Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Melaleuca alternifolia, ext. [1]
Melaleuca alternifolia, essential oil; tea tree oil [2]

EC Number: 285-377-1 [1] - [2]
CAS Number: 85085-48-9 [1] 68647-73-4 [2]

CLH-O-0000007380-79-01/F

Adopted

30 November 2023

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ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MELALEUCA ALTERNIFOLIA, EXT. [1] *MELALEUCA ALTERNIFOLIA*, ESSENTIAL OIL; TEA TREE OIL [2]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: Melaleuca alternifolia, ext. [1] *Melaleuca alternifolia*, essential oil; tea tree oil [2]
EC number: 285-377-1 [1] [2]
CAS number: 85085-48-9 [1] 68647-73-4 [2]
Dossier submitter: Poland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany	<confidential>	Company-Importer	1
Comment received				
We are a member of the REACH consortium of tea tree oil and support the considerations handed in by the Lead Registrant. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO.pdf				
Dossier Submitter's Response				
Thank you for commenting. Please see responses to comment no. 5, 23 and comment no. 10 and 11 by MS-SE and NL.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Sweden		MemberState	2
Comment received				
We note that respiratory sensitisation is not open for commenting, however the Swedish CA would like to point out the following: The Swedish CA agrees with the Dossier Submitter's proposal "no classification" for respiratory sensitisation. However, we consider that the basis to propose no classification should be lack of data for respiratory sensitisation rather than two negative GPMT studies which is the argument now.				

Dossier Submitter's Response
Noted and agreed. Thank you for commenting.
RAC's response
Noted and agreed.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Netherlands		MemberState	3
Comment received				
On page 99, the annotation of the corresponding tables in the summary table 35 are not correct. On page 75, a reference seems incorrect in Table 23 where 2017b was used twice.				
Dossier Submitter's Response				
Thank you for commenting. In fact, the reference of two generation study in the rat in Table 23 is incorrect, it should be: "Anonymous (2017a)" instead of "Anonymous (2017b)".				
RAC's response				
-				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	4
Comment received				
SID and physchem: - Typo: In Chapter 1.3.2 (Chemical name) the chemical name for 1,8-Cineole is stated as "1,33-trimethyl-2-oxabicyclo[2.2.2]octane" and should be corrected to "1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane" - From a formal point of view, the active substance seem to comprise two substances. A) CAS 68647-73-4 with the corresponding CAS name: Essential oils, Melaleuca alternifolia (13C, 14C, 16C); The CAS entry also contains the following notes: Extractives and their physically modified derivatives. Melaleuca alternifolia, Myrtaceae. A search with the CAS number on the ECHA-page leads to three entries. Two entries have the same CAS number but different list-numbers: 614-679-1 (extract form tea tree) and 641-387-1 (Melaleuca Alternifolia (Tea Tree) Leaf Oil. The third search results lists a formally different substance that is described in the next bullet point (EC 285-377-1). The CAS number 68647-73-4 was apparently incorrectly assigned to this entry. B) EC 285-377-1, CAS 85085-48-9, Melaleuca alternifolia, ext. The description of the EC-entry is as follows: "Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from Melaleuca alternifolia, Myrtaceae." It is not clear whether the CLH proposal addresses one or two substances. Next to this a comparison of the identity of both substances should be given. It is also not clear from which substance and under which method the stated substance composition in 1.3.7 was obtained. Please consider that a different method of manufacture (different extraction method) can lead to different compositions or even to different substance identities. According to				

REACH guidance for naming plant extracts with different extraction methods lead to different substances (see in guidance UVCB sub-type 3, where the source is biological and the process is refinement: Thus, refinements of extracts made by different processes, e.g. using different solvents or different purification steps, will result in different substances.) According to 1.3.6 of the CLH report, the method of manufacture is described separately as confidential information in Volume 4. Even though this specific data is confidential, please provide an explanation and/or justification in the non-confidential part of the CLH report what substance / what substances are covered and why they are covered in one CLH report. In case two formally different substances are covered, then two CLH entries should be created. We noticed that the proposed labelling does not comply with the proposed classification. In our view, the labelling should read as follows: GHS02, GHS07, GHS08, GHS09 Dgr H226, H302, H332, H315, H317, H304, H361f, H410 The ATE for Acute Tox. 4 H332 should address dusts and mists.
Dossier Submitter's Response
Noted. Thank you for commenting. Reply to comment on SID, provided by the ECHA Team: "The substance "tea tree oil" has been commonly described by the identifiers: 1. "Essential oils, Melaleuca alternifolia" CAS 68647-73-4 for describing the active substance according to the Commission Regulation (EC) 1107/2009 and 2. "Melaleuca alternifolia, ext.", EC 285-377-1, CAS 85085-48-9 for describing the substance registered under REACH (https://echa.europa.eu/registration-dossier/-/registereddossier/20921). The compositions and manufacturing processes reported in both cases have been analysed and concluded to be corresponding to the same substance. Standard ISO 4730 is used as a reference for setting specifications for this substance. Therefore the above identifiers that are used to describe the same substance are included in the proposed Annex VI entry. ECHA received C&L notifications reporting CAS 68647-73-4 and describing the substance with various names, for these submissions REACH-IT created different list numbers. However, the information provided on the identity and composition of these substances is limited. Therefore, we propose not to include these names in the CLH proposal. In conclusion, the notified active substance shall has been 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."
RAC's response
Noted. Dusts and mists is added to the derived ATE for inhalation.

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2023	France	Consortium HE	Industry or trade association	5

Comment received

The Consortium HE thanks the European Chemicals Agency for the opportunity to provide comments on the Tea Tree Oil (TTO) dossier. The Consortium HE calls on the regulatory authorities to assess the harmonised classification of TTO considering the following principles:
- The harmonized classification should deal with the substance itself rather than any impurities or substances that result from chemical reactions in unsuitable storage conditions.
- Only relevant and treatment-related biological effects from studies with a relevant route of exposure should be considered for classification purposes.
- Human-relevant New Approach Methodologies (NAMs) applicable to the hazard identification should be considered as part of the weight of evidence analysis.

The recent Fouyet et al. (2022) study with the hPlacentox assay should be mentioned in the data available on the Endocrine Disruption assessment.
According to Fouyet et al. (2022), TTO seems to be a hormone modulator rather than endocrine disruptor since it increases the placental hormone hPL but do not cause adverse cellular effects (TTO did not activate P2X7 receptor). Previous studies showed that the P2X7 receptor activation is a common cellular mechanism of toxicity for endocrine disruptors in placenta, as P2X7 receptor was activated by all the tested endocrine disruptors in JEG-Tox cells.

Furthermore, the key component of TTO (4-terpineol) do not have the same hormonal effect as whole TTO, proving the need to study the whole essential oil rather than its components individually to conclude on the potential toxic effects.

Fouyet, S; Olivier, E.; Leproux, P.; Dutot, M.; Rat, P. Evaluation of Placental Toxicity of Five Essential Oils and Their Potential Endocrine-Disrupting Effects. Curr. Issues Mol. Biol. 2022, 2, 2794–2810. https://doi.org/10.3390/ cimb44070192

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil.pdf

Dossier Submitter's Response

Thank you for your comments and additional data.
The classification criteria of Regulation (EC) No 1272/2008 will be taken into account during the RAC opinion-making process on the proposed CLH.
According to Art. 5 of CLP regulation: the available information, referred to in paragraph 1 of Art. 5, should '*relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used*'.
All available and reliable data as well as data on components of Melaleuca alternifolia, ext should be considered for classification purposes.
According to section 3.7.2.5.5 of Annex I to CLP regulation: *in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity.* Since Melaleuca alternifolia, ext. is used not only in PPPs, oral route of human exposure could not be excluded.

The CLP classification criteria take into account, first of all, the harmful effects shown in the available animal studies, while the mechanism of action, including endocrine disruption, is not a criterion for classifying a substance for reprotoxicity. In addition Fouyet study (2022) has been done in vitro using method which is neither recognised internationally and nor by OECD for classification of health hazards. In addition in the opinion of authors of this study the observed effects did not demonstrate any adverse effects, thus it is not useful for classification of TTO according to criteria set in Regulation 1272/2008.

RAC's response

Noted.
Most relevant animal studies used for classification are performed with Tea Tree Oil according to ISO 4730; 2004 or 2017, so relevant for classification. In case of the LLNA tests, well-storage conditions are noted.
All studies available are used for **hazard** classification purposes, also gavage studies. It is noted that dietary studies might show no effects or effects at higher doses compared to gavage studies, but that is no reason for no classification.
With regards to the endocrine disruptive assessment, this is part of the DRAR, not of the CLH report.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	France	Consortium HE	Industry or trade association	6

Comment received

The Consortium HE calls on the regulatory authorities to assess the harmonized classification of TTO considering the following principles:
- The harmonized classification should deal with the substance itself rather than any impurities or substances that result from chemical reactions in unsuitable storage conditions.
- Only relevant and treatment-related biological effects from studies with a relevant route of exposure should be considered for classification purposes.
- Human-relevant New Approach Methodologies (NAMs) applicable to the hazard identification should be considered as part of the weight of evidence analysis.

The Consortium HE's comments below relate to the following elements:
o Skin sensitization assessment
o Developmental toxicity assessment
o Endocrine disruption assessment

The recent Fouyet et al. (2022) study with the hPlacentox assay should be mentioned in the data available on the Endocrine Disruption assessment.
According to Fouyet et al. (2022), TTO seems to be a hormone modulator rather than endocrine disruptor since it increases the placental hormone hPL but do not cause adverse cellular effects (TTO did not activate P2X7 receptor). The results obtained (no alteration of estradiol release) appear in contradiction with in vitro studies mentioned that demonstrated estrogenic and anti-androgenic effects of TTO in MCF-7 human breast cells reported by Henley et al. (2007).

Furthermore, the key component of TTO (4-terpineol) do not have the same hormonal effect as the 4-terpineol at the same concentration naturally present in TTO, proving the need to study the whole essential oil rather than its components individually to conclude on the potential toxic effects. Indeed, 4-terpineol at 36.98% induced a higher

progesterone secretion and estradiol than the control, while 4-terpineol at the same concentration (36.98%) naturally present in TTO had no effect on progesterone and estradiol. Conversely, TTO stimulated the secretion of hPL but 4-terpineol did not. Fouyet, S; Olivier, E.; Leproux, P.; Dutot, M.; Rat, P. Evaluation of Placental Toxicity of Five Essential Oils and Their Potential Endocrine-Disrupting Effects. Curr. Issues Mol. Biol. 2022, 2, 2794–2810. https://doi.org/10.3390/ cimb44070192
Dossier Submitter's Response
Thank you for your comments and additional data. Please see DS's response to comment no. 5.
RAC's response
Noted. Please note that the ED classification is not part of this CLH dossier.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Belgium	Pranarom International	Company-Manufacturer	7
Comment received				
Pranarom International SA calls on the regulatory authorities to assess the harmonized classification of TTO considering the following principles: ☐ The harmonized classification should deal with the substance itself rather than any impurities or substances that result from chemical reactions in unsuitable storage conditions. ☐ Only relevant and treatment-related biological effects from studies with a relevant route of exposure should be considered for classification purposes. ☐ Human-relevant New Approach Methodologies (NAMs) applicable to the hazard identification should be considered as part of the weight of evidence analysis. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil_Final version.docx				
Dossier Submitter's Response				
Thank you for your comments and additional data. Please see DS's response to comment no. 5.				
RAC's response				
Noted. Most relevant animal studies used for classification are performed with Tea Tree Oil according to ISO 4730; 2004 or 2017, so relevant for classification. In case of the LLNA tests, well-storage conditions are noted. All studies available are used for **hazard** classification purposes, also gavage studies. It is noted that dietary studies might show no effects or effects at higher doses compared to gavage studies, but that is no reason for no classification. With regards to the endocrine disruptive assessment, this is part of the DRAR, not of the CLH report.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number

27.01.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	8
Comment received				
We now welcome the opportunity to comment on the consultation on the harmonized classification of Tea Tree Oil (TTO). In particular, we would like to introduce comments on the proposal to classify TTO as a Category 2 reprotoxin and as a skin sensitiser. These comments are made as the Lead Registrant of the REACH tea tree oil dossier. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO_Final.pdf				
Dossier Submitter's Response				
Thank you for your comment. Please see DS's response to comment no. 5 , 21 and comment no. 10 and 11 by MS-SE and NL.				
RAC's response				
Noted. Points are included in the discussion in the draft RAC opinion.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany	<confidential>	Company-Importer	9
Comment received				
As explained in the attachement: It has been proposed by the dossier submitter to classify TTO as a Category 2 reprotoxin based on observed male fertility effects observed in gavage studies on both rat and dog. In the conclusions of the STOT-RE classification proposal by the Dossier Submitter (DS) it states that: "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." This conclusion is also pertinent for other classification proposals where the conclusion relies on the use of gavage studies on TTO (or other terpenes), in this case Classification for Reproduction. Although gavage administration is a normal way to evaluate toxicity, in some cases it creates pharmacokinetic (and then pharmacodynamic) circumstances which cannot be encountered in real conditions of exposure and can be considered in these cases as a non-relevant route of exposure (as would be IV or IP mode of administration). This is shown in a series of studies with α-Terpineol. α-Terpineol is a constituent of TTO and very similar to its main component Terpinen-4-ol. A set of studies was carried out in order to evaluate the effects of Terpineol on reproduction. All these studies are reliable without restrictions (Volume 3 – B.6 (AS) PPPR combined renewal and assessment report on TTO). In a repeated dose gavage toxicity study in rats, the main effects at the top dose of 750				

mg/kg bw were reduced testis weight and an indication of reduced epididymal weights. Further, reduced numbers or complete absence of spermatozoa accompanied by the presence of degenerate spermatogenic cells were observed in the epididymis after a 5 week dosing period to 750 mg/kg bw with no apparent recovery within 2 weeks. Other related abnormalities were seen less frequent in some animals. In summary, following gavage administration a clear testicular toxicity was observed at 750 mg/kg bw/day, while no testicular effect was seen at 250 mg/kg bw/day.
This testicular toxicity was investigated more closely, and it was checked if the type of administration, i.e. gavage, had an impact on the results.

In a comparative two-week study, Terpineol multiconstituent was administered orally either by diet or by gavage to male rats. Two groups (5 male animals/group) received Terpineol orally by gavage at 500 and 750 mg/kg bw and two others via the diet, at concentrations of 8,000 or 12,000 ppm for two weeks. There were two control groups, one vehicle control gavage administration and one pure control. The results relevant in this case were: Negative effects on sperm mobility clearly confirmed previous gavage studies, while no effects were detected when Terpineol was administered via diet.

Such discrepancies of effects, depending on the mode of dose administration were confirmed in a 90-day toxicity study (i.e. a whole period of spermatogenesis). Terpineol multiconstituent was dissolved in corn oil, mixed in Ssniff powder feed at the dose level of 12000 ppm and fed to male Sprague-Dawley rats (10/dose) daily ad libitum for 13 weeks. A slight significant increase in the percentage of abnormal (4.8 %) sperms was noted at 12000 ppm as compared to the control group. However, the change was considered incidental as it was well within the range of normal biological variation noted among male rats [the range of the in-house historical control data for mean percentage of abnormal sperms: 0.1- 7.4%]. The sperm motility remained unaffected by dietary administration of test item. There were no test item-related changes observed in cauda epididymal weight/sperm count and testicular weight/spermatid count.

In conclusion: It is proposed that no classification is warranted for reproduction due to the unsuitability of the use of gavage studies on TTO for the purposes of classification.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO.pdf

Dossier Submitter's Response

Thank you for comment.
All available and reliable data as well as data on components of Melaleuca alternifolia, ext should be considered for classification purposes.
According to section 3.7.2.5.5 of Annex I to CLP regulation: *in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity.* Since Melaleuca alternifolia, ext. is used not only in PPPs, oral route of human exposure could not be excluded.
Please see DS's response to comment no. 5 and comment no. 10 and 11 by MS-SE and NL.

RAC's response

Exposure route and way of dosing might be of relevance in a risk assessment. In the case of hazard classification, all information is included. Gavage studies are not unsuitable for classification purposes.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MELALEUCA ALTERNIFOLIA, EXT. [1] *MELALEUCA ALTERNIFOLIA*, ESSENTIAL OIL; TEA TREE OIL [2]

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Sweden		MemberState	10
Comment received				
Fertility The Swedish CA does not support the proposal to classify tea tree oil in Repr. 2 for fertility but considers it to be a case for Repr. 1B classification. This is based on the clear and consistent evidence of effects on testes and spermatogenesis (also acknowledged by the Dossier Submitter) seen in short-term (28d) and subchronic studies, as well as in a 2-generation (OECD 416, GLP) study in the rat in combination with a decreased observed fertility. More specifically, the adverse effects include: ☐ Degenerative testes changes, smaller epididymides and testes, and oligospermia/aspermia at 125 and 250 mg/kg bw/day in a 28d study in rats (Anon., 2010b) ☐ ↓ Sperm counts and motility, ↑ percent abnormal sperms, ↓ testes and epididymides weights, degenerative changes of seminiferous tubules, cell debris in tubular lumen of testes and atrophic appearance, sertoli cell vacuolation, sperm granuloma and cellular debris in epidydimal duct lumen at 60 and 120 mg/kg bw/day in a 90-day study in rats (Anon., 2011b) ☐ ↓ Sperm counts and motility, ↑ Percent abnormal sperms, sperm granuloma, oligospermia, single cell necrosis, luminal cell debris and degeneration/atrophy of seminiferous tubules at 60 mg/kg bw/day in a 90-day study in rats (Anon., 2016a) ☐ ↓ viability and motility of spermatids at 75/60 and 180/120 mg/kg bw/day in a 90-day study in dogs (Anon., 2018a). ☐ ↓ progressive sperm motility (F0/F1), ↓ cauda epididymal sperm count (F0/F1), ↑ percent abnormal sperm (F0) together with ↓ male and female fertility indices, ↓ implantations, ↓ mean litter size, ↓ mean viable litter size and ↓ day 4 survival index at 38 and 50 mg/kg bw/day in a 2-generation study in the rat (Anon., 2017b). Altogether, we consider these clear and consistent evidence to warrant classification as Repr. 1B, H360F. We do not support the argumentation of the Dossier Submitter that the route of exposure (gavage as opposed to dietary exposure) should decrease the concern and relevance for humans, resulting in a category 2 classification for effects on fertility. The gavage route of exposure is a common exposure route to demonstrate the inherent properties of substances in animal studies. In addition, tea tree oil is used in cosmetic products, subject to topical application and dermal exposure/uptake. For your information, the Swedish CA has recently submitted a Repr. 1B CLH proposal that covers a group of substances, including the substance p-cymene, which is one of the constituents of tea tree oil. Interestingly, similar findings on testicular toxicity and spermatotoxicity are reported for this substance.				
Dossier Submitter's Response				
Thank you for comment. Noted				
RAC's response				
Noted. Please find the proposal of category 1B for fertility in the draft opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Netherlands		MemberState	11
Comment received				

Fertility:
A clear effect of TTO on male fertility was observed in a 2-generation study, 28-day study, and two 90-day studies in rats and a 90-d study in dogs. The fertility effects included decreased sperm count and mobility, sometimes associated with microscopic changes in the testes (germ cell degeneration/atrophy and sertoli cell vacuolation) and epididymides (sperm, oligospermia/chronic active inflammation, oligospermia, single cell necrosis, luminal cell debris). These effects on sperm parameters contributed to the significantly lower mean litter size and viable litter size in the 2-generation study and a clear reduction in male fertility index. In addition, there was a significant reduction of the mean number of corpora lutea, implantations and an increase in pre-implantation loss indicative of an effect on female fertility. The systemic toxicity at the dose of 50-60 mg/kg bw/day at which the fertility effects were observed in the 2-gen and 90-d studies was limited to changes in body weight gain, which is marginally even toxicity and cannot explain such strong effects on fertility parameters. Also noteworthy is that the top dose was reduced in the F1 generation due to the severity of the fertility effects rather than systemic toxicity and that even the lower top dose of 38 mg/kg bw/day still induced sperm toxicity.

Based on the clear effects on fertility in males and females, we propose to classify in category 1B for effects on fertility rather than category 2.

Development:
The dossier submitter claims that the developmental effects observed in two prenatal developmental toxicity studies in the rat and 1 in the rabbit, and one 2-generation toxicity study in rats were caused by to maternal toxicity.
The effects on development in the 2-gen rat study include a decrease of pup mean body weight at 38 mg/kg bw/day and increase of post-implantation loss, mean viable litter size, and a significant decrease in Day 4 survival index. These effects could not be linked to maternal toxicity (see also fertility) and are relevant for classification for development.
The effects on development in the prenatal developmental toxicity study (PNDT) in the rat include significant delay in skeletal ossification, incomplete/poor ossification at 150/60 and/or 300/120 mg/kg bw/day. At these doses severe maternal toxicity was observed, in particular mortality of 3 rabbits after 3 days of treatment at the top dose. Due to their nature, these developmental effects are likely to be secondary to the maternal toxicity.
The effects on development in the PNDT study in the rabbit include a significant increase of post-implantation loss at 75 mg/kg bw/day resulting in a reduction of the number of foetuses. This effect was observed without maternal toxicity, therefore is relevant for classification.
The effects on development in the second PNDT study in the rat include malformations, a statistically higher incidence of skeletal malformations (short, bent scapula or humerus, short and bent femur, malformed vertebrae were noted at 100 and 250 mg/kg bw/day). Furthermore, visceral variations were statistically higher at 250 mg/kg bw/day which include dilated brain ventricles and displaced gonads associated with the intrauterine growth retardation. 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. These effects occurred together with clear maternal toxicity such as severely reduced maternal body weight gain (-20% and -45% respectively at 100 and 250 mg/kg bw/day) and food consumption resulting in the

mortality of 7 females between GD8 and GD11 exposed to 250 mg/kg bw/day of TTO. In this study the dosing makes it difficult to ascertain whether there is an effect on development, as the high and mid dose were relatively high and no effects occurred at the low dose. The most relevant effect for developmental toxicity is the increase in post-implantation loss in the rat 2-gen study and the rabbit PNDT. However, the increases were relatively small and in the rat 2-gen study it is difficult to separate developmental toxicity from the fertility effects. The two rat PNDTs showed malformations and foetal growth retardation, but these were likely associated with maternal toxicity. Although it is a borderline case for Cat 1B, due to the uncertainties, we suggest a classification in category 2 for Developmental toxicity.
Dossier Submitter's Response
Thank you for comment. Noted
RAC's response
Noted. Please find the proposal of category 1B for fertility and category 2 for developmental toxicity in the draft opinion.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany	Stockton Europe Ltd.	Please select organisation type..	12
Comment received				
Vol 1 Point 2.6.6.1: The applicant is aware of the difference between hazard and risk and the applicability of either during assessment and classification of the active substance. However, TTO is a natural substance, for which the Regulations 1107/2009 and 1272/2008 is deemed not suitable in every aspect. The applicant therefore requests an in-depth consideration of the non-standard situation for TTO.				
Dossier Submitter's Response				
Thank you for your comment. The classification criteria of Regulation (EC) No 1272/2008 have to be taken into account during the RAC opinion-making process on the proposed CLH. According to Art. 5 of CLP regulation: the available information, referred to in paragraph 1 of Art. 5, should '*relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used*'. All available and reliable data as well as data on components of Melaleuca alternifolia, ext should be considered for classification purposes.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	13
Comment received				
It has been proposed by the dossier submitter to classify TTO as a Category 2 reprotoxin based on observed male fertility effects observed in gavage studies on both rat and dog. In the conclusions of the STOT-RE classification proposal by the Dossier Submitter (DS) it states that:				

"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."

This conclusion is also pertinent for other classification proposals where the conclusion relies on the use of gavage studies on TTO (or other terpenes), in this case Classification for Reproduction.

Although gavage administration is a normal way to evaluate toxicity, in some cases it creates pharmacokinetic (and then pharmacodynamic) circumstances which cannot be encountered in real conditions of exposure and can be considered in these cases as a non-relevant route of exposure (as would be IV or IP mode of administration).

This is shown in a series of studies with alpha-Terpineol. alpha-Terpineol is a constituent of TTO and very similar to its main component Terpinen-4-ol. A set of studies was carried out in order to evaluate the effects of alpha-Terpineol on reproduction. All these studies are reliable without restrictions (Volume 3 – B.6 (AS) PPPR combined renewal and assessment report on TTO).

In a repeated dose gavage toxicity study in rats, the main effects at the top dose of 750 mg/kg bw alpha-Terpineol were reduced testis weight and an indication of reduced epididymal weights. Further, reduced numbers or complete absence of spermatozoa accompanied by the presence of degenerate spermatogenic cells were observed in the epididymis after a 5 week dosing period to 750 mg/kg bw with no apparent recovery within 2 weeks. Other related abnormalities were seen less frequent in some animals. In summary, following gavage administration a clear testicular toxicity was observed at 750 mg/kg bw/day, while no testicular effect was seen at 250 mg/kg bw/day.

This testicular toxicity was investigated more closely, and it was checked if the type of administration, i.e. gavage, had an impact on the results.

In a comparative two-week study, Terpineol multiconstituent was administered orally either by diet or by gavage to male rats. Two groups (5 male animals/group) received Terpineol orally by gavage at 500 and 750 mg/kg bw and two others via the diet, at concentrations of 8,000 or 12,000 ppm for two weeks. There were two control groups, one vehicle control gavage administration and one pure control. The results relevant in this case were: Negative effects on sperm mobility clearly confirmed previous gavage studies, while no effects were detected when Terpineol was administered via diet.

Such discrepancies of effects, depending on the mode of dose administration were confirmed in a 90-day toxicity study (i.e. a whole period of spermatogenesis). Terpineol multiconstituent was dissolved in corn oil, mixed in Ssniff powder feed at the dose level of 12,000 ppm and fed to male Sprague-Dawley rats (10/dose) daily ad libitum for 13 weeks. A slight significant increase in the percentage of abnormal (4.8 %) sperms was noted at 12,000 ppm as compared to the control group. However, the change was

considered incidental as it was well within the range of normal biological variation noted among male rats [the range of the in-house historical control data for mean percentage of abnormal sperms: 0.1- 7.4%]. The sperm motility remained unaffected by dietary administration of test item. There were no test item-related changes observed in cauda epididymal weight/sperm count and testicular weight/spermatid count.

In conclusion: It is proposed that no classification is warranted for reproduction due to the unsuitability of the use of gavage studies on TTO for the purposes of classification.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO_Final.pdf

Dossier Submitter's Response

Thank you for comment.
Please see DS's response to comment no. 5 and comment no. 10 and 11 by MS-SE and NL.

RAC's response

Exposure route and way of dosing might be of relevance in a risk assessment. In the case of hazard classification, all information is included. Gavage studies are not unsuitable for classification purposes.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	United Kingdom	Health and Safety Executive	National Authority	14

Comment received

Sexual function and fertility

The classification of Repr. 2 for sexual function and fertility is based on evidence from animal studies, indicating a treatment related effect on fertility, testes, epididymides and sperm (in two species – rats and dogs) in the absence of severe maternal toxicity, and the DS states that such effects are not reported in humans exposed to components of Tea Tree Oil (TTO) at relatively high doses with food. Taking into account the uncertainty regarding the relevance of the effects to humans, the DS has proposed Repr. 2. Would the DS be able to provide the human evidence referred to in this conclusion alongside an explanation as to why this evidence decreases the concern.

Lactation

During the assessment of lactation, the DS noted that the mean pup body weights in the F2 litter were reduced at 38 mg/kg bw/d but had recovered by the end of the lactation period. This data does not appear to be provided. For completeness, it would be useful to see the data on pup body weights during the lactation period for both the F1 and F2 pups.

Dossier Submitter's Response

Sexual function and fertility :

The findings reported in rats and dogs have not been reported in humans so far. In the revised assessment report for Tea Tree Oil it is already acknowledged that there are no targeted epidemiological studies on this issue.

Nevertheless, for Tea Tree Oil special emphasis has to be put on the natural occurrence of the components, largely even in everyday food items such as carrots, citrus (grapefruit). This a situation which is rarely observed in the plant protection sector when synthetic substances are discussed for authorization but rather common for natural substances such as Tea Tree Oil. As the components are mainly terpenes a natural exposure is hardly avoidable and has been documented for hundreds of years.

A summary of the natural occurrence of the Tea Tree Oil components and its comparison to the exposure through the proposed use as plant protection product is presented in the tables on the following pages. Please note that both tables on TMDI and IESTI calculations have already been presented in the submitted dossier section MCA-6.

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.

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 1) 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.

Both tables show the calculations from consumer risk assessment and demonstrate the massive natural exposure via natural exposure. Keeping this in mind it is highly unlikely that a toxicity as observed in rats and dogs would have been undetected during all the past years. Thus, the missing of targeted epidemiological studies by itself can be seen as proof of the absence of effects in humans.

Therefore, due to the high natural exposure and no effects reported in humans and considering the long timespan for which the natural exposure already occurs this is enough evidence to conclude that there are no effects in human comparable to those in rats and dogs.

Table 2: Chronic exposure levels of TTO components in comparison to regulatory status and uptake limits

TMDI calculations	Exposure level [mg/kg bw/day]						
	Name	Natural exposure in food	Exposure from application (Measured / calculated)	Appl. exp. in % of natural exposure in food	US EPA food additive	Cramer class acc. to Toxtree classification scheme (EUR 24898 EN – 2011)	Chronic uptake from fungicidal use below general toxicity threshold of 30 / 9 / 1.5 µg/kg bw/day (Cramer class 1 / 2 / 3) (EFSA 2016, 2019[1])
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	0.00079	0.00042	53%	**Y**	Common terpenes = Cramer class 1 (monocyclic terpenes)	< 0.03 mg/kg bw/day
	α-Terpinene	0.015	0.007	47%	**Y**		< 0.03 mg/kg bw/day
	α-Terpinolene	0.00476	0.002	42%	**Y**		< 0.03 mg/kg bw/day
	Limonene	0.793	0.001	0%			< 0.03 mg/kg bw/day
	p-Cymene	0.00008	0.001	1250%	**Y**		< 0.03 mg/kg bw/day
	Sum	**0.814**	**0.0114**	1%			**< 0.03 mg/kg bw/day**
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.00468	0.00019	4%	**Y**		< 0.03 mg/kg bw/day
	α-Terpineol	8.873	0.004	0%	**Y**		< 0.03 mg/kg bw/day
	Sum	**8.878**	**0.0042**	0%			**< 0.03 mg/kg bw/day**
Bicyclic monoterpenes	1,8-Cineole	-	0.00019	--	**Y**		< 0.03 mg/kg bw/day
	α-Pinene	0.279	0.003	1%	**Y**		< 0.03 mg/kg bw/day
	Sabinene	0.589	0.0001	0%			< 0.03 mg/kg bw/day
	Sum	**0.868**	**0.0033**	0%			**< 0.03 mg/kg bw/day**
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	0.372	0.0001	**0%**		Bicyclo-compound = Cramer class 2	**< 0.009 mg/kg bw/day**
Polycyclic sesquiterpenes, Aromadendrene group	Aromaden-drene	0.242	0.0001	0%		Common component of food (Cramer class 2)	**< 0.009 mg/kg bw/day**
	Ledene	-	0.0001	--		Cramer Class 3	< 0.0015 mg/kg bw/day
	Sum	**0.242**	**0.0002**	0%			

Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	1.198	0.00001	0%		Common component of food (Cramer class 2)	< 0.009 mg/kg bw/day
	Viridiflurol	0.183	0.00001	0%			< 0.009 mg/kg bw/day
	Sum	**1.381**	**0.00002**	0%			**< 0.009 mg/kg bw/day**

Table 3: Acute exposure levels of TTO components in comparison to regulatory status and uptake limits

IESTI calculations	Exposure level [mg/kg bw/day]						
	Name	Natural exposure in food	Exposure from application (Measured / calculated)	Appl. exp. In % of natural exposure in food	US EPA food additive	Cramer class acc. to Toxtree classification scheme (EUR 24898 EN – 2011)	Acute uptake from fungicidal use below general toxicity threshold of 30 / 9 / 5 µg/kg bw/day (Cramer class 1 / 2 / 3) (EFSA 2016, 2019[2])
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	1.696	0.006	0%	**Y**	Common terpenes = Cramer class 1 (monocyclic terpenes)	< 0.03 mg/kg bw/day
	α-Terpinene	0.036	0.189	525%	**Y**		
	α-Terpinolene	0.220	0.051	23%	**Y**		
	Limonene	89.333	0.024	0%			< 0.03 mg/kg bw/day
	p-Cymene	0.0036	0.027	750%	**Y**		< 0.03 mg/kg bw/day
	Sum	**91.289**	**0.297**	0%			
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	0.0017	0.003	176%	**Y**		< 0.03 mg/kg bw/day
	α-Terpineol	8.480	0.119	1%	**Y**		
	Sum	**8.482**	**0.122**	1%			
Bicyclic monoterpenes	1,8-Cineole	-	0.003		**Y**		< 0.03 mg/kg bw/day
	α-Pinene	0.266	0.081	30%	**Y**		
	Sabinene	0.557	0.002	0%			< 0.03 mg/kg bw/day
	Sum	**0.823**	**0.086**	361			
Polycyclic sesquiterpenes,	δ-Cadinene	**52.00**	0.002	0%		Bicyclo-compound	< 0.009 mg/kg bw/day

[1] EFSA Scientific Committee, More SJ et al, 2019. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal 2019;17(6):5708, 17 pp. https://doi.org/10.2903/j.efsa.2019.5708 **and** EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues),2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549, 129 pp. doi:10.2903/j.efsa.2016.4549

[2] EFSA Scientific Committee, More SJ et al, 2019. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal 2019;17(6):5708, 17 pp. https://doi.org/10.2903/j.efsa.2019.5708 **and** EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues),2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549, 129 pp. doi:10.2903/j.efsa.2016.4549

Cadinane group						= Cramer class 2	
Polycyclic sesquiterpenes, Aromadendrene group	Aromaden-drene	0.231	0.006	3%		Common component of food (Cramer class 2)	
	Ledene	-	0.002			Cramer Class 3	< 0.005 mg/kg bw/day
	Sum	**0.231**	**0.008**	3%			
Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	1.145	0.001	0%		Common component of food (Cramer class 2)	< 0.009 mg/kg bw/day
	Viridiflurol	0.175	0.001	1%			< 0.009 mg/kg bw/day
	Sum	**1.320**	**0.002**	0%			< 0.009 mg/kg bw/day

Lactation:

KCA 5.6.1/01	Anonymous	2017a	Tea Tree Oil: Two generation reproduction toxicity study in Wistar rats ADVINUS THERAPEUTICS LIMITED, India Report No.: G11090 Document No.: 400395 Report Date: 2017-11-30 GLP, not published

F1 pup weight during lactation

Group	Dose (mg/kg bw/day)	Mean weight ± SD of pubs on day				
		1	4	7	14	21
Male pubs						
G1	0	6,19 ± 0,76	8,02 ± 1,56	1,96 ± 2,21	17,41 ± 3,57	26,85 ± 5,51
G2	10	5,10* ± 0,50	7,29 ± 1,14	9,67 ± 1,53	15,25 ± 5,38	22,58* ± 5,38
G3	25	5,65* ± 0,42	7,21 ± 0,85	9,67 ± 1,31	15,03 ± 2,61	22,42* ± 4,00
G4	50	5,63 ± 0,69	7,15 ± 1,48	10,14 ± 2,09	17,78 ± 3,80	27,55 ± 5,89
Female pubs						
G1	0	5,77 ± 0,63	7,61 ± 1,29	10,49 ± 1,96	17,00 ± 3,42	26,50 ± 5,10
G2	10	5,56 ± 0,59	7,25 ± 1,11	9,52 ± 1,63	15,25 ± 3,41	22,10* ± 4,99
G3	25	5,42 ± 0,40	7,03 ± 0,89	9,40 ± 1,45	15,02 ± 2,73	22,25* ± 3,85
G4	50	5,32 ± 0,51	6,36 ± 1,08	9,32 ± 1,95	16,34 ± 3,58	26,18 ± 5,80
Combined sex						
G1	0	5,98 ± 0,67	7,81 ± 1,38	10,70 ± 1,99	17,19 ± 3,42	26,61 ± 5,14
G2	10	5,65 ± 0,54	7,27 ± 1,05	9,57 ± 1,50	15,14 ± 3,09	22,24* ± 4,96
G3	25	5,58 ± 0,39	7,14 ± 0,86	9,51 ± 1,42	15,14 ± 2,71	22,47* ± 3,91
G4	50	5,56 ± 0,65	6,81 ± 1,35	9,77 ± 2,08	17,14 ± 3,72	26,93 ± 5,81

Statistical Test: ANOVA & Dunnett's Test
*statistically significant

F2 pub weight during lactation

Group	Dose (mg/kg bw/day)	Mean weight ± SD of pubs on day				
		1	4	7	14	21
Male pubs						
G1	0	6,21 ± 0,73	8,17 ± 1,50	11,42 ± 1,84	19,77 ± 2,93	30,94 ± 4,84

G2	10	5,96 ± 0,87	7,73 ± 1,76	10,81 ± 1,81	19,53 ± 3,61	30,24 ± 5,18
G3	25	5,93 ± 0,60	7,54 ± 1,12	10,76 ± 1,56	19,41 ± 2,96	29,38 ± 5,13
G4	50	5,51* ± 0,62	6,73* ± 1,01	10,00 ± 1,25	18,29 ± 2,13	27,70 ± 4,43
Female pubs						
G1	0	5,82 ± 0,75	7,90 ± 1,47	11,05 ± 1,66	19,36 ± 3,13	30,75 ± 4,69
G2	10	5,59 ± 0,73	7,10 ± 1,24	10,25 ± 1,79	19,65 ± 3,57	29,07 ± 4,75
G3	25	5,70 ± 0,60	7,31 ± 1,10	10,53 ± 1,49	18,38 ± 2,68	28,60 ± 4,25
G4	50	5,09* ± 0,50	6,51* ± 0,87	9,62* ± 0,93	17,82 ± 2,11	27,26 ± 4,41
Combined sex						
G1	0	6,00 ± 0,73	8,04 ± 1,44	11,23 ± 1,71	19,59 ± 2,94	30,93 ± 4,62
G2	10	5,83 ± 0,85	7,53 ± 1,69	10,50 ± 1,83	19,12 ± 3,87	29,76 ± 5,24
G3	25	5,83 ± 0,58	7,43 ± 1,08	10,66 ± 1,49	18,88 ± 2,93	29,32 ± 5,02
G4	50	5,28* ± 0,45	6,63* ± 0,93	9,81* ± 1,07	18,10 ± 2,11	27,45 ± 4,44

Statistical Test: ANOVA & Dunnett's Test
*statistically significant

RAC's response

Noted.
Thank you for the additional information on the 2-generation study which is included in the RAC opinion.
The additional information with regards to exposure is not relevant for hazard classification.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	15

Comment received

The proposed classification as Repr. 2 (H361f - Suspected of damaging fertility) is supported based on observed fertility effects on sperm integrity, testicular damage and epididymis impairment in multiple studies in rats and dogs.

Dossier Submitter's Response

Thank you for your comment.
Noted

RAC's response

Noted. However, in the draft RAC opinion, category 1B for fertility and category 2 for developmental toxicity is proposed.

Date	Country	Organisation	Type of Organisation	Comment number
26.01.2023	France	Puressentiel	Company-Downstream user	16

Comment received

As extensively discussed under Point 10.10., it is most likely that adverse effects on fertility were due to the administration type (by gavage). For other terpenes (which were also part of TTO) it was shown that sperm damage does not occur after dietary administration:

According to Guidance on the Application of the CLP Criteria (Version 5.0 July 2017), part 3.1.3.5. Decision on classification, 'The assessment on classification has to be performed with respect to all the relevant routes of exposure (oral, dermal, inhalation) on the basis of all adequate reliable data, data on Terpineol multiconstituent (α-Terpineol is a constituent of Tea tree oil and very similar to its main component Terpinen-4-ol) has been submitted to ECHA which give strong indication that reproductive effects can be accounted to the type of administration i.e. gavage, and that an administration via diet,

which represents a realistic human exposure, does not reveal reprotoxic effects at same doses. Still according to Guidance on the Application of the CLP Criteria (Version 5.0 July 2017), part 1.3.2.1. Human health hazards, 'there are a few specific cases in which bioavailability may have an influence on hazard classification'.
For terpineol, toxicokinetic analysis demonstrated that gavage led "to biologically non-relevant effects that should not be considered for classification purposes " (p.106)
When α-terpineol was given in the diet to male rats at the same dose of 750 mg/kg bw/d the sperm motility remained unaffected (Tea Tree Oil - Draft Renewal Assessment Report, page 196). This study demonstrates that oral gavage at high dose clearly resulted in much higher systemic exposure than expected, leading to biologically non-relevant effects that should not be considered for classification purposes.
Gavage exposure creates pharmacokinetic circumstances which cannot be encountered in real conditions of exposure and can be considered in this case as a non-relevant route of exposure (as would be IV or IP mode of administration).

Additionally, the stressful nature of the gavage method can alter the hypothalamic-pituitary-adrenal axis endocrine system. Because the endocrine system has complex positive and negative feedback loops, the effects of a stressful event may not be limited to endpoints associated with the hypothalamic-pituitary-adrenal axis challenging the use of gavage for the assessment of any endocrine-responsive endpoint (i.e. reprotoxicity) .

The classification Repr. 2 with hazard statement H361f-Suspected of damaging fertility, based on the significantly lower male and female mating and fertility indices in the two-generation study (Anonymous 2017a, Cf. Table 34 below) of TTO in rats, can therefore be questioned in relation to the gavage method of administration.

The recent Fouyet et al. (2022) study with the hPlacentox assay should be mentioned in the data available on the Endocrine Disruption assessment.
The hPlacentox assay, based on the use of human placental cells for the measurement of P2X7 activation, estradiol, progesterone, hPlacental Lactogen, and hyperglycosylated ßhCG secretions, could be described as addressing early/intermediate Key Events and a knowledge gap on female reproduction/fertility via placental function .
Indeed, hormone-associated pregnancy disorders in clinics share a common cellular biomarker: the P2X7 receptor activation. Previous studies showed that the P2X7 receptor activation is a common cellular mechanism of toxicity for endocrine disruptors in placenta, as P2X7 receptor was activated by all the tested endocrine disruptors in JEG-Tox cells , .
The hPlacentox has been ranked 1st out of 256 tests evaluated by PEPPER (which is a public private platform dedicated to the pre-validation of endocrine disruptors characterization methods) and is planned for an OECD submission in 2023.
According to Fouyet et al. (2022), TTO seems to be a hormone modulator rather than endocrine disruptor since it increases the placental hormone hPL but do not cause adverse cellular effects (TTO did not activate P2X7 receptor). The results obtained (no alteration of estradiol release) appear in contradiction with in vitro studies mentioned that demonstrated estrogenic and anti-androgenic effects of TTO in MCF-7 human breast cells reported by Henley et al. (2007).

Furthermore, the key component of TTO (4-terpineol) do not have the same hormonal effect as whole TTO, proving the need to study the whole essential oil rather than its components individually to conclude on the potential toxic effects. Indeed, 4-terpineol induced a higher progesterone secretion and estradiol than the control, while TTO had no

effect on progesterone and estradiol. Conversely, TTO stimulated the secretion of hPL but 4-terpineol did not. The above new studies (Fouyet et al, 2022) should be included in the report as part of the weight of evidence analysis.
Dossier Submitter's Response
Thank you for comment. Please see DS's response to comment no. 5 and comment no. 10 and 11 by MS-SE and NL.
RAC's response
Noted. Exposure route and way of dosing might be of relevance in a risk assessment. In the case of hazard classification, all information is included. Gavage studies are not unsuitable for classification purposes.

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2023	France	Consortium HE	Industry or trade association	17
Comment received				
As extensively discussed under Point 10.10. (page 84 of the CLH report), it is most likely that adverse effects on fertility were due to the administration type (by gavage). For other terpenes (which were also part of TTO) it was shown that sperm damage does not occur after dietary administration: Data on Terpineol multiconstituent (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/22822/7/9/1) (α-Terpineol is a constituent of Tea tree oil and very similar to its main component Terpinen-4-ol) give strong indication that reproductive effects can be accounted to the type of administration i.e. gavage, and that an administration via diet, which represents a realistic human exposure, does not reveal reprotoxic effects at same doses. When Terpineol multiconstituent (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/22822/7/9/1) was given in the diet to male rats at the same dose of 750 mg/kg bw/d the sperm motility remained unaffected. This study demonstrates that oral gavage at high dose clearly resulted in much higher systemic exposure than expected, leading to biologically non-relevant effects that should not be considered for classification purposes. Gavage exposure creates pharmacokinetic circumstances which cannot be encountered in real conditions of exposure and can be considered in this case as a non-relevant route of exposure (as would be IV or IP mode of administration). Additionally, the stressful nature of the gavage method can alter the hypothalamic-pituitary-adrenal axis endocrine system. Because the endocrine system has complex positive and negative feedback loops, the effects of a stressful event may not be limited to endpoints associated with the hypothalamic-pituitary-adrenal axis challenging the use of gavage for the assessment of any endocrine-responsive endpoint (i.e. reprotoxicity) . In conclusion, the classification Repr. 2 with hazard statement H361f-Suspected of damaging fertility, based on the significantly lower male and female mating and fertility indices in the two-generation study (Anonymous 2017a, Cf. Table 34 below) of TTO in rats, can therefore be questioned in relation to the gavage method of administration.				

In the prenatal developmental toxicity study (Anonymous 2018b) performed according to OECD 414 and in GLP conditions (Table 51 on page 113 of the CLH report), we agree with the Rapporteur Member State that the effects observed in this study (Anonymous 2018b) does not indicate that TTO developmental toxicity in rabbits meets classification criteria for this health hazard. In fact, a small mean increase of post implantation loss (1.76±1.84) in 21 females at 75 mg/kg bw/d in comparison with post implantation loss in 21 control females (0.52±0.81) is rather due to one dam with resorption of all foetuses which does not seem to be treatment related since this effect was not observed in any other dams exposed 75 mg/kg bw/d (Table 54 on page 116 of the CLH report).

Futhermore, according to Fouyet et al. (2022), TTO do not cause adverse cellular effects (TTO did not activate P2X7 receptor) in the hPlacentox assay. (Hormone-associated pregnancy disorders in clinics share a common cellular biomarker: the P2X7 receptor activation.)
The hPlacentox assay, based on the use of human placental cells for the measurement of P2X7 activation, estradiol, progesterone, hPlacental Lactogen, and hyperglycosylated ßhCG secretions, could be described as addressing early/intermediate Key Events and a knowledge gap on female reproduction/fertility via placental function.

Fouyet, S; Olivier, E.; Leproux, P.; Dutot, M.; Rat, P. Evaluation of Placental Toxicity of Five Essential Oils and Their Potential Endocrine-Disrupting Effects. Curr. Issues Mol. Biol. 2022, 2, 2794–2810. https://doi.org/10.3390/ cimb44070192

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil.pdf

Dossier Submitter's Response

Thank you for comment.
Please see DS's response to comment no. 5 and comment no. 10 and 11 by MS-SE and NL.

RAC's response

Noted.
Exposure route and way of dosing might be of relevance in a risk assessment. In the case of hazard classification, all information is included. Gavage studies are not unsuitable for classification purposes.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	France	Consortium HE	Industry or trade association	18

Comment received

In the prenatal developmental toxicity study in rabbits (Anonymous 2018b) performed according to OECD 414 and in GLP conditions (Cf. Table 51 of the CLH report):
- Main developmental parameters such as number of early resorptions, late resorptions, live fetuses, weight of fetuses, incidence of malformations and skeletal anomalies were not affected.

- At a dose of 75 mg/kg bw/d a significant increase in post implantation loss was observed. However, this small mean increase of post implantation loss (1.76±1.84) in 21 females at 75 mg/kg bw/d in comparison with post implantation loss in 21 control females (0.52±0.81) is rather due to one dam with resorption of all fetuses which does not seem

to be treatment related since this effect was not observed in any other dams exposed 75 mg/kg bw/d (Cf. Table 54 of the CLH report), as reported by the Rapporteur Member State. We agree with the Rapporteur Member State that the effects observed in this study (Anonymous 2018b) does not indicate that TTO developmental toxicity in rabbits meets classification criteria for this health hazard (Cf. Tables 51 and 54 of the CLH report).
Dossier Submitter's Response
Thank you for comment. Noted
RAC's response
On page 90 of the CLH report it was noted: "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." And: "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."

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	19
Comment received				
The proposed classification as Acute Tox. 4 (H302, H332) is supported based on ATE values of 300 < ATE ≤ 2000 mg/kg bw for oral exposure and 1 < ATE ≤ 5 mg/L for inhalative exposure via mists/dusts.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	20
Comment received				
The proposed classification as Skin Irrit. 2 (H315) is supported based on reversible acute dermal irritation effects in rabbits with mean values of ≥ 2,3 - ≤ 4.0 for erythema or oedema at 24, 48 and 72 hours after patch removal.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany	<confidential>	Company-Importer	21

Comment received

As explained in the attachement:
This discussion on the skin sensitisation potential is taken from the REACH dossier on TTO:
A total of four murine Local Lymph Node Assays (LLNA) are available for tea tree oil. EC3 values obtained in the LLNAs ranged between 25.5% and 4.4%, suggesting that tea tree oil has weak to moderate skin sensitising potential. However, a principal confounding factor for the LLNA test concerns the fact that tea tree oil is classified as a Cat. 2 irritant in contact with skin. It is known that both sensitisers and irritants can induce lymphocyte proliferation. Whereas true sensitisers stimulate the proliferation of antigen-specific lymphocytes, the response for irritants is nonspecific. Measurement of lymphocyte proliferation in the LLNA using 3H-T incorporation does not allow for a differentiation of these effects. Because of this, it is recognised that, taken in isolation, testing of non-sensitising, irritating substances using the LLNA can give rise to false positive results.
A Guinea Pig Maximisation Test (GPMT) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were seen in any of the twenty test animals evaluated. The guinea pig provides a better model for the human immune system than does the mouse. Given the strengths of the GPMT method, and its ability to differentiate between specific and non-specific lymphocyte proliferations with a degree of confidence not possible in the LLNA, the results of the existing study should be taken into account when a GPMT study is already available.
In the PPPR renewal and assessment report of TTO, a similar conclusion was reached by the DS who stated that a further GPMT study was performed according to OECD TG 406 under GLP conditions. It was concluded that since during a challenge no skin reactions were observed 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control (10 guinea pigs) and treatment group (20 guinea pigs) it is concluded that TTO is not a skin sensitiser.
In view of the very clear negative results obtained in the GPMTs, it is concluded that the ISO Standard Tea Tree Oil (as placed on the market) does not meet the criteria for classification as a skin sensitiser.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO.pdf

Dossier Submitter's Response

Thank you for comment.
It is important to point out that limitations of LLNA for testing skin sensitisation effects of skin irritating substances are not unique to the LLNA and have also been associated with GPT for skin sensitization (Basketter DA, Kimber I. Skin sensitization, false positives and false negatives: experience with guinea pig assays. Journal of Applied Toxicology. 2010;30(5):381–386). Therefore, positive results of reliable four positive mouse LLNA (GLP) studies cannot be completely omitted.

RAC's response

Noted.
RAC agrees with the DS. Further human data also support the skin sensitisation potential of TTO.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Sweden		MemberState	22
Comment received				
The Swedish CA supports the classification of tea tree oil as Skin sens. 1B, based on consistent results of four positive mouse LLNA (GLP) studies.				
Dossier Submitter's Response				
Thank you for support and comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany	Stockton Europe Ltd.	Please select organisation type..	23
Comment received				
Applicant is of the opinion that no classification for skin sensitization is warranted for tea tree oil 1. Vol 1, 1.5.2 / 2.11.2; Page 69 /page 275; versus Vol 3 B.6.0 / B.6.2.6; Page 11 / page 96: Inconsistency in classification information: In Vol 3 B.6, RMS has proposed no classification for skin sensitization, whereas in Vol 1, Skin sensitization 1B (H317) is stated for Tea tree oil. 2. Vol 3 B.6.0 / B.6.2.6; Page 11 / page 100: While the Tea tree oil component Limonene (0.5 – 1.5% of TTO) is classified as Skin sensitizer, a M&K test with TTO does not show any sensitizing effect. It should be noted however that R-Limonene was stated to have weak sensitizing properties (B.6.2.6/04, LLNA; EC3 value 30%). 3. Vol 1, 1.5.2 / 2.11.2, Page 66ff and B.6.2.6: Please note that results from ex vivo LLNA sensitization tests are less specific for sensitization than Guinea Pig Maximisation Test (GPMT) (in vivo) testing. It is known that both sensitisers and irritants can induce lymphocyte proliferation. Whereas true sensitisers stimulate the proliferation of antigen-specific lymphocytes, the response for irritants is nonspecific. Measurement of lymphocyte proliferation in the LLNA using 3H-T incorporation does not allow for a differentiation of these effects. Because of this testing of non-sensitising but irritating substances using the LLNA test can result in false positive results. For tea tree oil (according to ISO standard), various tests are available, including two GPMT conducted in accordance with the Magnusson and Kligman method are available. No positive reactions were seen in any of the 40 test animals evaluated in these two studies. This is relevant because TTO is classified as skin irritant (H315).				
Dossier Submitter's Response				
Thank you for comment. Regarding that all available data should be used for classification purposes the data from REACH registration dossier of Melaleuca alternifolia, ext.(CAS No. 85085-48-9) were included in Vol. 1. The proposed classification for Skin sensitization 1B (H317) is based on reliable results of four positive mouse LLNA (GLP) studies. It should be noted that component of TTO (α-Terpinene 5-13%), not only limonene, was classified as skin sensitiser. It is important to point out that limitations of LLNA for testing skin sensitisation effects of skin irritating substances are not unique to the LLNA and have also been associated with GPT for skin sensitization (Basketter DA, Kimber I. Skin sensitization, false positives and false negatives: experience with guinea pig assays. Journal of Applied Toxicology.				

2010;30(5):381–386). Therefore, positive results of reliable four positive mouse LLNA (GLP) studies cannot be completely omitted.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	24

Comment received

This discussion on the skin sensitisation potential is taken from the REACH dossier on TTO:

A total of four murine Local Lymph Node Assays (LLNA) are available for tea tree oil. EC3 values obtained in the LLNAs ranged between 25.5% and 4.4%, suggesting that tea tree oil has weak to moderate skin sensitising potential. However, a principal confounding factor for the LLNA test concerns the fact that tea tree oil is classified as a Cat. 2 irritant in contact with skin. It is known that both sensitisers and irritants can induce lymphocyte proliferation. Whereas true sensitisers stimulate the proliferation of antigen-specific lymphocytes, the response for irritants is nonspecific. Measurement of lymphocyte proliferation in the LLNA using 3H-T incorporation does not allow for a differentiation of these effects. Because of this, it is recognised that, taken in isolation, testing of non-sensitising, irritating substances using the LLNA can give rise to false positive results.

A Guinea Pig Maximisation Test (GPMT) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were seen in any of the twenty test animals evaluated. The guinea pig provides a better model for the human immune system than does the mouse. Given the strengths of the GPMT method, and its ability to differentiate between specific and non-specific lymphocyte proliferations with a degree of confidence not possible in the LLNA, the results of the existing study should be taken into account when a GPMT study is already available.
In the PPPR renewal and assessment report of TTO, a similar conclusion was reached by the DS who stated that a further GPMT study was performed according to OECD TG 406 under GLP conditions. It was concluded that since during a challenge no skin reactions were observed 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control (10 guinea pigs) and treatment group (20 guinea pigs) it is concluded that TTO is not a skin sensitiser.

In view of the very clear negative results obtained in the GPMTs, it is concluded that the ISO Standard Tea Tree Oil (as placed on the market) does not meet the criteria for classification as a skin sensitiser.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to the Proposed Classification of TTO_Final.pdf

Dossier Submitter's Response
Thank you for comment. Please see response to comment no. 23 (above)
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
26.01.2023	France	Puressentiel	Company-Downstream user	25
Comment received				
For TTO there is a clearly negative fully valid GPMT, OECD 406/GLP (Anonymous 2015e) which has equal weight of evidence to the LLNA, OECD 429/GLP (ECHA dissemination site), Cf. Table 18 below. It is recognized that a GPMT is the most sensitive and stringent test to detect skin sensitization and that the LLNA is prone to give false positive answers. FDA recommends the Buehler assay instead of the LLNA which is no further considered since at least 6 years. In July 2021, the OECD expert group on Defined Approaches for Skin Sensitisation (DASS) warned that the LLNA is not suitable for all high-log Kow substances. Some substances (such as limonene, linalool, citronellol) are rated as sensitisers by LLNA, but are non-sensitisers in humans based on a weight of evidence analysis. (OECD: Annex 6: Analysis of LLNA reference data to conclude on predictivity of alternative methods for skin sensitization for lipophilic chemicals) It is extensively reported in published literature that after aging, oxidized forms of terpene substances act as skin sensitizing substances. Since artificially aged/oxidized terpenes do not represent the active substance TTO, those study types should not be considered relevant for the TTO harmonized classification. As supportive information, the positive response in LLNA test of limonene (component of TTO) was submitted by applicant . However, limonene itself could not be considered as allergenic in humans because in the human patch tests only products of limonene air oxidation were used. Most human studies were performed with air-oxidised limonene after at least 10 weeks of air exposure (4 h/day stirred). Therefore, the conclusions of the positive responses in LLNA tests of TTO in terms of classification for skin sensitisation may be questioned, even more as experimental studies show diverging results (GPMT vs. LLNA) and the patch tests studies on human skin did not consider the potential oxidation of the tested sample.				
Dossier Submitter's Response				
Thank you for comment. It should be noted that stable under storage conditions Melaleuca alternifolia, ext., were used in all LLNA studies. Please see response to comment no. 23 (above).				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2023	France	Consortium HE	Industry or trade association	26
Comment received				
For TTO there is a clearly negative fully valid GPMT, OECD 406/GLP (Anonymous 2015e) which has equal weight of evidence to the LLNA, OECD 429/GLP (ECHA dissemination site) (Table 18 on pages 60-62 of the CLH report). In July 2021, the OECD expert group on Defined Approaches for Skin Sensitisation				

(DASS) warned that the LLNA is not suitable for all high-log Kow substances. Some substances (such as limonene, linalool, citronellol) are rated as sensitisers by LLNA, but are non-sensitisers in humans based on a weight of evidence analysis: https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-CBC-MONO(2021)11/ann6%20&doclanguage=en.
It is extensively reported in published literature that after aging, oxidized forms of terpene substances act as skin sensitizing substances. Since artificially aged/oxidized terpenes do not represent the active substance TTO, those study types should not be considered relevant for the TTO harmonized classification.
As supportive information, the positive response in LLNA test of limonene (component of TTO) was submitted by applicant (Table 19 on page 66 of the CLH report). However, limonene itself could not be considered as allergenic in humans because in the human patch tests only products of limonene air oxidation were used. Most human studies were performed with air-oxidised limonene after at least 10 weeks of air exposure (4 h/day stirred). This is considered unrealistic for most situations (RAC Opinion d-limonene, 15 March 2019).
Therefore, the conclusions of the positive responses in LLNA tests of TTO in terms of classification for skin sensitisation may be questioned, even more as experimental studies show diverging results (GPMT vs. LLNA) and the patch tests studies on human skin did not consider the potential oxidation of the tested sample.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil.pdf

Dossier Submitter's Response

Thank you for comment.
It should be noted that stable under storage conditions Melaleuca alternifolia, ext., were used in all LLNA studies.
Please see response to comment no. 23 (above).

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	France	Consortium HE	Industry or trade association	27

Comment received

For TTO there is a clearly negative fully valid GPMT, OECD 406/GLP (Anonymous 2015e) which has equal weight of evidence to the LLNA, OECD 429/GLP (ECHA dissemination site), Cf. Table 18 of the CLH report. However, it is recognized that the LLNA tends to overestimate the sensitization potential of certain types of compounds. Therefore, results obtained from LLNAs should not automatically be considered as the gold standard; rather it is necessary to carefully evaluate the applicability domains of this test method . (D.W. Roberts, T.W. Schultz, A.M. Api. Chemical applicability domain of the Local Lymph Node Assay (LLNA) for skin sensitisation potency. Regul. Toxicol. Pharmacol., 80 (2016), pp. 260-267 10.1016/j.yrtph.2016.07.018)

- In July 2021, the OECD expert group on Defined Approaches for Skin Sensitization (DASS) warned that the LLNA is not suitable for all high-log Kow substances. Some substances (such as limonene, linalool, citronellol) are rated as skin sensitizers by LLNA, but are non-skin sensitizers in humans based on a weight of evidence analysis . The LLNA protocol is particularly favorable for autoxidation. (OECD: Annex 6: Analysis of LLNA

reference data to conclude on predictivity of alternative methods for skin sensitization for lipophilic chemicals) - It is extensively reported in published literature that after aging, oxidized forms of terpene substances act as skin sensitizing substances. Since artificially aged/oxidized terpenes do not represent the active substance TTO, those study types should not be considered relevant for the TTO harmonized classification. As supportive information, the positive response in LLNA test of limonene (component of TTO) was submitted by applicant . However, limonene itself could not be considered as allergenic in humans because in the human patch tests only products of limonene air oxidation were used. Most human studies were performed with air-oxidized limonene after at least 10 weeks of air exposure (4 h/day stirred). This is considered unrealistic for most situations: RAC Opinion d-limonene – 15 March 2019 Therefore, the conclusions of the positive responses in LLNA tests of TTO in terms of classification for skin sensitization may be questioned, even more as experimental studies show diverging results (GPMT vs. LLNA) and the patch tests studies on human skin did not consider the potential oxidation of the tested sample.
Dossier Submitter's Response
Thank you for comment. It should be noted that stable under storage conditions Melaleuca alternifolia, ext., were used in all LLNA studies. Please see response to comment no. 23 (above).
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2023	France	Consortium HE	Industry or trade association	28
Comment received				
For 90-day studies in rats (Anonymous 2011 and 2016a), we note that in Table 23 (page 76 of the CLH report) it says "feeding" and not , as described on pages 105-108 where it says: "Tea Tree Oil administered by gavage". The Rapporteur Member State stated that a detrimental effect on spermatogenesis was seen in studies where Tea Tree Oil was administered by gavage (page 84). Therefore, the method of administration by gavage should be indicated instead of "feeding" in Table 23 (page 76). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil.pdf				
Dossier Submitter's Response				
Thank you for commenting. Noted, however "Administration: gavage" is indicated in second column of table 23 (page 76).				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	29
Comment received				
The proposed classification as Asp. Tox. 1 (H304) is supported, since it is a hydrocarbon and has a kinematic viscosity of 1.71 mm2/s measured at 40 °C, which is sufficient according to Regulation (EC) No 1272/2008.				
Dossier Submitter's Response				
Thank you for support and commenting.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	France		MemberState	30
Comment received				
FR agrees with the classification proposal for environmental hazard and with the acute M factor.				
Dossier Submitter's Response				
Thank you for support and commenting.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	United Kingdom	Health and Safety Executive	National Authority	31
Comment received				

1. It is somewhat unclear which CAS and EC number 'substance' is being classified here. Whilst the DS indicates they intend to clarify this later on, it will be important to determine, as far as possible, exactly which version(s) of Metaleuca alternifolia tea tree oil extract are being classified and whether these proposed harmonised classifications cover other similar substances with different registered uses. This CLH focusses on a particular form the substance used in plant protection products (PPP), there are other similar variations apparently also used in biocides, pharmaceuticals and cosmetics - and registered under REACH. It would be best to avoid different classifications based on different regulatory data sets, unless justified by them having substantively different compositions (noting the % w/w composition of individual components in this substance is also quite variable).
For example, from a brief, non-exhaustive, search on ECHA's Information on Chemicals site, we find the following substances all based on Metaleuca alternifolia or tea tree oil (excluding reaction masses):

Name	EC / List no.	CAS no.
Tea tree essential oil	942-904-6	-
essential oil tea tree - Melaleuca Alternifolia leaf oil	943-662-4	-
Melaleuca alternifolia, ext.		
IUPAC name: TEA TREE OIL	285-377-1	85085-48-9

Tea Tree Oil Pharmaceutical grade	941-669-7 -
Tea Tree Oil standard grade	941-670-2 -
Extract from tea tree	
Process related name: Tea tree oil	614-679-1 68647-73-4
Melaleuca Alternifolia (Tea Tree) Leaf Oil	641-387-1 68647-73-4
Melaleuca Alternifolia Leaf Oil, Fractionated	924-611-5 -
Melaleuca Alternifolia Leaf Oil	928-656-1 -
Melaleuca Tea Tree Oil	
Synonym: TEA TREE OIL	617-013-8 8022-72-8
Melaleuca Tea Tree Oil	617-013-8 8022-72-8
Melaleuca Tea Tree Oil	617-013-8 8022-72-8

These last three all have the same EC and CAS no.s but may be based on different data sets and variable compositions. We note (just from the ECHA database) that 'Extract from tea tree' - with EC No. 614-679-1 and CAS 68647-73-4 also appears to be listed under PPP Regulations; there may be others registered as pesticides, biocides, pharmaceutical ingredients or cosmetics - we have not checked.

2. Referring to Section 2.9.2.1 of the CLH Report, relating to the overall bioaccumulation potential of TTO. We note there are no experimental BCF data available for the complete UVCB substance, or for individual components. The log Kow values for a substantial proportion of components (potentially comprising over 50% of the complete substance) are > the CLP trigger of 4, indicating a possible bioaccumulation concern. The DS considers this concern to be low based on estimated (Episuite v4.11) BCFs for the majority of components being < 500. In the absence of experimental data, log Kow values (especially where experimentally measured themselves) are usually considered more reliable than estimated BCFs to determine bioaccumulation potential. It is also not made clear whether all these component substances fit within the applicability domain of the QSAR model used and we note some may also be surface active.

We think there is still uncertainty over the bioaccumulation potential of TTO. This may become important depending whether the substance is considered rapidly degradable or not and if new acute toxicity data became available and a surrogate approach to chronic classification were subsequently used. Could further information be supplied please to clarify the potential bioaccumulation concern.

Dossier Submitter's Response

Noted.
Thank you for commenting.

1. Reply to comment on CAS and EC number 'substance', provided by the ECHA Team:

"The substance "tea tree oil" has been commonly described by the identifiers:
1. "Essential oils, Melaleuca alternifolia" CAS 68647-73-4 for describing the active substance according to the Commission Regulation (EC) 1107/2009 and
2. "Melaleuca alternifolia, ext.", EC 285-377-1, CAS 85085-48-9 for describing the substance registered under REACH (https://echa.europa.eu/registration-dossier/-/registereddossier/20921).
The compositions and manufacturing processes reported in both cases have been analysed and concluded to be corresponding to the same substance. Standard ISO 4730 is used as a reference for setting specifications for this substance.
Therefore the above identifiers that are used to describe the same substance are included in the proposed Annex VI entry.

ECHA received C&L notifications reporting CAS 68647-73-4 and describing the substance with various names, for these submissions REACH-IT created different list numbers. However, the information provided on the identity and composition of these substances is limited. Therefore, we propose not to include these names in the CLH proposal.

In conclusion, the notified active substance shall has been 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."

2. Reply to comment on bioaccumulation potential of TTO.
All available data are presented. Dossier submitter do not have any additional data.

RAC's response

RAC agrees with UK, in contrast to the proposal by the DS, to conclude to consider TTO for the purpose of environmental hazard classification as having a high potential to bioaccumulate. 12 of the 15 known constituents of TTO have an experimental measured log K_{OW} above the trigger of 4.0 and 6 of the 12 have additionally an estimated BCF value above the trigger of 500. Only 3 of the 15 known constituents have an experimental measured log K_{OW} below the trigger of 4.0 and an estimated BCF value below the trigger of 500.

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2023	Germany		MemberState	32

Comment received

We agree to the proposed classification.

To: 2.8.2.2.5 Hydrolysis: It was concluded that Tea Tree Oil can be considered as rapidly biodegradable in light of hydrolysis.
But, hydrolysis is abiotic degradation and not biodegradation. Further the hint on degradation in the gas phase under the influence of light gives no information about hydrolysis.

To Table 2.8.2.1-1: Summary of relevant information on rapid degradability
First row OECD 301: Method / Results: method is given, but not the results.
Next row OECD 307: Results of carbon dioxide evolution could be given too, not only DT50 values.

To Page 190: Degradation
"As summarised in section 10.1., …" What section 10.1?

Dossier Submitter's Response

Noted.
Thank you for commenting.

Typo: Instead of "As summarised in section 10.1., …" it should be ""As summarised in section 2.8.2., …"

It should be noted, that the subject of this commenting phase was the document: Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonized Classification and Labeling (CLH Report)

according to Regulation (EC) N° 1272/2008, Tea Tree Oil (TTO) Volume 1, August 2022 not prepared by dossier submitter.
RAC's response
RAC agrees with DE that no hydrolysis study is available and consequently Hydrolysis has not been evaluated by the DS.

PUBLIC ATTACHMENTS
1. Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil_Final version.docx [Please refer to comment No. 7]
2. Response to the Proposed Classification of TTO.pdf [Please refer to comment No. 1, 9, 21]
3. Response to the Proposed Classification of TTO_Final.pdf [Please refer to comment No. 8, 13, 24]
4. Consortium HE - Comments on the dossier proposing a harmonised classification and labelling for Tea tree oil.pdf [Please refer to comment No. 5, 17, 26, 28]