoning for the proposed test designs is given in the respective sections in which the open points are discussed. The resulting studies are summarized in the table Table 2.10.1.2.4- 1 below.

Table 2.10.1.2.4- 1 Summary of study recommendations

Guideline	Assay Name	Reasoning
Mammalian cell lines or species		
OECD TG 456 or OPPTS 890.1200	Steroidogenesis or aromatase	To sufficiently investigate endocrine activity on S modality Either of the proposed tests can be conducted
OECD TG 440	Uterotrophic bioassay	To sufficiently investigate endocrine activity on E modality
OPPTS 890.1500	male pubertal assay	To asses androgen activity with an intact HPG axis and to investigate thyroid parameters
OECD TG 408	90-day rat	Investigation of hormone levels *in vivo* especially to conclude on thyroid activity

2.10.1.2.5. Overall conclusion

After repeated exposure of TTO to rats and dogs, there is apparent presence of testicular damage, most probably causative for epididymal lesions and abnormalities in sperm motility and morphology parameters. An evaluation of existing *in vivo* information of TTO does not indicate that the lesions originate from an imbalance of sexual hormones but rather from damage of the spermatogenic system caused by the cytotoxic properties of the substance. *In vitro* tests showing estrogenic and anti-androgenic activity of TTO were reported in the open literature, however due to restrictions in study reliability these tests results should not be used for WoE, especially since the OECD guideline-conformed studies were performed demonstrating no estrogen or (anti)androgen activity as possible underlying mechanisms for endocrine disruption.

As no endocrine activity (see lines of evidence Annex 1) has been observed, a biologically plausible link to observed adversity cannot be established. It is therefore not reasonable to consider TTO as an endocrine disrupting substance.

Annexes 1-4 to section 10, vol 1 demonstrating line of evidence for mechanistic parameters, line of evidence for EATS mediated parameters, line of evidence for parameters sensitive to, but not diagnostic of, EATS and line of evidence for general toxicity are presented below.

All the parameters which are useful for the ED assessment, identified in each relevant and reliable study, have been reported in the tabular form using the Excel template provided in the EU endocrine GD. These data are attached as a separate document named

A_TTO for ED assessment

ANNEX 1 LINE OF EVIDENCE FOR MECHANISTIC PARAMETERS

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated line of evidence for endocrine activity	In vitro mechanistic	in vitro assays	Androgen receptor	MDA-kb2	0.14	Uptake from the medium (in vitro)	0.005%	Decrease	Anti androgen effect detected	no conclusive evidence for endocrine activity but cytotoxicity	No AR/ER (ant)agonistic activity in vitro.	E.A.S.
			Estrogen receptor	MCF-7	0.1	Uptake from the medium (in vitro)	0.025%	Increase	weak estrogen binding detected with cytotoxicity at higher doses			E.A.S
				MCF-7	1	Uptake from the medium (in vitro)	0.00125%	Increase	weak estrogen binding detected			E.A.S
				human, cervix, cell line	0.14	Uptake from the medium (in vitro)		No effect				E.A.S
	In vivo mechanistic	mechanistic studies	Adrenals weight (Hershberger)	rats, orchidoepididymectomized	1	Oral		No effect		no conclusive evidence for endocrine activity	No AR (ant)agonistic activity in vitro.	E.A.S
			Cowpers glands weight (Hershberger)	rats, orchidoepididymectomized	1	Oral	120	Increase	Within HCD; organ weights of other relevant organs remain unaffected; not considered sufficient evidence of an androgenic effect based on guideline requirements			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rats, orchidoepididymectomized	1	Oral	60	Decrease	Antagonistic effect during co-treatment with testosterone;out of HCD; dose dependent; a decrease in a single androgen-dependent organ (bulbourethral gland) was not considered sufficient evidence of an anti-androgenic effect based on guideline requirements			E.A.S
			Glans penis weight (Hershberger)	rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			E.A.S
			LABC weight (Hershberger)	rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			E.A.S
			Seminal vesicles weight (Hershberger)	rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			E.A.S

Annex 2 Line of evidence for EATS mediated parameters

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated line of evidence for adversity	EATS-mediated parameter	Male reproductive organs	Testis histopathology	rat	4	Oral	125	Increase	degenerative changes	sufficient evidence of cytotoxic effects on the sperms, dose dependent degeneration of testis correlating decline in testis and epididymis weight, decline in sperm number and motility in all species, changed sperm morphology supports cytotoxic effect	Evidence of testes toxicity	E.A.S
				rat	13	Oral	120	Increase	degeneration, Sertoli cell vacuolation, sperm stasis			E.A.S
				rat	13	Oral	60	Increase	degeneration			E.A.S
				dog	13	Oral	120	Increase	Tubule with elongated spermatides.			E.A.S
				rat	16	Oral	25+50	Increase	atrophy, degeneration; only two animals (mid (25) and high (50) dose); associated with sperm abnormalities			
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 50			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	17	Oral	25+38	Increase	atrophy / degenerative changes-seminiferous tubules, degeneration; only two animals (mid (25) and high (38) dose);associated with sperm abnormalities			
			Testis weight	rat	4	Oral	250	Decrease	absolute weight			E.A.S
				rat	13	Oral	120	Decrease	seen in recovery			E.A.S
				rat	13	Oral		No effect	highest dose tested 60			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
				dog	13	Oral	120	Decrease	relative weight			E.A.S
			Epididymis histopathology	rat	4	Oral	125	Increase	Aspermia, oligospermia, cell debris in lumen			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	13	Oral	120	Increase	sperm granuloma, cell debris, Aspermia, oligospermia			E.A.S
				rat	13	Oral	60	Increase	sperm granuloma			E.A.S
				rat	16	Oral	25+50	Increase	cell debris, oligospermia; only two animals (mid (25) and high (50) dose)associated with sperm abnormalities			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 50			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	17	Oral	25+38	Increase	atrophy, aspermia; only two animals (mid (25) and high (38) dose) associated with sperm abnormalities			
				dog	13	Oral	120	Increase	Immature spermatozoa			E.A.S
			Epididymis weight	rat	4	Oral	250	Decrease	absolute weight			E.A.S
				rat	13	Oral	120	Decrease	seen in recovery			E.A.S
				rat	13	Oral	60	Decrease	seen in 8 weeks recovery			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
				dog	13	Oral	120	Decrease	absolute weight			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Sperm morphology	rat	13	Oral	60	Increase	Number of abnormal sperms headless tail, tailless head			E.A.S
				rat	13	Oral	60	Increase	number of abnormal sperms			E.A.S
				rat	16	Oral	50	Increase	% of abnormal sperm (headless tails, tailless heads, abnormal flagellum with normal head			
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
				dog	13	Oral		No effect	Highest dose tested 120			E.A.S
			Sperm motility	rat	13	Oral	60	Decrease				E.A.S
				rat	13	Oral	60	Decrease				E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	16	Oral	50	Decrease	% progressive motile sperm + motile sperm; decreased at highest dose			E.A.S
				rat	17	Oral	38	Decrease	% progressive motile sperm; decreased at highest dose			E.A.S
				dog	13	Oral	60	Decrease				E.A.S
			Sperm numbers	rat	13	Oral	60	Decrease				E.A.S
				rat	13	Oral	60	Decrease				
				rat	16	Oral	50	Decrease	epididymal sperm number; decreased at highest dose			E.A.S
				rat	17	Oral	38	Decrease	epididymal sperm number; decreased at highest dose			E.A.S
				dog	13	Oral	60	Decrease				E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Avian fertility	Japanese quail	23	Oral	300	Decrease	% fertile eggs of eggs laid; Decrease at 300, strong decrease 1000/600			E.A.S
		supporting evidence for sperm cytotoxicity	Fertility (mammals)	rat	16	Oral	50	Decrease	occurrence of pregnancy; 14 (of 25) dams did not deliver litters	fertility and number of implantations are impaired due to the loss of sperms		E.A.S
				rat	13	Oral	38	Decrease	occurrence of pregnancy; within HCD; 3 (of 23) dams did not deliver litters			E.A.S
			Small and large intestines histopathology	dog	13	Oral	120	Increase	lymphoid hyperplasia, autolysis, haemorrhage in the Peyers patches	supporting evidence for cytotoxic mechanism		E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Age at balanopreputial separation	rat	16	Oral	50	Increase	Dose dependent increase; considered toxicologically insignificant in the absence of any adverse effects on male fertility and histopathology of male reproductive organs.	No conclusive evidence for endocrine mechanism; findings regarded as incidental		E.A.S
			Ano-Genital distance	rat	3 (during Gestation)	Oral	38	Decrease	Decrease was considered incidental since values were within HCD; no clear dose dependency.			E.A.S
			Gestation length	rat	16	Oral	50	Decrease	significant at highest dose; statistical significance not of biological relevance			E.A.S
				rat	13	Oral		No effect	highest dose tested 38			E.A.S
			Genital abnormalities	rat	2	Oral		No effect	highest dose tested 120			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rabbit	3	Oral		No effect	highest dose tested 75			
		male reproductive organs without effects	Coagulating gland histopathology	rat	13	Oral		No effect	Highest dose tested 60	supporting evidence against an endocrine mechanism as there are no relevant changes detected in any other male reproductive organ		E.A.S
			Coagulating gland weight	rat	13	Oral		No effect	Highest dose tested 120			E.A.S
				rat	13	Oral		No effect	Highest dose tested 60			
			Mammary gland histopathology (male)	rat	13	Oral		No effect	Highest dose tested 120			E.A.S
				rat	13	Oral		No effect	Highest dose tested 60			E.A.S
				dog	13	Oral		No effect	Highest dose tested 120			E.A.S
			Prostate histopathology (with seminal vesicles and coagulating glands)	rat	13	Oral		No effect	Highest dose tested 120			E.A.S
				rat	13	Oral		No effect	Highest dose tested 60			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 50			E.A.S
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 38			E.A.S
				dog	13	Oral	120	Increase	reduced in size according to study author related to juvenile condition of test animals; in the absence of further histopathological findings without biological relevance			E.A.S
			Prostate weight	rat	13	Oral		No effect	Highest dose tested 120			E.A.S
				rat	13	Oral		No effect	Highest dose tested 60			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
			Seminal vesicles histopathology	rat	13	Oral		No effect	Highest dose tested 120			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	13	Oral		No effect	Highest dose tested 60			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 50			E.A.S
				rat	3 (during Gestation)	Oral		No effect	highest dose tested 38			E.A.S.
			Seminal vesicles weight	rat	13	Oral		No effect	Highest dose tested 120			E.A.S
				rat	13	Oral		No effect	Highest dose tested 60			E.A.S
				rat	16	Oral		No effect	highest dose tested 50			E.A.S
				rat	17	Oral		No effect	highest dose tested 38			E.A.S
		female reproductive organs	Age at Vaginal opening	rat	16	Oral		No effect		no evidence for endocrine effects as all female reproductive organs are not		E.A.S
			Estrus cyclicity	rat	10	Oral		No effect				E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	7	Oral		No effect		impaired in any species up to the highest dose.		E.A.S
			Genital abnormalities	rabbit	3	Oral		No effect				E.A.S
				rat	2	Oral		No effect				E.A.S
			Mammary gland histopathology (female)	rat	13	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				dog	13	Oral		No effect				E.A.S
			Ovary histopathology	rat	4	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	16	Oral		No effect				E.A.S
				rat	17	Oral		No effect				E.A.S
				rat	3(during gestation)	Oral		No effect				E.A.S
				rat	3(during gestation)	Oral		No effect				E.A.S
				dog	13	Oral		No effect				E.A.S
			Ovary weight	rat	4	Oral		No effect				E.A.S
				rat	13	Oral	120	Increase	seen in 28-day recovery; according to study author in the absence of further histopathological			E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
									findings without biological relevance			
				rat	13	Oral		No effect				E.A.S
				rat	16	Oral		No effect				E.A.S
				rat	17	Oral		No effect				E.A.S
				dog	13	Oral		No effect				E.A.S
			Oviduct histopathology	rat	13	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
			Uterus histopathology (with cervix)	rat	4	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	16	Oral		No effect				E.A.S
				rat	17	Oral		No effect				E.A.S
				rat	3(during gestation)	Oral		No effect				E.A.S
				rat	3(during gestation)	Oral		No effect				E.A.S
				dog	13	Oral		No effect				E.A.S
			Uterus weight (with cervix)	rat	4	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	2	Oral		No effect				E.A.S
				rat	16	Oral		No effect				E.A.S
				rat	17	Oral		No effect				E.A.S

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				dog	13	Oral		No effect				E.A.S
				rabbit	3	Oral		No effect				E.A.S
			Vagina histopathology	rat	13	Oral		No effect				E.A.S
				rat	13	Oral		No effect				E.A.S
				rat	16	Oral		No effect				E.A.S
				rat	17	Oral		No effect				E.A.S
				dog	13	Oral		No effect				E.A.S
		Thyroid effects	Thyroid histopathology	rat	13	Oral		No effect		secondary effect to higher enzyme depletion in the liver		T
				rat	13	Oral		No effect				T
				dog	13	Oral		No effect				T
												T
			Thyroid weight	rat	13	Oral	120	Increase	seen in 28-day recovery			T
				rat	13	Oral	60	Increase	seen in 16 weeks recovery			T
				rat	16	Oral		No effect				T
				rat	17	Oral		No effect				T
				dog	13	Oral	30	Increase	not dose dependent not present at higher doses			T

Annex 3 Line of evidence for parameters sensitive to but not diagnostic of EATS

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated line of evidence for adversity	Parameter sensitive to, but not diagnostic of, EATS	Other endocrine affected tissues	Brain weight	rat	4	Oral		No effect	highest dose 250	sufficient evidence that other endocrine tissues are not affected which supports the theory that the effects found follow a toxicity mechanism rather than an endocrine mechanism. Findings in the adrenals are not consistent and conclusive and can be regarded as incidental		
				rat	13	Oral	120	Decrease	seen in 28-day recovery			
				rat	13	Oral		No effect	highest dose 120			
				rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			
				rat	3 (during gestation)	Oral		No effect	highest dose tested 50			
				rat	3 (during gestation)	Oral		No effect	highest dose tested 38			
				dog	13	Oral		No effect	highest dose 120			
			Pituitary histopathology	rat	13	Oral		No effect				
				rat	13	Oral		No effect				
				rat	16	Oral		No effect				
				rat	17	Oral		No effect				

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				dog	13	Oral	120	Increase	Cyst pars distalis probably incidental			
			Pituitary weight	rat	13	Oral		No effect				
				rat	13	Oral		No effect				
				rat	16	Oral		No effect				
				rat	17	Oral		No effect				
			Adrenals histopathology	rat	4	Oral	250	Increase	hypertrophy zona fasciculata regarded as incidental			
				rat	13	Oral	120	Change	hypertrophy zona fasciculata regarded as incidental			
				rat	13	Oral		No effect				
				rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			
				dog	13	Oral		No effect				

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Adrenals weight	rat	4	Oral	250	Increase	probably incidental (Within HCD)			
				rat	13	Oral	120	Decrease	seen in 28 days recovery			
				rat	13	Oral	60	Increase	seen in 12- and 16-weeks recovery			
				rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			
				dog	13	Oral		No effect				
		further developmental parameters	Cracked eggs	Japanese quail	23	Oral		No effect	highest dose tested 1000/600	Lack of dose dependence; data not conclusive		
			Eggshell thickness	Japanese quail	23	Oral	300	Decrease	Significant decrease at 300, no clear dose-dependence at highest concentrations			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Litter size	rabbit	3	Oral		No effect	highest dose tested 75	supportive information for cytotoxic effect on sperms as effects can result from failed fertilization due to poor sperm measures (as found for TTO); no effect seen in studies were undosed males were used for fertilisation		
				rat	2	Oral		No effect	highest dose tested 120			
				rat	3 (during gestation)	Oral	50	Decrease	significant at highest dose			
				rat	3 (during gestation)	Oral		No effect	highest dose tested 38			
				rat	3 (during gestation)	Oral		No effect	during lactation; highest dose tested 38			
			Litter viability	rat	3 (during gestation)	Oral	50	Decrease	significant at highest dose			
			Egg production	Japanese quail	23	Oral	300	Decrease	Decrease at 300 and 1000/600	Due to maternal toxicity		
				Japanese quail	23	Oral	300	Decrease	Decrease at 300 and 1000/600			
			Litter/pup weight	rat	2	Oral	60	Decrease	at 60 within HCD, at 120 treatment related			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Litter/pup weight	rabbit	3	Oral		No effect	highest dose tested 75	General toxicity due to extensive exposure of pups via breast milk; no effect seen in studies where pups were not suckled		
				rat	3 (during gestation)	Oral	10+25	Decrease	during lactation; transient and not of toxicological relevance; highest dose tested 50			
				rat	3 (during gestation)	Oral	38	Decrease	during lactation; significant at highest dose			
			Number of live births	rabbit	3	Oral		No effect	highest dose tested 75	regarded as incidental		
				rat	3 (during gestation)	Oral	10	Decrease	contributed to one dam			
			Number of ovarian follicles	rat	17	Oral	38	Increase	considered incidental	regarded as incidental		
			Numbers of embryonic or foetal deaths	rabbit	3	Oral	75	Increase	early and late resorptions, within HCD	sufficient evidence for a general toxic mechanism; due to lack of findings in sex		

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			and viable foetuses	rat	2	Oral	120	Increase	early and late resorptions, slight	ratio and developmental parameters the overall picture does not reflect an endocrine pattern. Many Effects are within historical control data or statistically not significant		
			Number of dams with resorptions	rat	2	Oral	120	Increase				
			Post implantation loss	rabbit	3	Oral	75	Increase	Not significant			
				rat	2	Oral		No effect	highest dose tested 120			
			Pre implantation loss	rabbit	3	Oral		No effect	highest dose tested 75			
				rat	2	Oral		No effect	highest dose tested 120			
				rat	16	Oral	50	Increase	significant at highest dose			
				rat	17	Oral		No effect	highest dose tested 38			
			Number of implantations, corpora lutea	rabbit	3	Oral		No effect	highest dose tested 75			
				rat	2	Oral		No effect	highest dose tested 120			
				rat	16	Oral	50	Decrease				
				rat	17	Oral		No effect	highest dose tested 38			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Presence of anomalies (external, visceral, skeletal	rabbit	3	Oral		No effect	highest dose tested 75			
				rat	2	Oral		No effect	highest dose tested 120			
			Pup development	rat	3 (during gestation)	Oral		No effect	incisor eruption, ear opening and eye opening; observed delays considered incidental			
				rat	3 (during gestation)	Oral		No effect	incisor eruption, ear opening and eye opening; observed delays considered incidental			
			Pup survival index	rat	3 (during gestation)	Oral	50	Decrease	Day 4; significant at highest dose			
				rat	3 (during gestation)	Oral		No effect	highest dose tested 38			
			Sex ratio	rabbit	3	Oral		No effect	highest dose tested 75			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	2	Oral		No effect	highest dose tested 120			
				Japanese quail	23	Oral	1000/600	Decrease	Egg laying hens; Only 3/16 at 1000/600			
			Egg viability (% viable embryo of egg set)	Japanese quail	23	Oral	300	Decrease	Decrease at 300 and 1000/600			
				Japanese quail	23	Oral	300	Decrease	Decrease at 300 and 1000/600			
			Embryo viability (embryonic day 15)	Japanese quail	23	Oral		No effect	highest dose tested 1000/600			
				Japanese quail	23	Oral	300	Decrease	Viable 15-day embryos/viable 8-day embryos ratio; Decrease at 300 and 1000/600			
			Hatchability	Japanese quail	23	Oral	300	Decrease	Dose-dependent decrease			

	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
			Time to mating	rat	16	Oral	50	Increase	dose dependent; not significant	Sufficient evidence for the increase not to be caused by estrus cyclicity disruption; lack of endocrine pattern		E.A.S.
				rat	10	Oral		No effect	highest dose tested 38			E.A.S.
												E.A.S.
			Estrus cyclicity	rat	10	Oral		No effect	highest dose tested 50			E.A.S.
				rat	7	Oral		No effect	highest dose tested 38			E.A.S.

ANNEX 4 LINE OF EVIDENCE FOR GENERAL TOXICITY

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
		General signs of toxicity	Body weight	rat	4	Oral	250	Decrease	statistically significant	Dose dependent decrease in bodyweight; conclusive for general toxicity	Decreased food consumption consistent with decreased body weight in dog	
				rat	13	Oral		No effect	Highest dose 120			
				rat	13	Oral		No effect	Highest dose 60			
				rat	16	Oral	50	Decrease	9%; males			
				rat	13	Oral		No effect	during gestation; highest dose tested 50			
				rat	16	Oral		No effect	during lactation; highest dose tested 50			
				rat	13	Oral		No effect	until mating: highest dose tested 50			
				rat	16	Oral		No effect	during gestation; highest dose tested 38			
				rat	19	Oral		No effect	during lactation; highest dose tested 38			
				rat	10	Oral		No effect	until mating; highest dose tested 50			
				rat	17	Oral		No effect	no effect: highest dose tested 38			
				rat	16	Oral	50	Decrease	bw gain; 13%; males			
				rat	13	Oral	10	Decrease	gain; during gestation;toxicologicall y not relevant			
				rat	16	Oral	25	Decrease	gain; during lactation; considered incidental			
				rat	13	Oral		No effect	gain; until mating; highest dose tested 38			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	16	Oral		No effect	gain; during gestation; highest dose tested 38			
				rat	19	Oral		No effect	gain; during lactation; highest dose tested 38			
				rat	10	Oral		No effect	gain; until mating; highest dose tested 50			
				rat	17	Oral		No effect	gain; highest dose tested 38			
				rat	2	Oral	60	Decrease	signif. at 60 and 120 (d17 + d20)			
				rat	2	Oral	60	Decrease	bw gain; ca 15 / 30% (gd 5-20/0-20)			
				rat	2	Oral	60	Decrease	bw gain-corrected for uterine weight; signif. at 60 and 120			
				rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			
				rats, orchidoepididymectomized	1	Oral		No effect	bw gain; highest dose tested 120			
				rabbit	3	Oral		No effect	highest dose tested 75			
				rabbit	3	Oral	30	Decrease	bw gain; 40 / 42% (gd 6-29/0-29)			
				rabbit	3	Oral		No effect	bw gain -corrected for uterus weight; highest dose tested 75			
				dog	13	Oral		No effect	After Dose Reduction from 180 to 120			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				Japanese quail	23	Oral	300	Decrease	Dose-dependent decrease; Significant for males at 300, for females at 1000/600; also decreased body weight gain			
			Food consumption	rat	4	Oral	250	Decrease	statistically significant	Dose dependent decrease in food consumption ; conclusive for general toxicity		
				rat	13	Oral	120	Decrease	statistically significant			
				rat	13	Oral		No effect	Highest dose 250			
				rat	10	Oral	10	Decrease	until mating; treatment related, non-adverse since no effect on bw			
				rat	13	Oral	10	Decrease	during gestation; treatment related, non-adverse since no effect on bw			
				rat	16	Oral	10	Decrease	during lactation; treatment related, non-adverse since no effect on bw			
				rat	16	Oral		No effect	during gestation; highest dose tested 38			
				rat	19	Oral		No effect	during lactation; highest dose tested 38			
				rat	16	Oral	10	Decrease	males; treatment related, non-adverse since no effect on bw			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	12	Oral		No effect	until mating: highest dose tested 38; males			
				rat	12	Oral		No effect	until mating: highest dose tested 38; females			
				rat	2	Oral	120	Decrease	from d8 to d20			
				rabbit	3	Oral	30	Decrease	within HCD; 28 / 36% (gd 6-29/0-29)			
				dog	13	Oral		No effect	After Dose Reduction from 180 to 120			
				Japanese quail	23	Oral	300	Decrease	Adult food consumption; Decrease at 300 and 1000/600			
			mortality	rat	4	Oral		No effect	Highest dose	Dose dependent increase in Mortality as sign for general toxicity		
				rat	13	Oral	120	Increase	statistically significant			
				rat	13	Oral		No effect	Highest dose 60			
				rat	16	Oral		No effect	highest dose tested 50			
				rat	13	Oral		No effect	highest dose tested 38			
				rat	2	Oral		No effect	highest dose tested 120			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			
				rabbit	3	Oral		No effect	Highest dose tested 75			
				dog	13	Oral		No effect	After Dose Reduction from 180 to 120			
				Japanese quail	23	Oral	1000/600	Increase	3/16 m, 1/16 f at 1000/600			
				Japanese quail	23	Oral	1000/600	Decrease	% of hatchlings surviving on a time period of 14 days			
			clinical signs	rat	16	Oral	25	Increase	slight salivation, not considered treatment related	supporting evidence for general toxicity		
				rat	13	Oral		No effect	highest dose tested 38			
				rat	2	Oral		No effect	highest dose tested 120			
				rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			
				rabbit	3	Oral		No effect	highest dose tested 75			
				Japanese quail	23	Oral	1000/600	Change	Clinical signs; m+f at 1000/600 reduced motility, staggering gait, ruffled feathers			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				Japanese quail	23	Oral		No effect	Macroscopic findings			
		Supportive target organ toxicity	Kidney histopathology	rat	16	Oral		No effect	highest dose tested 50	No evidence for target organ toxicity		
				rat	17	Oral		No effect	highest dose tested 38			
				rat	3(during gestation)	Oral		No effect	highest dose tested 50			
				rat	3(during gestation)	Oral		No effect	highest dose tested 38			
			Kidney weight	rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			
				rats, orchidoepididymectomized	1	Oral		No effect	highest dose tested 120			
			Spleen histopathology	rat	3(during gestation)	Oral		No effect	highest dose tested 50			
				rat	3(during gestation)	Oral		No effect	highest dose tested 38			
			Spleen weight	rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				rat	3(during gestation)	Oral		No effect	highest dose tested 50			
				rat	3(during gestation)	Oral		No effect	highest dose tested 38			
			Thymus weight	rat	3(during gestation)	Oral		No effect	highest dose tested 50			
				rat	3(during gestation)	Oral		No effect	highest dose tested 38			
		Target organ toxicity	Liver histopathology	rat	4	Oral	125	Increase	Vacuolation	sufficient evidence for general mild liver toxicity as metabolising organ; Thyroid weight increase secondary effect to higher enzyme depletion in the liver		
				rat	13	Oral	120	Increase	Vacuolation			
				rat	16	Oral		No effect	highest dose tested 50			
				rat	17	Oral		No effect	highest dose tested 38			
				dog	13	Oral		No effect	After Dose Reduction from 180 to 120			
			Liver weight	rat	4	Oral	125	Increase	statistically significant			
				rat	13	Oral	60	Increase	statistically significant			
				rat	13	Oral	60	Increase	statistically significant			
				rat	13	Oral		No effect	highest dose tested 50			
				rat	16	Oral		No effect	highest dose tested 38			
				rats, orchidoepididymectomized	17	Oral		No effect	highest dose tested 120			

Evidence of general toxity	Grouping	Line(s) of evidence	Effect Target	Species	Exposure weeks	Route of exposure	Effect dose mg/kg/day	Effect direction	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
				dog	1	Oral		No effect	After Dose Reduction from 180 to 120			
			Thyroid histopathology	rat	13	Oral		No effect				T
				rat	13	Oral		No effect				T
				dog	13	Oral		No effect				T
					0							
			Thyroid weight	rat	13	Oral	120	Increase	seen in 28-day recovery			T
				rat	13	Oral	60	Increase	seen in 16 weeks recovery			T
				rat	16	Oral		No effect				T
				rat	17	Oral		No effect				T
				dog	13	Oral	30	Increase	not dose dependent not present at higher doses			T
			Small and large intestines histopathology	dog	13	Oral	120	Increase	lymphoid hyperplasia, autolysis, haemorrhage in the Peyers patches	supporting evidence for cytotoxic mechanism		

RMS: The assessment of endocrine disrupting properties of TTO was done according to the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

The adverse effects which could potentially be related to endocrine disrupting properties of TTO, which were identified in the standard toxicity assessment studies, were confined mainly to male reproductive system. TTO has been demonstrated in several repeated dose toxicity studies in male rats and dogs and in 2-generation study in rats to cause decrease in sperm count and motility, increase in sperm abnormalities, decrease in weight of testes and epididymides, histopathological changes in testes and epididymides, reduction of mean litter size and reduced pups survival by day 4 (P-Generation), reduced male and female mating and fertility indexes (P-Generation), reduction of no. of corpora lutea and no. of implantations at 50 mg/kg bw/d. The highest dose level at which these effects were not observed in 2-generation study was 25 mg/kg bw/d, which thus considered as No Observed Adverse Effect Level (NOAEL) for reproductive toxicity.

In literature search on potential endocrine disruptiong properties of TTO, performed according to the relevant guidance (EFSA, 2011), four *in vitro* studies were identified showing either no estrogenic response, or weak-estrogenic response, weak anti-androgen response, and no anti-estrogenic response. The reliability of the studies is low since they were not performed according to recognised guidelines, had poor reporting not allowing to assess key elements of design and results of studies. In some studies a cytotoxicity of TTO at concentration of 0.025% was observed.

To investigate potential endocrine disruptiong properties of TTO, noting that TTO have decreased fertility and affected sperm cells in rats and dogs the applicant has submitted the results of two studies: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties and the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists, which were done to clarify whether these effects could be mediated by interaction of TTO with androgen and estrogen receptor in germ and somatic cells.

In the Hershberger assay performed according to OECD 441 and in GLP conditions TTO at doses of 60-120 mg/kg, sufficient to affect the seminiferous epithelium in male rats and dogs, did not interact with androgen receptor in androgen sensitive tissues of male rats, therefore it was possible to conclude that it does not have the androgenic or antiandrogenic activity in this test system .

In the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists performed on the (h)ERα-HeLa-9903 cell line according to OECD TG 455 and in GLP conditions Tea Tree Oil tested in seven concentrations ranging from 100 pg/mL to 100 µg/mL was found to be nontoxic for (h)ERα-HeLa-9903 cells, and it did not induce a response specific for estrogen receptor agonist.

The results of both studies demonstrate that TTO does not have androgenic or anti-androgenic activity as well as estrogenic activity therefore these results do not provide evidence that the effect on testes and sperm cells are mediated by endocrine disrupting mechanism. This conclusion is further supported by lack of effect of TTO on histopathology or weight of prostate and seminal vesicles or male rat mammary glands, lack of effect of TTO on oestrus cycle of females exposed to this substance in 2-generation study at doses up to 50 mg/kg bw/d, lack of effect in non-reproductive endocrine organs such as pituitary or adrenals.

There were no histopathological changes in thyroid in any repeated dose toxicity study in rats and dogs, no increase in thyroid weight was observed in 2-generation reproduction toxicity study in rats , but increase in absolute thyroid weight was seen in rats exposed to TTO in the repeated dose 90-day oral toxicity study at 60 mg/kg bw/d after 4 and 16 weeks recovery period and in dogs in the repeated dose 90-day oral toxicity study at 120 mg/kg bw/d. These data do not provide clear evidence that TTO may affect HPT axis, since no histopathological changes were seen in hypothalamus, pituitary and thyroid in any study in rats, dogs, and rabbits and the effect on thyroid weight was seen only in some, but not in all studies. However, the potential endocrine effect on thyroid may need to be investigate further, since thyroid hormones were not measured in any study.

The applicant made a thorough analysis of the mode of action (MoA) providing a description of a series of potential biological events, i.e. key events (KEs) that may result in degeneration of spermatogenic epithelium in testis and alterations in epididymis, concluding that cytotoxic destruction of sperm cells may be responsible for all effects observed in male reproductive system of mammals. Tea Tree oil (TTO), and its major active terpene component, terpinen-4-ol, have been shown to have significant anti-proliferative activity against two tumour cell lines by causing primary necrotic cell death and cell cycle arrest in MTT assay (Greay et al. 2010).

The known cytotoxicity of TTO could thus be responsible for all the effects found in the male reproductive system of mammals, without a need to initiate these effects by disruption of endocrine system, particularly of the HPG axis. In the opinion of RMS, based on the existing data reviewed in this report and based on a fact that no estrogenic and/or anti-androgenic properties of TTO were detected in reliable tests, it is plausible to assume that TTO does

not have endocrine disrupting properties and it affects the male germ cells by direct cytotoxic mechanism. It seems to be important to note the effect of TTO on reproductive system has a clear threshold, since at a dose level of 25 mg/kg bw/d this effect is not observed, what does not seem probable for the substance with endocrine disrupting properties. It is however admitted that this conclusion is based on limited number of studies.

To remove the uncertainties of the above assessment of potential ED properties of TTO, if considered necessary, the recommendation on further testing of the OECD Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD 2018) can be applied.

In case of TTO the recommendations listed in scenario D, E, F, G, H and I of the OECD Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption for interpretation of two-generation study (OECD TG 416) may apply, since:

- TTO was positive in OECD TG 416 test and in repeated dose toxicity studies for effects in testis, but negative in in vitro Estrogen Receptor OECD TG 455 without added metabolising system (scenario D) or
- TTO was positive in OECD TG 416 and in repeated dose toxicity studies for effects in testis, but negative in Hershberger Bioassay in Rats (OECD TG 441) and in in vitro Estrogen Receptor OECD TG 455 (scenario E)

In these scenarios the advised steps which could be taken to strengthen weight of evidence is to perform in vitro thyroid hormone receptor (TR), steroidogenesis (S) assays with added metabolising system. *In vitro* androgen receptor (AR) and *in vitro* estrogen (ER) receptor assays does not seem to be needed since the negative results in Estrogen Receptor assay (OECD TG 455) and Hershberger Bioassay in Rats (OECD TG 441) were found. These recommendations are in principle compatible with tests proposed by the applicant:

- OECD TG 456 or OPPTS 890.1200 to sufficiently investigate endocrine activity on S modality,
- OECD TG 440 uterotrophic bioassay to sufficiently investigate endocrine activity on E modality,
- OPPTS 890.1500 male pubertal assay to asses androgen activity with an intact HPG axis and to investigate thyroid parameters, and/or
- OECD TG 408 to investigate hormone levels in vivo especially to conclude on thyroid activity.

2.10.2 ED assessment for non-target species

The evaluation regarding endocrine disruptive properties of Tea Tree Oil has been performed in line with the new ECHA/EFSA guidance on evaluation of endocrine disruptive properties.

2.10.2.1 ED assessment for T-modality

2.10.2.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

See tables in point 2.10.1 above for lines of evidence related to the T-modality

2.10.2.1.1.1 Assessment of the integrated lines of evidence and weight of evidence

Mammals

The data package regarding endocrine disruptive properties of TTO in mammals was evaluated by the RMS toxicology expert and considered potentially incomplete. Below final conclusions of the RMS toxicology expert regarding T-mediated adversity are presented.

Conclusions of the ED evaluation taken by the RMS toxicology expert with regard to T-modality (for details see Vol. 3CA, B.6.8.3):

There were no histopathological changes in thyroid in any repeated dose toxicity study in rats and dogs, no increase in thyroid weight was observed in 2-generation reproduction toxicity study in rats , but increase in absolute thyroid weight was seen in rats exposed to TTO in the repeated dose 90-day oral toxicity study at 60 mg/kg bw/d after 4 and 16 weeks recovery period and in dogs in the repeated dose 90-day oral toxicity study at 120 mg/kg bw/d. These data do not provide clear evidence that TTO may affect HPT axis, since no histopathological changes were seen in hypothalamus, pituitary and thyroid in any study in rats, dogs, and rabbits and the effect on thyroid weight was seen only in some, but not in all studies. However, the potential

endocrine effect on thyroid may need to be investigate further, since thyroid hormones were not measured in any study.

Summary of key findings regarding T-mediated modalities is presented in table below (Table B.6.8.3.2-1 in Vol. 3CA, B.8.6.3):

Endpoints for T-mediated adversity as listed in ED GD	Endpoints for T-mediated adversity available for TTO	Species
HDL/LDL ratio	not measured	rat, dog
Liver Weight	no effect	dog
	increase	rat
Thyroid histopathology	no effect	rat, dog
Thyroid weight	increase	dog, rat
T3/T4 level	not measured	dog, rat
TSH level	not measured	rat, dog

In general, effects on thyroid were investigated in limited number of studies. Therefore further discussion in area of toxicology is deemed necessary to decide if the dataset is complete or further studies are required.

Non-mammalian species

No specific, validated guidelines and criteria regarding evaluation of endocrine disruption potential in birds are available and the ECHA/EFSA guidance indicates two types studies performed for birds that may be used in the evaluation of ED properties:

- avian reproduction test (OECD 206),
- US EPA avian two-generation study (OCSPP 890.2100).

For TTO the reproductive toxicity study in line with OECD 206 was performed with Japanese by [redacted] 2018 (summarised in this document under B.9.1.1.3/01). The study was accepted by the RMS with NOEL value of 100 mg a.s./kg bw/d, i.e. the lowest dose tested at which no adverse impact on any of the reproductive parameters investigated was observed. At remaining test doses (300 and 1000/600 mg a.s./kg bw/d) clear treatment related effects were seen on almost all investigated reproductive parameters. However, in case of the maximum dose effects observed on reproductive performance could be attributed to the overall toxicity in adult birds (increased motility, staggering gait, exophtalamus, abdominal and gasping breathing, increased water consumption, emesis).

In case of the middle dose (300 mg a.s./kg bw/d) no overt signs of toxicity were observed in most of birds, so effects on reproductive parameters were clearly treatment related.
However, it is not fully clear how to relate these effects to potential endocrine MoA, as according the ECHA/EFSA guidance document all parameters investigated in avian reproduction test performed according to OECD 206 are sensitive to, but not diagnostic of EATS.
The guidance indicates that in absence of relevant parameters to be investigated, the gross pathology results may be helpful in determination of potentially EATS mediated effects. In the study by [redacted] (2018) gross pathology was performed on all surviving birds at test termination as well as on prematurely deceased birds. According to the gross pathology report, no test item-related pathological changes were noted at macroscopic inspection in the organs and tissues of the surviving adult male and female birds from all test groups at test termination. None of the surviving adult quails revealed any lesions, organ or tissue changes at any of the tested concentrations. In case of prematurely deceased birds in test group 1000/600 mg a.s./kg bw/d, 4 birds were examined and no pathological changes were found in two of them. In remaining two birds pathological changes included autolytic intestines, anus soiled with faeces, fragile kidneys, little content in gastro-intestinal tract and discoloured/milky sacci abdominales. In neither bird (prematurely deceased or sacrificed at test termination) any pathological changes of potential endocrine target organs were recorded.

In absence of any respective criteria regarding linking of effects on reproductive performance with EATS-mediated effects, it is not possible to conclude whether effects observed in reproductive study with Japanese quail ([redacted], 2018) were caused by disruption of the hormonal system. As ECHA/EFSA guidance does not indicate what further testing might be required to address ED properties of active substance in birds, no further assessment of this issue is possible at this stage.

With regard to aquatic species, no specific studies to address issue of potential for endocrine disruption in fish are available for Tea Tree Oil.

The only study investigating parameters sensitive to, but not diagnostic of EATS, is the fish early life stage toxicity test with *Pimephales promelas* performed by Sheerbaum, 2017 (summarised in this document under B.9.2.2/01). In the study, no delay in swim up time (i.e. the time taken for the fish fry to be free feeding) was observed up to the maximum test concentration of 3.15 mg a.s./L (mean measured). Effects were observed on hatching success (at three highest concentrations tested) and growth (stimulation of length and weight of fish in two highest concentrations tested). However, these test concentrations also affected fry survival, so observed effects could be due to the general toxicity and not due to effects on EATS-mediated modalities. Nevertheless, it is noted that ELS test is not sufficient to conclude on ED potential in fish, as it provides only limited information on T-mediated effects and no information on EAS-modalities.

Overall, further studies must be performed in order to conclude on impact of TTO on T-modality. Testing strategy is proposed in point 2.10.2.1.3.2 below.

2.10.2.1.2 Initial analysis of the evidence and identification of the relevant scenario

Table 2.10.2.1.2-1: Selection of relevant scenario

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	

2.10.2.1.3 MoA analysis for T-modality

2.10.2.1.3.1 Postulate MoA

Data not sufficient, MoA cannot be postulated.

2.10.2.1.3.2 Further information to be generated to postulate MoA

Mammals

The data package regarding endocrine disruptive properties of TTO in mammals was evaluated by the RMS toxicology expert and considered potentially incomplete. List of studies to be generated in presented in point 2.10.1 above.

Non-mammalian species

The testing strategy proposed by the Applicant is presented below (text in italics).

In order to have EATS-mediated adversity sufficiently investigated with regard to ecotoxicology, the GD recommends the following set of studies to be performed:
[…] In terms of T-mediated parameters, an Amphibian metamorphosis assay (AMA, OECD TG 231) or a Larval amphibian growth and development assay (LAGDA; OECD TG 241, with the option to also measure EAS) are recommended.

Summary of study recommendations is provided in table below:

Guideline	*Assay Name*	*Reasoning*
OECD TG 231 *or* *OECD TG 241*	*Amphibian metamorphosis assay* *or* *Larval amphibian growth and development assay*	*To assess thyroidal effects in non-target organisms*

As a standard approach, Amphibian Metamorphosis Assay (AMA, OECD TG 231) or Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD TG 248) are considered relevant in order to investigate effects on T-modality.
Limited information is available from the mammalian dataset, but increased thyroid weight was observed in some studies. As TH levels were not measured in any of the studies, it cannot be concluded if TTO would potentially have impact on thyroid hormone synthesis and for this reason XETA (Xenopus Eleutheroembryo Thyroid Assay, OECD TG 248) could result with false-negative result in case TTO has some effects on TH synthesis. Taking this into account, it seems that AMA (Amphibian Metamorphosis Assay, OECD TG 231) might be more relevant to address potential effects of TTO on T-modality. Nevertheless, in case in area of toxicology it will be concluded that TTO is not expected to have effects on TH synthesis and transport, Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD TG 248) may be sufficient.

2.10.2.1.3.3 Empirical support of the postulated MoA

Not relevant, data insufficient to perform the MoA analysis.

2.10.2.1.3.4 Conclusion on MoA analysis

Not relevant, data insufficient to perform the MoA analysis.

2.10.2.1.4 Conclusion on the ED assessment for T-modality

Further data needs to be generated in order to conclude on T-activity and T-adversity of TTO. Taking all presented information into account, the RMS recommends to perform the following studies to investigate effects of TTO on T-modality in non-mammalian species:

- Amphibian Metamorphosis Assay (AMA, OECD 231) or Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD TG 248) in order to investigate effects on T-modality, depending on the outcome of the discussion in area of toxicology.

2.10.2.2 ED assessment for EAS-modality

2.10.2.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

See tables in point 2.10.1 above for lines of evidence related to the T-modality.

2.10.2.2.1.1 Assessment of the integrated lines of evidence and weight of evidence

Mammals

The results of available mechanistic studies were ambiguous and for this reason inconclusive for EAS-mediated activity of TTO. However, in the available *in vivo* studies potentially EAS-mediated adversity has been observed, mainly on the male reproductive system of tested species (rats and dogs).
The full study summaries together with RMS evaluation may be found in Vol. 3CA, B.6, while below summary of potentially EAS-mediated effects is provided in the tabular form to aid Member States and EFSA evaluation.

Table 2.10.2.2.1.1: EAS-adversity observed in mammalian studies

Study / reference	Dose levels	NOEL/NOAEL [mg/kg bw/day]	Potentially EAS-mediated effects	Remarks
Short-term studies				
28-days, administration by gavage, rats (Wistar rats) B.6.3.1/01 [redacted] (2010) N896 Non-GLP	0, 62.5, 125, 250 mg/kg bw/day	NOEL = 62.5 mg/kg body weight/day	<u>At 125 mg/kg bw/day</u> • Degenerative changes in testes • Oligospermia • Epididymal cell debris <u>At 250 mg/kg bw/day</u> ↓ absolute and relative weights of testes and epididymides • Small sized epididymides and testes • Degenerative changes in testes • Aspermia	Decreased bodyweight and food consumption at 250 mg/kg bw/d (statistically not significant, both sexes)
90-days, feeding, rats (Wistar rats – HsdCpb) B.6.3.2/01 [redacted] (2011) G7153	0, 3, 60, 120 mg/kg bw/day	Males: NOAEL (90 days) = 30 mg/kg bw/day Females: NOAEL (91 days) = 60 mg/kg bw/day	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms <u>At 120 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms ↓ Absolute and relative weights of testes and epididymides ↑Ovary weight (during recovery phase) • Degenerative changes in seminiferous tubules • Cell debris in tubular lumen of testes and atrophic appearance • Sertoli cell vacuolation • Sperm granuloma • Cell debris in epidydimal duct lumen	Decreased food consumption at all doses (statistically significant, males) Increased bodyweight gain (statistically significant, males) Due to clinical signs, two rats sacrificed at 120 mg/kg bw/d
90-days, feeding, rats (Wister rat - Hsd Han) B.6.3.2/02 [redacted] (2016) G11089	0, 60 mg/kg bw/day	Not estimated	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms • Sperm granuloma • Oligospermia, • Single cell necrosis, • Luminal cell debris • Degeneration/atrophy of seminiferous tubules	No effects on bodyweight, bodyweight gain and food consumption
90-days oral, dogs (Beagle) B.6.3.2/03 [redacted] (2018) 34433	0, 30, 75/60, 180/120 mg/kg bw/day	NOAEL (90 days) = 30 mg/kg bw/day	<u>At 75/60 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids <u>At 180/120 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids	Reduced bodyweight, bodyweight gain and food consumption in the top dose 180 mg/kg bw/d with recovery after reduction to 120 mg/kg bw/d
Generational studies				

Study / reference	Dose levels	NOEL/NOAEL [mg/kg bw/day]	Potentially EAS-mediated effects	Remarks
Two generation study in the rat B.6.6.1/01 [redacted] (2017) G11090	Generation-P: 0, 10, 25 and 50 mg/kg day by gavage. Generation-F1: 0, 10, 25 and 38 mg/kg day	Reproduction/ offspring NOAEL= 25 mg/kg bw/day	38 mg/kg day: ↓Progressive motile sperm (F1) Delayed preputial separation (F1) Increased anogenital distance (F1 males, anogenital distance index not affected) 50 mg/kg day: ↓Corpora lutea (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P)	P-generation Decreased bodyweight and bodyweight gain (7-9%, days 78 to 113) at 50 mg/kg bw/d (males) No bodyweight effects in females F1-generation Reduced bodyweight at 10 mg/kg bw in males (statistically significant, 4.5%) and at 38 mg/kg bw/d in males and females (statistically significant, 10.3 and 12.6%, respectively)

Although rather limited dataset is available to evaluate ED properties of TTO in mammals, from the available data clear adversity on male reproductive system may be seen, including decreased weight of testes and epididymides accompanied by histopathological changes and effects on sperm. Based on clinical signs and bodyweight effects it seems that in majority of the studies effects were observed at doses not overcoming MTD, but more detailed discussion in this area is required in area of toxicology.
Effect on anogenital distance seen in generational study in F1 males at the top dose was most probably related with significant (>10%) effect on bodyweight at the time when measurements were taken (). However, the delayed preputial separation does not seem to be related to the retarded development since at the time of the measurement F1 males in the top dose were correctly developed. In addition to that, slight delay of preputial separation was also seen at the low and mid dose, although it was statistically not significant.

Besides clear effects on male reproductive system, exposure to TTO resulted with reduction of the mean litter size, pup survival as well as mating and fertility indices. Furthermore, reduced number of corpora lutea and implantations were observed in females in the 2-generation study, without any effects on bodyweight, bodyweight gain or food consumption. The increased ovary weight was observed during the recovery period at the top dose (120 mg/kg bw/d) during the 90-day dietary toxicity study with rats without histopathological findings. No effects on ovaries were observed in dog study. No other treatment related effects related to the female reproductive system were observed in the performed studies (including developmental toxicity studies with rats and rabbits). In none of the studies hormone levels (estrogen, progesterone, testosterone) were measured.

The data package regarding endocrine disruptive properties of TTO in mammals was evaluated by the RMS toxicology expert and considered incomplete. Below final conclusions of the RMS toxicology expert regarding EAS-mediated adversity together with explanation of potential reasons for effects seen on male reproductive system are presented.

Conclusions of the ED evaluation taken by the RMS toxicology expert with regard to EAS-modalities (for details see Vol. 3CA, B.6.8.3):

The assessment of endocrine disrupting properties of TTO was done according to the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

The adverse effects which could potentially be related to endocrine disrupting properties of TTO, which were identified in the standard toxicity assessment studies, were confined mainly to male reproductive system. TTO has been demonstrated in several repeated dose toxicity studies in male rats and dogs and in 2-generation study in rats to cause decrease in sperm count and motility, increase in sperm abnormalities, decrease in weight of testes and epididymides, histopathological changes in testes and epididymides, reduction of mean litter size and reduced pups survival by day 4 (P-Generation), reduced male and female mating and fertility indexes (P-Generation), reduction of no. of corpora lutea and no. of implantations at 50 mg/kg bw/d. The highest dose level at which these effects were not observed in 2-generation study was 25 mg/kg bw/d, which thus considered as No Observed Adverse Effect Level (NOAEL) for reproductive toxicity.

In literature search on potential endocrine disruption properties of TTO, performed according to the relevant guidance (EFSA, 2011), four *in vitro* studies were identified showing either no estrogenic response, or weak-estrogenic response, weak anti-androgen response, and no anti-estrogenic response. The reliability of the studies is low since they were not performed according to recognised guidelines, had poor reporting not allowing to assess key elements of design and results of studies. In some studies a cytotoxicity of TTO at concentration of 0.025% was observed.

To investigate potential endocrine disruption properties of TTO, noting that TTO have decreased fertility and affected sperm cells in rats and dogs the applicant has submitted the results of two studies: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties and the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists, which were done to clarify whether these effects could be mediated by interaction of TTO with androgen and estrogen receptor in germ and somatic cells.

In the Hershberger assay performed according to OECD 441 and in GLP conditions TTO at doses of 60-120 mg/kg, sufficient to affect the seminiferous epithelium in male rats and dogs, did not interact with androgen receptor in androgen sensitive tissues of male rats, therefore it was possible to conclude that it does not have the androgenic or antiandrogenic activity in this test system.

In the Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists performed on the (h)ERα-HeLa-9903 cell line according to OECD TG 455 and in GLP conditions Tea Tree Oil tested in seven concentrations ranging from 100 pg/mL to 100 μg/mL was found to be nontoxic for (h)ERα-HeLa-9903 cells, and it did not induce a response specific for estrogen receptor agonist.

The results of both studies demonstrate that TTO does not have androgenic or anti-androgenic activity as well as estrogenic activity therefore these results do not provide evidence that the effect on testes and sperm cells are mediated by endocrine disrupting mechanism. This conclusion is further supported by lack of effect of TTO on histopathology or weight of prostate and seminal vesicles or male rat mammary glands, lack of effect of TTO on oestrus cycle of females exposed to this substance in 2-generation study at doses up to 50 mg/kg bw/d, lack of effect in non-reproductive endocrine organs such as pituitary or adrenals.

Summary of key findings regarding EAS-mediated modalities is presented in table below (Table B.6.8.3.2-2 in Vol. 3CA, B.8.6.3):

Endpoints for EAS-mediated adversity as listed in ED GD	Endpoints for EAS-mediated adversity available for TTO	Species
Accessory sex organs histopathology	not measured	dog, rat
Age at first oestrus	not measured	rat
Age at balanopreputial separation	increase	rat
Age at vaginal opening	no effect	rat
Anogenital distance	increase	rat
Cervix histopathology	no effect	rat
	not measured	dog
Coagulating gland histopathology	no effect	rat

	not measured	dog
Coagulating gland weight	no effect	rat
	not measured	dog
Cowper's gland weight	not measured	rat, dog
Epididymis histopathology	various sperm effects	rat, dog
Epididymis weight	decrease	rat, dog
Oestrus cyclicity	no effect	rat
Glans penis weight	not measured	rat
Genital abnormalities	no effect	rat, rabbit
LABC weight	not measured	rat
Mammary gland histopathology (male)	no effect	rat, dog
Mammary gland histopathology (female)	no effect	rat, dog
Nipple Development (A)	not measured	rat
Ovary histopathology	no effect	dog, rat
Ovary weight	no effect	dog
	increase (only in recovery)	rat
Oviduct histopathology	not measured	dog
	no effect	rat
Prostate histopathology (with seminal vesicles and coagulating glands)	reduced in size	dog
	no effect	rat
Prostate weight	no effect	dog, rat
Seminal vesicles histopathology	no effect	rat, dog
Seminal vesicles weight	no effect	rat, dog
Sperm morphology	increase in damaged sperms	dog, rat
Sperm motility	decrease	dog, rat
Sperm numbers	decrease	dog, rat
Testis histopathology	degenerative changes	dog, rat
Testis weight	decrease	dog, rat
Uterus histopathology (with cervix)	no effect	dog, rat, rabbit
Uterus weight (with cervix)	no effect	dog, rat, rabbit
Vagina histopathology	no effect	dog, rat, rabbit
Vaginal smears	not measured	dog, rat

The applicant made a thorough analysis of the mode of action (MoA) providing a description of a series of potential biological events, i.e. key events (KEs) that may result in degeneration of spermatogenic epithelium in testis and alterations in epididymis, concluding that cytotoxic destruction of sperm cells may be responsible for all effects observed in male reproductive system of mammals. Tea Tree oil (TTO), and its major active terpene component, terpinen-4-ol, have been shown to have significant anti-proliferative activity against two tumour cell lines by causing primary necrotic cell death and cell cycle arrest in MTT assay (Greay et al. 2010). The known cytotoxicity of TTO could thus be responsible for all the effects found in the male reproductive system of mammals, without a need to initiate these effects by disruption of endocrine system, particularly of the HPG axis. In the opinion of RMS, based on the existing data reviewed in this report and based on a fact that no estrogenic and/or anti-androgenic properties of TTO were detected in reliable tests, it is plausible to assume that TTO does not have endocrine disrupting properties and it affects the male germ cells by direct cytotoxic mechanism. It seems to be important to note the effect of TTO on reproductive system has a clear threshold, since at a dose level of 25 mg/kg bw/d this effect is not observed, what does not seem probable for the substance with endocrine disrupting properties. It is however admitted that this conclusion is based on limited number of studies.

Non-mammalian species

No studies enabling evaluation of effects of TTO on EAS-modalities were provided in support of this evaluation. The available data for birds and aquatic organisms were already discussed in point 2.10.2.1.1.1 above and derived conclusions are valid for T-modality as well as for EAS-modalities. Discussion is thus not repeated here.

Overall, further studies must be performed in order to conclude on impact of TTO on EAS-modalities. Testing strategy is proposed in point 2.10.2.2.3.2 below.

2.10.2.2.2 Initial analysis of the evidence and identification of the relevant scenario

Table 2.10.2.2.2-1: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
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	

2.10.2.2.3 MoA analysis for EAS-modalities

2.10.2.2.3.1 Postulate MoA

Data not sufficient, MoA cannot be postulated

2.10.2.2.3.2 Further information to be generated to postulate MoA

Mammals

The data package regarding endocrine disruptive properties of TTO in mammals was evaluated by the RMS toxicology expert and considered potentially incomplete. List of studies to be generated in presented in point 2.10.1 above.

Non-mammalian species

The testing strategy proposed by the Applicant is presented below (text in italics).

In order to have EATS-mediated adversity sufficiently investigated with regard to ecotoxicology, the GD recommends the following set of studies to be performed:

The Medaka extended one-generation test (MEOGRT, OECD TG 240) or the fish life cycle toxicity test FLCTT (US EPA TG OPPTS 850.1500) cover the 'EAS-mediated' parameters. Recently, concerns have been raised as to the suitability of Medaka as test species for ED, referring to sometimes overly variable reproduction rates also in the control groups and suspected low sensitivity to some endocrine effects. Hence, alternatively the Zebrafish extended one-generation reproduction test (ZEOGRT) can be performed, however, only a draft OECD test guideline is available as yet. […]

Summary of study recommendations is provided in table below:

Guideline	***Assay Name***	***Reasoning***
OECD TG 240 *or* *OPPTS 850.1500*	*Medaka extended one-generation test* *or* *fish life cycle toxicity test*	*To sufficiently investigate EAS modalities on endocrine disruption in non-target organisms*

As a standard approach, Fish Short-Term Reproduction Assay (FSTRA, OECD TG 229) is considered relevant in order to investigate effects on EAS-modalities

However, the RMS has some concerns as in this test only adult animals are exposed. However, available literature data on effects on non-target arthropods clearly indicate that eggs and early larvae (L_1-L_2) are stages most sensitive to particular TTO constituents. It is possible that in case of fish the same relation will be observed with eggs, embryos and larvae being most sensitive stages that may develop adverse effects following exposure to TTO.
It is noted that in none of level 3 studies the early fish stages are exposed to the tested chemical. The Applicant proposed to perform Medaka extended one-generation test (OECD 240) or fish full life cycle test (OPPTS 850.1500). However, both these test are level 5 tests according to OECD CF and should not be performed without information from level 3 or 4 studies. The only test in which the exposure starts from eggs and is continued until completion of sexual differentiation level 4 Fish Sexual Development Test (FSDT, OECD TG 234) and in opinion of the RMS this test would be most suitable to address the EAS-mediated effects in fish, covering stages probably most sensitive to TTO.

2.10.2.2.3.3 Empirical support of the postulated MoA

Not relevant, data insufficient to perform the MoA analysis.

2.10.2.2.3.4 Conclusion on MoA analysis

Not relevant, data insufficient to perform the MoA analysis.

2.10.2.2.4 Conclusion on the ED assessment for EAS-modality

Further data needs to be generated in order to conclude on EAS-activity and EAS-adversity of TTO. Taking all presented information into account, the RMS recommends to perform the following studies to investigate effects of TTO on EAS-modalities in non-mammalian species:

- Fish Sexual Development Test (OECD 234).

2.10.3 Overall conclusion on the ED assessment

Overall, the data available are not sufficient to conclude on ED properties of TTO in humans and non-target species. Following studies are proposed by the RMS to be performed in order to complete the available dataset and finalise assessment of ED properties of Tea Tree Oil:

- OECD TG 456 or OPPTS 890.1200 to sufficiently investigate endocrine activity on S modality,
- OECD TG 440 uterotrophic bioassay to sufficiently investigate endocrine activity on E modality,
- OPPTS 890.1500 male pubertal assay to asses androgen activity with an intact HPG axis and to investigate thyroid parameters, and/or
- OECD TG 408 to investigate hormone levels in vivo especially to conclude on thyroid activity,
- Fish Sexual Development Test (OECD 234) in order to investigate effects on EAS-modalities,
- Amphibian Metamorphosis Assay (AMA, OECD 231) or Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD TG 248) in order to investigate effects on T-modality, depending on the outcome of the discussion in area of toxicology.

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

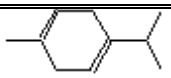
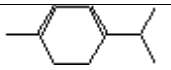
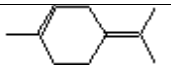
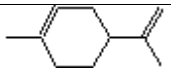
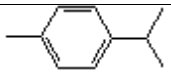
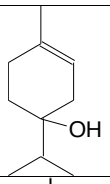
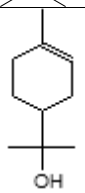
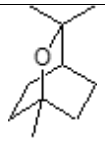
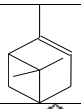
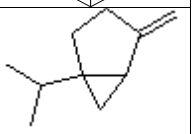
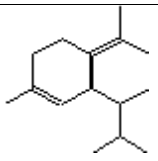
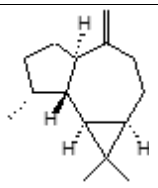
Table 58: Substance identity and information related to molecular and structural formula of the substance

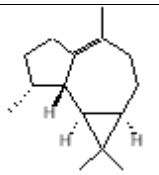
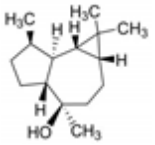
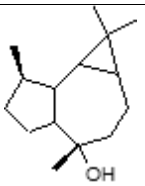
<table>
<tr><td>Name(s) in the IUPAC nomenclature or other international chemical name(s)</td><td>Melaleuca alternifolia, ext. [1]
Melaleuca alternifolia, essential oil; tea tree oil [2]</td></tr>
<tr><td>Other names (usual name, trade name, abbreviation)</td><td>Tea Tree Oil, Oil of Melaleuca alternifolia (Terpinen-4-ol Type)
Essential oil of melaleuca alternifolia</td></tr>
<tr><td>ISO common name (if available and appropriate)</td><td>-</td></tr>
<tr><td>EC number (if available and appropriate)</td><td>285-377-1 [1][49]
- [2]</td></tr>
<tr><td>CAS number (if available)</td><td>85085-48-9 [1]
68647-73-4 [2]
Tea Tree Oil (TTO) is naturally occurring substance having complex composition (UVCB substance[50]). TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols.
For the components please refer to following table:
<table>
<tr><th>Name</th><th>CAS No.</th><th>EC No.</th></tr>
<tr><td>Terpinen-4-ol</td><td>562-74-3</td><td>209-235-5</td></tr>
<tr><td>γ-Terpinene</td><td>99-85-4</td><td>202-794-6</td></tr>
<tr><td>α-Terpinene</td><td>99-86-5</td><td>202-795-1</td></tr>
<tr><td>α-Terpineol</td><td>98-55-5</td><td>202-680-6</td></tr>
<tr><td>α-Terpinolene</td><td>586-62-9</td><td>209-578-0</td></tr>
<tr><td>α-Pinene</td><td>80-56-8</td><td>201-291-9</td></tr>
<tr><td>p-Cymene</td><td>99-87-6</td><td>202-796-7</td></tr>
<tr><td>1,8-Cineole (Eucalyptol)</td><td>470-82-6</td><td>207-431-5</td></tr>
<tr><td>Limonene</td><td>138-86-3</td><td>205-341-0</td></tr>
<tr><td>Aromadendrene</td><td>489-39-4</td><td>207-694-6</td></tr>
<tr><td>δ-Cadinene</td><td>483-76-1</td><td>--</td></tr>
<tr><td>Sabinene</td><td>3387-41-5</td><td>222-212-4</td></tr>
<tr><td>Globulol</td><td>489-41-8</td><td>207-696-7</td></tr>
<tr><td>Viridiflorol</td><td>552-02-3</td><td>209-003-3</td></tr>
<tr><td>Ledene</td><td>21747-46-6</td><td>244-565-3</td></tr>
</table></td></tr>
<tr><td>Other identity code (if available)</td><td>Not available.</td></tr>
<tr><td>Molecular formula</td><td>No molecular formula and mass can be assigned to Tea Tree Oil because it is a substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The specified components according to ISO 4730:2004 are displayed in the</td></tr>
</table>

[49] The substance tea tree oil has also been commonly described by the identifiers EC 285-377-1, CAS 85085-48-9 corresponding to Melaleuca alternifolia, ext., registered under REACH (https://echa.europa.eu/registration-dossier/-/registered-dossier/20921). Therefore these identifiers are also included in the proposed Annex VI entry.

following table. Furthermore, the components contained in Tea Tree Oil can be grouped according to their chemical structure as several components have very similar structures. Consequently, their chemical properties within the assigned groups are comparable.

Structural formula

	Name	Molecular weight	Molecular formula	Structural formula
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	136.24	$C_{10}H_{16}$	
	α-Terpinene	136.24	$C_{10}H_{16}$	
	α-Terpinolene	136.24	$C_{10}H_{16}$	
	Limonene	136.24	$C_{10}H_{16}$	
	p-Cymene	134.22	$C_{10}H_{14}$	
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	154.25	$C_{10}H_{18}O$	
	α-Terpineol	154.25	$C_{10}H_{18}O$	
Bicyclic monoterpenes	1,8-Cineole (Eucalyptol)	154.25	$C_{10}H_{18}O$	
	α-Pinene	136.24	$C_{10}H_{16}$	
	Sabinene	136.24	$C_{10}H_{16}$	
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	204.35	$C_{15}H_{24}$	
Polycyclic sesquiterpenes Aromadendrene group	Aromadendrene	204.35	$C_{15}H_{24}$	

		Ledene (Viridiflorene)	204.35	$C_{15}H_{24}$	
	Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	222.37	$C_{15}H_{26}O$	
		Viridiflorol	222.37	$C_{15}H_{26}O$	

SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	No molecular formula and mass can be assigned to Tea Tree Oil because it is a substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols.
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	TTO is manufactured by steam distillation of the leaves of the tea tree.
Degree of purity (%) (if relevant for the entry in Annex VI)	

Name	**Min. %**	**Max. %**
Terpinen-4-ol	30	48
γ-Terpinene	10	28
α-Terpinene	5	13
α-Terpineol	1.5	8
α-Terpinolene	1.5	5
α-Pinene	1	6
p-Cymene	0.5	8
1,8-Cineole (Eucalyptol)	trace	15
Limonene	0.5	1.5
Aromadendrene	0.5	3
δ-Cadinene	trace	3
Sabinene	trace	3.5
Globulol	trace	1
Viridiflorol	trace	1
Ledene	trace	3

source: ISO 4730:2004[51]

[51] Oil of Melaleuca, Terpinen-4-ol type (Tea Tree Oil); International standard; ISO 4730:2004(E); Second edition 2004-10-01

2.11.1.2 Composition of the substance

Table 59: **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)
Terpinen-4-ol	30 - 48	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
γ-Terpinene	10 - 28	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Terpinene	5 - 13	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
α-Terpineol	1.5 - 8	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Terpinolene	1.5 - 5	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Pinene	1 - 6	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
p-Cymene	0.5 - 8	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
1,8-Cineole (Eucalyptol)	trace - 15	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
Limonene	0.5 - 1.5	Flam. Liq. 3 H226 Skin Irrit. 2 H315 Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410	Flam. Liq. 3 H226 Skin Irrit. 2 H315 Aquatic Acute 1 H400 Aquatic Chronic 1 H410
Aromadendrene	0.5 - 3	No entry in Annex VI	**None**
δ-Cadinene	trace - 3	No entry in Annex VI	**None**
Sabinene	trace - 3.5	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
Globulol	trace - 1	No entry in Annex VI	**None**
Viridiflorol	trace - 1	No entry in Annex VI	**None**
Ledene	trace - 3	No entry in Annex VI	**None**

Table 60: **Impurities (non-confidential information) if relevant for the classification of the substance**

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 impurity exists for Tea Tree Oil.				

Table 61: **Additives (non-confidential information) if relevant for the classification of the substance**

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
None.					

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 62: **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 and ATEs	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	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	TBD	*Melaleuca alternifolia*, ext. [1] *Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1B Asp. Tox. 1 Repr. 2 Aquatic Acute 1 Aquatic Chronic 3	H226 H302 H332 H315 H317 H304 H361f H400 H412	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H302 H332 H315 H317 H304 H361f H400 H412		oral: ATE = 1049 mg/kg bw inhalation: ATE= 3.64 mg/L (mist) M=1	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	*Melaleuca alternifolia*, ext. [1] *Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] [2]	85085-48-9 [1] 68647-73-4 [2]							

2.11.2.2 Additional hazard statements / labelling

Not applicable

Table 63: **Reason for not proposing harmonised classification and status under CLH public consultation**

Hazard class	Reason for no classification	Within the scope of 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	harmonised classification proposed	Yes
Flammable solids	hazard class not applicable	No
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	data conclusive but not sufficient for classification	Yes
Pyrophoric solids	hazard class not applicable	No
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	data conclusive but not sufficient for classification	Yes
Oxidising solids	hazard class not applicable	No
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	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	harmonised classification proposed	Yes
Skin corrosion/irritation	harmonised classification proposed	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	hazard class not assessed in this dossier; data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	hazard class not assessed in this dossier; data lacking	Yes
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier; data lacking	No

Tea Tree Oil is an active substance in the scope of the Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). The substance is not currently listed in Annex VI of CLP, and there have been no previous

classification and labelling discussions of this substance. The substance is therefore subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required.

2.11.3 History of the previous classification and labelling

Tea Tree Oil (TTO) has not previously been assessed for harmonized classification by RAC or TC C&L.
TTO is not currently listed in Annex VI of Regulation (EC) 1272/2008.

2.11.4 Identified uses

Tea Tree Oil is intended to be used large-scale in the field (e.g. tomato and grape) and in greenhouse (e.g. tomato) to control fungal diseases, e.g. powdery mildew and grey mold.

2.11.5 Data sources

Source of data are studies which have been submitted for Annex I renewal under 1107/2009 and studies that were evaluated under 91/414/EEC. Combined Renewal Assessment Report for TTO prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008. Vol 3. December 2021

REACH registration dossier for melaleuca alternifolia, ext. (EC no 285-377-1), CSR, 2020.

Cosmetic Ingredient Review: https://cir-safety.org/sites/default/files/melalt092020SLR.pdf

BfR assessment of Tea tree oil used in cosmetics:
https://www.bfr.bund.de/cm/349/use_of_undiluted_tea_tree_oil_as_a_cosmetic.pdf

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

Not relevant (no metabolites were detected in the soil metabolism studies

2.12.1 STEP 1: Exclusion of degradation products of no concern

Not relevant.

2.12.2 STEP 2: Quantification of potential groundwater contamination

Not relevant.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

Not relevant.

2.12.3.1 STEP 3, Stage 1: screening for biological activity

Not relevant.

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Not relevant.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

Not relevant.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

Not relevant.

2.12.5 STEP 5: Refined risk assessment

Not relevant.

2.12.6 Overall conclusion

No metabolites were detected in soil metabolism studies, hence the evaluation of the relevance in groundwater is obsolete.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.13.1 Identity and physical chemical properties

Tea Tree Oil (TTO) is naturally occurring substance of biological origin having complex composition (UVCB substance). It does not have optically resolvable isomers. Therefore, no isomeric forms exist which have to be considered for risk assessment.

2.13.2 Methods of analysis

Not relevant since Tea Tree Oil technical is not composed of isomers.

2.13.3 Mammalian toxicity

Not applicable

2.13.4 Operator, Worker, Bystander and Resident exposure

Not applicable

2.13.5 Residues and Consumer risk assessment

Not applicable

2.13.6 Environmental fate

Not applicable

2.13.7 Ecotoxicology

Not applicable

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: Not applicable

Food of animal origin: Not applicable

Soil: TTO and its constituents

Groundwater: TTO and its constituents

Surface water: TTO and its constituents

Sediment: TTO and its constituents

Air: TTO and its constituents

2.14.2 Definition of residues for monitoring

Monitoring data are deemed not necessary due to the natural occurrence of TTO constituents.

Level 3

Tea Tree Oil (TTO)

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.	X		It is considered that Article 4 of Regulation(EC) No 1107/2009 is complied with Tea Tree Oil for the representative uses. A safe use has been demonstrated for the product containing Tea Tree Oil (Timorex Gold (BM 608), formulation EC) for use as an fungicide.
3.1.1.2	**Submission of further information**			
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
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.		X	
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		See Level 2, Point 2.6.11 to 2.6.13

	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		-
	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.	X		For all representative uses.
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		
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.	X		
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		Sufficient information has been presented by notifier to support the declared technical specification of Tea Tree Oil with respect to the identity and content of lead components in the respective technical specifications. The analytical methods provided are acceptable.

	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			No FAO specification
	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			No FAO specification
Methods of analysis				
		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 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.	X		Adequate analytical methods are available for the determination of Tea Tree Oil (lead components). Analytical methodology is available for the technical compound as well as for the formulated product.
	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.			Not required
	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		
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	
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	
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.		X	
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.**		X	
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.		X	
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		X	
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.	n.a.	n.a.	Not applicable.
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	All studies on degradation in soil and aquatic systems demonstrated that TTO constituents are not persistent in the environment with DT_{50} values <40 days and DT_{90} values <100 days.
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	**Persistence** All studies on degradation in soil and aquatic systems demonstrated that TTO constituents are not persistent in the environment with DT_{50} values <40 days and DT_{90} values <100 days. **Bioaccumulation** Estimated BCF submitted in support of CLP classification was < 500 for all monoterpene components, which account on average for > 95% of Tea Tree Oil. For the sesquiterpenes, BCF > 500 has been estimated, however, for the majority of these below 600, i.e. close to the trigger of 500. The sesquiterpene content of Tea tree oil is traces to max. 3.5% (individually), and cumulatively usually < 5%. Cumulative content of components with BCF > 600 (Cadinene, Aromadendrene and Ledene, BCF range 5000-7000) usually is below 2%. On this basis Tea Tree Oil is considered to be not bioaccumulative. **Toxicity** The long-term toxicity to aquatic species is clearly above 0.01 mg/L, so TTO does not fulfil criteria for toxic substances

Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	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 justification above for PBT criteria.
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.		X	The risk to all of potentially exposed non-target species was demonstrated to be acceptable from the intended uses of TTO in glasshouse. With regard to field uses, the risk was concluded to be acceptable for birds, soil organisms and non-target plants. However no acceptable risk to small herbivorous mammals could be concluded from both field uses in vineyard and tomatoes. In addition to that, the risk to aquatic organisms and bees could not be finalised. Although acceptable risk to non-target arthropods was demonstrated based on standard Tier I and Tier II studies, the RMS is of the opinion that due to specific mode of action the effects on most sensitive arthropod species (eggs and larvae) were not sufficiently investigated. Hence, in line with indications of ESCORT 2 guidance document, studies performed with most sensitive stages should be performed.
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	Available data were not sufficient to conclude on ED potential of TTO in non-target species. Further studies to address EATS-mediated modalities must be provided.
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.		X	
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			Based on the performed evaluation low acute contact and oral risk could be concluded. However, no valid chronic and larvae studies were provided, hence the chronic risk could not be finalised.

	− has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			
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		No residue definitions are required
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 groundwater exposure has been estimated for the "virtual compound", constructed on the basis of worst case input parameters derived in regulatory studies for selected TTO constituents. The whole application rate was attributed to this compound as representing worst case. Since PEC_{gw} calculated with these assumptions were <0.1 µg/L for all crops, scenarios and models, no unacceptable exposure of groundwater is anticipated.

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		X	

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;	X		

	(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). 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				
None				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
None				
3.1.4.6 Toxicology and metabolism				
None				
3.1.4.7 Residue data				
None				
3.1.4.8 Environmental fate and behaviour				
None				
3.1.4.9 Ecotoxicology				
Study on chronic toxicity to adult bees	Relevant for field uses of TTO (tomatoes and vineyards)	X		
Study on chronic toxicity to bee larvae (repeated exposure study performed to adult emergence)	Relevant for field uses of TTO (tomatoes and vineyards)	X		
Study to investigate effects on biological methods for sewage treatment	Formal data gap based on data requirements. Study is not used in the risk assessment.	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)
Aquatic risk assessment could not be finalised	Relevant for both field uses (tomatoes and vineyards)
Chronic and larvae risk assessment for bees	Relevant for both field uses (tomatoes and vineyards)

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)
None identified in the assessment	Not applicable

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		Vineyards	Tomatoes (field)	Tomatoes (glasshouse)
Operator risk	Risk not identified	None	None	None
	Assessment finalised	None	None	None
Worker risk	Risk not identified	None	None	None
	Assessment finalised	None	None	None
Bystander risk	Risk not identified	None	None	None
	Assessment finalised	None	None	None
Consumer risk	Risk not identified	None	None	None
	Assessment finalised	None	None	None
Risk to wild non target terrestrial vertebrates	Risk identified			
	Assessment not finalised	X	X	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified			
	Assessment not finalised	X	X	
Risk to aquatic organisms	Risk identified			
	Assessment not finalised	X	X	
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
Surface water exposure	Approach for calculation of the surface water exposure needs to be discussed since soil degradation data for the lead compound considered in modelling are available for single soil only (at least 4 are required in line with data requirements) resulting with most rapid degradation of other tested compounds.
Refinement of the risk to frugivorous mammals	Approach taken for refinement of the MAF and fTWA in tomatoes should be discussed. No respective residue decline studies were performed, but based on the information available from the residue trials where residues were measured at several time points it was possible to conclude that DT50 for TTO in tomato fruits would be <1 day. It should be thus discussed if such an estimation may be agreed for the regulatory risk assessment.
Non-target arthropods	Apart of being fungicide, TTO exhibits also insecticidal mode of action targeted on eggs and larvae. These most sensitive stages are not included in design of standard toxicity tests with NTA. In opinion of the RMS further studies including these most sensitive stages should be performed, in line with recommendations of ESCORT 2. However, this issue should be further discussed before a formal data requirement is set.

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
Not applicable		

[redacted]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

4 APPENDIX 1. GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

- EFSA. 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
- EC (European Commission). Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the Renewal Regulation). SANCO/2012/11251 rev.1.2, July 2012.
- EC (European Commission). 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) 283/2013 and Regulation (EU) No 284/2013. SANCO/10181/2013– rev. 2, May 2013.
- EC (European Commission). Guidance document on rules for revision of assessment reports. SANCO/10180/2013– rev. 1, March 2013.
- EC (European Commission). Template to be used for Assessment Reports. SANCO/12592/2012– rev. 0, November 2012.
- EC (European Commission). Template to be used for the List of Endpoints. SANCO/12483/2014– rev. 3, 29 May 2015.

Volume 3 – B1: Identity

None

Volume 3 - B2: Physicochemical properties

- FAO/WHO, FAO Plant Production and Protection Paper 228. Manual on Development and use of FAO and WHO specifications for pesticides 1st edition – third revision. Pesticide specifications. Rome, 2016

Volume 3 - B5: Analytical methods

- EC (European Commission). Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. SANCO/3030/99 rev.4, 11/07/00.
- EC (European Commission). Residues: Guidance for generating and reporting methods of analysis in support of preregistration data requirements for Annex II (part A, section 4) and Annex III (part A, Section 5) of directive 91/414. SANCO/3029/99 rev. 4, 11/07/00.
- EC (European Commission).: Guidance document on pesticide residues analytical methods. SANCO/825/00 rev.8.1, 16/11/2010.

Volume 3 - B6: Toxicology and metabolism of the active substance

- European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009;7(12):1438.
- EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874
- OECD Guidance document on semiochemical active substances and plant protection products, Series on Pesticides. No. 93. ENV/JM/MONO(2017)33
- Guidance 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
- OECD (2018), Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, OECD Publishing, Paris.

https://doi.org/10.1787/9789264304741-en

- Guidance of EFSA. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092
- OECD series of testing and assessment No. 43, Guidance document on mammalian reproductive toxicity testing and assessment. ENV/JM/MONO(2008)16
- OECD series of testing and assessment No. 106, Guidance document for histologic evaluation of endocrine and reproductive tests in rodents. Part 1, ENV/JM/MONO(2009)11
- Guidance on Evaluation of Reproductive Toxicity Data. Monograph No. 31. European Centre for Ecotoxicology and Toxicology of Chemicals. ECETOC, 2002
- Guidance on dermal absorption. EFSA Journal 2017;15(6):4873

Volume 3 - B7: Residues

- EC (European Commission), Appendix D. Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs; SANCO 7525/VI/95 - rev.9 March 2011.
- OECD (Organisation for Economic Co-operation and Development), OECD MRL Calculator: User Guide. In: Series on Pesticides No 56. ENV/JM/MONO(2011)2, 01 March 2011.
- Uwe Meier (editor), Growth Stages of mono-and dicotyledonous plants. BBCH Monograph, 2. Edition. Federal Biological Research Centre for Agriculture and Forestry, 2001
- EC (European Commission), Working document on the nature of residue in fish. SANCO/1187/2013, rev. 3, 31 January 2013.
- OECD (Organisation for Economic Co-operation and Development), Guidance document on residues in livestock. In: Series on Pesticides No 73. ENV/JM/MONO(2013)8, 04-Sep-2013.
- OECD (Organisation for Economic Co-operation and Development), Metabolism in Crops. OECD guideline for the testing of chemicals No 501, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Metabolism in Rotational Crops. OECD guideline for the testing of chemicals No 502, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Metabolism in Livestock. OECD guideline for the testing of chemicals No 503, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Residues in Rotational Crops (Limited Field Studies). OECD guideline for the testing of chemicals No 504, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Residues in Livestock. OECD guideline for the testing of chemicals No 505, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Stability of Pesticide Residues in Stored Commodities. OECD guideline for the testing of chemicals No 506, 16 October 2007.
- OECD (Organisation for Economic Co-operation and Development), Nature of the Pesticide Residues in Processed Commodities – High Temperature Hydrolysis. OECD guideline for the testing of chemicals No 507, 16 October 2007.
- OECD (Organisation for Economic Co-operation and Development), Magnitude of the pesticide residues in processed commodities. OECD guideline for the testing of chemicals No 508, 3 October 2008.
- OECD (Organisation for Economic Co-operation and Development), Crop Field Trial. OECD guideline for the testing of chemicals No 509, 7 September 2009.
- EC (European Commission), Appendix A. Metabolism and distribution in plants. 7028/IV/95-rev.3, 1997.
- EC (European Commission), Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev.6, 1997.
- EC (European Commission), Appendix C. Testing of plant protection products in rotational crops. 7524/VI/95-rev.2, 1997.
- EC (European Commission), Appendix F. Metabolism and distribution in domestic animals. 7030/VI/95-rev.3, 1997.
- EC (European Commission), Appendix I. Calculation of maximum residue level and safety intervals. 7039/VI/95, 1997. As amended by the document: classes to be used for the setting of EU pesticide maximum residue levels (MRLs). SANCO 10634/2010.

- EFSA, Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, September 2015

Volume 3 - B8: Environmental fate and behaviour

- Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, Version 1.1, December 2014.
- Soil persistence models and EU registration, 1997.
- FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies in EU Registration, Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference SANCO/10058/2005, version 2.0.
- FOCUS (2014): Generic Guidance for Tier 1 FOCUS Ground Water Assessments. version 2.2, May 2014.
- FOCUS (2009): Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU; Report of the FOCUS Ground Water Work Group, EC Document Reference SANCO/13144/2010, version 3, October 2014.
- Generic guidance for FOCUS surface water scenarios. Version 1.4, May 2015.
- FOCUS (2007): Landscape and Mitigation Factors in Aquatic Risk Assessment, Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2015, ver.2.0
- 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.

Volume 3 - B9: Ecotoxicology

- European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009;7(12):1438.
- Guidance for identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311.
- EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 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.
- European Food Safety Authority, 2013. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295
- Guidance Document on Terrestrial Ecotoxicology. SANCO/10329/2002 rev 2 final. 17th October 2002.
- Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods. Candolfi, M.P. et al. (2000) from the ESCORT 2 Workshop.
- European Food Safety Authority: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA Supporting publication 2015:EN-924.
- European Food Safety Authority: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA Supporting publication 2019:EN-1673.

Volume 4 Annex C:

- SANCO/10597/2003 –rev. 10.1, 13 July 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under regulation (EC) No 1107/2009.

4.1 REFERENCE LIST

Section identity, physical chemical and analytical methods

None

Section data on application and efficacy

None

Section toxicology

None

Section residue and consumer risk assessment

None

Section fate and behaviour in environment

None

Section ecotoxicology

None