Committee for Risk Assessment

RAC

Annex 3
Records
of the targeted consultation for

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 3 - RECORDS OF THE TARGETED PUBLIC CONSULTATION 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

The proposal for the harmonised classification and labelling (CLH) of Melaleuca alternifolia, ext. [1] Melaleuca alternifolia, essential oil; tea tree oil [2], EC 285-377-1 [1]; - [2]; CAS 85085-48-9 [1]; 68647-73-4 [2] was submitted by Poland and was subject to a consultation, from 28 November 2022 to 27 January 2023. The comments received by that date are compiled in Annex 2 to the opinion.

The CLH report subject to the ad hoc consultation contains additional information that was not included in the version subject to consultation from 28 November 2022 until 27 January 2023.
For this reason an ad hoc consultation was launched from 27 March to 17 April 2023 and the comments received are listed below.

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
17.04.2023	France	<confidential>	Company-Manufacturer	1
Comment received				
The <confidential> thanks the European Chemicals Agency for the opportunity to provide further comments on the targeted consultation proposing a harmonized classification and labelling for Malaleuca alternifolia, ext, -essential oil; Tea tree oil, CAS number: 85085-48-9 / 68647-73-4, hereinafter called TTO. The <confidential> 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 Sexual function and fertility assessment
o Developmental toxicity assessment
o Endocrine disruption assessment

Dossier Submitter's Response

Thank you for your comment.
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 by a normal physiological route available are used for **hazard** classification purposes, including 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
17.04.2023	Belgium	Pranarom International S.A.	Company-Manufacturer	2

Comment received

Pranarom International S.A., as member of 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. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Essential Oils Consortium.docx
Dossier Submitter's Response
Thank you for your comments and additional data. Please see DS's response to comment no. 1 (above).
RAC's response
See response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Germany		MemberState	3
Comment received				
The German CA has reviewed the CLH proposal from the targeted consultation and has no comments. However, we wonder why the publication of the proposal in an alternative format was necessary and why this procedure was not explained further.				
Dossier Submitter's Response				
During the sanitisation there was a misunderstanding that the RAR vol 1. was the combined CLH dossier and RAR, and therefore it was published by mistake as a CLH report.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	FLORAME	Company-Downstream user	4
Comment received				
See attached file ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2023.04.14 EO Consortium - Comments harmonized C and L for Tea tree oil_Final version.pdf				
Dossier Submitter's Response				
Thank you for your comments and additional data. Please see DS's response to comment no. 1 (above).				
RAC's response				
See response to comment no. 1.				

ANNEX 3 - RECORDS OF THE TARGETED PUBLIC CONSULTATION ON CLH PROPOSAL ON MELALEUCA ALTERNIFOLIA, EXT. [1] *MELALEUCA ALTERNIFOLIA*, ESSENTIAL OIL; TEA TREE OIL [2]

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2023	France	Laboratoire Puressentiel	Company-Downstream user	5
Comment received				
The Consortium HE thanks the European Chemicals Agency for the opportunity to provide further comments on the targeted consultation proposing a harmonized classification and labelling for Malaleuca alternifolia, ext, -essential oil; Tea tree oil, CAS number: 85085-48-9 / 68647-73-4, hereinafter called TTO. 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 Sexual function and fertility assessment o Developmental toxicity assessment o Endocrine disruption assessment				
Dossier Submitter's Response				
Thank you for your comments and additional data. Please see DS's response to comment no. 1 (above).				
RAC's response				
See response to comment no. 1.				

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2023	United Kingdom		Individual	6
Comment received				
Tea Tree oil is a high volume essential oil with a world wide production of 700 -900 tons /years. It is mostly used as an ingredient in cosmetics and traditional pharmaceutical applications. Usage in the UK alone is approximately 15 – 40 tons / year.				
Dossier Submitter's Response				
Thank you for your comment and information.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	COSMED - CONSORTIUM HE	Industry or trade association	7
Comment received				
COSMED and the Consortium HE (Essential Oils Consortium) thank the European Chemicals Agency for the opportunity to provide further comments on the targeted consultation proposing a harmonized classification and labelling for Malaleuca alternifolia, ext, essential oil; Tea tree oil, CAS number: 85085-48-9 / 68647-73-4, hereinafter called TTO. 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 Sexual function and fertility assessment o Developmental toxicity assessment				
Dossier Submitter's Response				
Thank you for your comments and additional data. Please see DS's response to comment no. 1 (above).				
RAC's response				
See response to comment no. 1.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Germany	<confidential>	Company-Importer	8
Comment received				
We welcome the opportunity to feed into the consultation on the harmonized classification of Tea Tree Oil (TTO) and support the response of the REACH Lead registrant. For matters of completeness we repeat our answer of the consulation in January 2023				
Dossier Submitter's Response				
Thank you for comment. Please see response to comment no. 1 (above) and DS's answer after consultation in January 2023.				
RAC's response				
See response to comment no. 1.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	9

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 comment.
Please see response to comment no. 1 (above) and DS's answer after consultation in January 2023.

RAC's response

1. Reproductive toxicity.

Thank you for your comments and for the agreement that TTO has a detrimental effect on spermatogenesis. With regards to the studies performed with α-Terpineol, one of the components of TTO, with different exposure routes: there might be a difference in potency shown with α-Terpineol in studies after gavage (with effects on testis and sperm at 750 mg/kg bw/day, not at 250 mg/kg bw/day) or dietary treatment (tested up to 623 mg/kg bw/day, only a slight significant increase in the percentage of abnormal sperm). All studies available are relevant for **hazard** classification purposes, thus including gavage studies. It is noted that dietary studies might show effects at higher doses compared to gavage studies, but that is no reason for no classification.

2. Skin sensitisation

The two negative GPMT tests (without a positive control, and with a not clear positive control) are not enough to dismiss the positive results in four positive LLNA tests (with two of them without any irritation) as well as positive responses in several human cases.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Switzerland	Givaudan	Company-Importer	10

Comment received

Givaudan supports the proposal of PL for a CMR Rep. 2 classification of Extract from tea tree; Tea Tree Oil (TTO); Oil of Melaleuca alternifolia (Terpinen-4-ol Type) on the following basis:

Gavage treatment of rats with Melaleuca Alternifolia essential oil resulted in degenerative changes in the testes and aspermia/oligospermia in epididymis which were linked with decreased weights of testes and epididymis in studies in rats. Reversible effects were also noted in dogs. We agree that these effects warrant classification for reproductive toxicity, yet we specifically do agree with the proposal of the dossier submitter, that these data justify a classification as Rep 2 . We consider these effects of doubtful relevance for the human situation due to the following observations:

1) Melaleuca Alternifolia essential oil is composed of monocyclic terpenes. These compounds are widely present in the human diet from multiple exposures from fruits,

herbal remedies, spices, teas and infusions and oral care products containing such terpenes. Therefore, there is a long history of safe use of monocyclic terpenes as natural components of a plant-based diet and as food additives.

Thus, Melaleuca Alternifolia shares for example p-cymene (1-isopropyl-4-methyl-benzene) as a constituent with 350 – 400 other natural complex substances which are widely used in different preparations. Also γ-terpinene and terpinolene contained in the oil are widely present in the natural diet. Thus EFSA (EFSA Journal 2015;13(4):4067) estimated the daily intake for p-cymene, γ-terpinene and terpinolene at 926 / 660 / 1200 µg / per capita per day. Based on assessment by EFSA, these constituents are safe for human ingestion at doses currently used.
Based on the wide occurrence of p-cymene and other cyclic terpenes with identical carbon skeleton, it is expected that there is a regular exposure and that a constant low level of p-isopropyl benzoic acid (p-iPBA) is formed metabolically upon consumption of a standard diet and is well tolerated. The quantitative level of p-iPBA in human urine is a subject which would warrant further study.

2) The detailed mode-of-action of the effects on male reproduction of tea tree oil has not been investigated. However, tea tree oil contains p-cymene among other monocyclic terpenes with the same carbon skeleton. Very similar effects as for tea tree oil had been noted in rats after gavage treatment with p-cymene. p-cymene is metabolized to p-isopropyl benzoic acid (p-iPBA) by preferential oxidation at the methyl group (unpublished data, <confidential>) which then is further converted to p-isopropyl benzoyl Coenzyme A. This CoA-conjugate is accumulating in rat hepatocytes to stable levels, but is cleared over time in human hepatocytes with a very clear quantitative species difference in metabolite accumulation (Food Chem. Tox. 153 (2021) 112243, https://doi.org/10.1016/j.fct.2021.112243).
There is now ample evidence from structure-activity studies (Archives of Toxicology (2020) 94:4115–4129; https://doi.org/10.1007/s00204-020-02918-9) and studies in ex vivo rat testes tissue (Archives of Toxicology; https://doi.org/10.1007/s00204-022-03379-y; Food Chem. Tox. 153 (2021) 112243, https://doi.org/10.1016/j.fct.2021.112243), that p-iPBA-CoA-conjugate accumulation in liver and testes tissue is a hallmark of the toxicity of p-iPBA, p-iPBA precursors and related substances in rats. Furthermore, this is a threshold based biochemical mode-of-action, whereby only above a certain threshold of CoA-conjugate accumulation, an impact on the lipid metabolism is observed which then may lead to the apical effects. Hence, this is not a mode-of-action where a no-threshold assumption has to be applied.

3) Further confirmation that tea tree oil may act by this specific mode of action comes from the fact that liver effects (increased weight of liver, pale liver, vacuolar degeneration of hepatocytes) were noted at the same dose where the male reproductive effects were noted. Liver effects in rats were also observed in parallel to testicular toxicity for other molecules metabolized to p-iPBA and related substances (Food Chem. Tox. 153 (2021) 112243, https://doi.org/10.1016/j.fct.2021.112243; Archives of Toxicology (2020) 94:4115–4129; https://doi.org/10.1007/s00204-020-02918-9) which may be explained by the accumulation of p-iPBA-CoA in the hepatocytes and disruption of lipid homeostasis.

4) While it is not clear to which extend the effects observed for tea tree oil are due to p-cymene, the common carbon skeleton of all terpenes in tea tree oil and the very similar macroscopic and microscopic effects on male reproductive organs and liver of rats noted for other chemicals leading to iPBA indicates that effects may be explained by this biochemical mode of action as the most likely explanation, of course it remains scientifically impossible to exclude another MoA.

5) In conclusion:
a. Terpenes in tea tree oil are widely occurring in natural food and are considered safe by EFSA, hence they are considered safe under current use levels for humans
b. The most likely mode of action is metabolic formation of p-iPBA and accumulation of p-iPBA-CoA in rat hepatocytes from tea tree oil.
c. This is a biochemical mode of action with a threshold. Only beyond the threshold lipid homeostasis is disrupted.
d. Accumulation of p-iPBA-CoA in hepatocytes has been found to be rat-specific and was not observed in human cells to a similar degree.
Based on (i) occurrence in the natural diet of humans of monocyclic terpenes, (ii) a likely threshold-dependent MoA and (iii) species difference in metabolism, we consider there is sufficient evidence that effects observed in rats and dogs are not relevant to the human situation.

Dossier Submitter's Response

Thank you for your comment and additional data.

RAC's response

Thank you for your comment. We agree on the degenerative changes in the testes and spermia/oligospermia in epididymis in studies with TTO in rats, warranting classification for reproductive toxicity.

1. Noted. History of safe use of monocyclic terpenes via the diet is not a valid argument in the discussion on the classification of TTO. Animal studies performed with the substance TTO are evaluated and result in clear effects on fertility. p-Cymene is only present in 0.5-8% in TTO, so studies with p-cymene are not covering all components present in TTO.
2. Agreed that the mode-of-action for the effects on spermatogenesis is not known. The clearance of the p-cymene metabolite in human hepatocytes might be higher compared to rat hepatocytes, still the overall metabolisation in vivo is not known for this component, let alone that (quantitative) differences in metabolisation are known for the full TTO mixture.
3. Noted.
4. In full agreement with the statement that it is not clear to which extent the effects observed for TTO are due to p-cymene (presence in the range of 0.5-8%), and that therefore it remains scientifically impossible to exclude another MoA.
5. See above.

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2023	France	Laboratoire Puressentiel	Company-Downstream user	11

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 in gavage studies. However, we note that these effects have not been confirmed by TTO administration via diet (which is a relevant route of exposure for humans). Such effects are not reported in humans and Tea Tree Oil is generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients.

Developmental toxicity assessment :

In the prenatal developmental toxicity study in rabbits (Anonymous 2018b) performed according to OECD 414 and in GLP conditions :
- 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 to 75 mg/kg bw/d , 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 .

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.
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 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.
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 and additional data.
Noted, however 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

No studies are available with TTO administered via the diet. So, classification is based on the available studies with TTO, with administration via oral gavage.
As noted "such effects are not reported in humans", in the CLH report, it is noted that there are no human studies to that effect available. So, human relevance cannot be excluded.

The effect on post-implantation loss at the highest dose of 75 mg/kg bw/day in the rabbit PNDT study is not only driven by one dam with 100% post-implantation loss. More dams with several resorptions contribute to the higher mean post-implantation loss. See for illustration in the Table below the individual data on percentage post-implantation loss in the rabbit PNDT study.

Control group	15 mg/kg bw/day	30 mg/kg bw/day	75 mg/kg bw/day
-	14.3	12.5	100.0
		50.0	18.2
-	11.1		42.9
-	-	-	16.7
10.0	14.3	28.6	-
-	-	66.7	25.0
	-	33.3	16.7
-	12.5	12.5	-
20.0	33.3	-	22.2
-	33.3	-	57.1
12.5		-	-
37.5	-	14.3	22.2
12.5	-	-	16.7
-	-	12.5	
	-	-	30.0
-	12.5	25.0	
-		-	-
-	14.3		20.0
-	14.3	-	-
-	-		25.0
-	33.3	-	42.9
12.5	-	11.1	
50.0		-	50.0
14.3	50.0	11.1	20.0

Agreed with the reply by the DS, there is no need to include the Fouyet study for the assessment of the classification for reproductive toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2023	United Kingdom		Individual	12
Comment received				
IFRA UK agree with the Polish proposal. However, we do not agree with the response of the Dutch and Swedish government agencies for reprotoxic effects.				
Dossier Submitter's Response				
Thank you for comment. Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2023	Germany	Stockton Europe Ltd.		13
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 comment. Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	COSMED - CONSORTIUM HE	Industry or trade association	14
Comment received				
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 in gavage studies. However, we note that these effects have not been confirmed by TTO administration via diet (which is a relevant route of exposure for humans). Such effects are not reported in humans and Tea Tree Oil is generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients. In the prenatal developmental toxicity study in rabbits (Anonymous 2018b) performed according to OECD 414 and in GLP conditions : - 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 to 75 mg/kg bw/d , 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.

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.
- According to Fouyet et al. (2022), tea tree oil do not cause adverse cellular effects in human placental cells (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. 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).

Dossier Submitter's Response

Thank you for comment and additional data.
Noted, however 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

Please see response to comment 11.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Germany	<confidential>	Company-Importer	15

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 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.
Dossier Submitter's Response
Thank you for comment. Please see response to comment no. 1 (above) and DS's answer after consultation in January 2023.
RAC's response
With regard to the studies performed with α-Terpineol, one of the components of TTO, with different exposure routes: there might be a difference in potency shown with α-Terpineol in studies after gavage (with effects on testis and sperm at 750 mg/kg bw/day, not at 250 mg/kg bw/day) or dietary treatment (tested up to 623 mg/kg bw/day, only a slight significant increase in the percentage of abnormal sperm). All studies available are used for **hazard** classification purposes, thus including gavage studies. It is noted that dietary studies might show effects no effect or effects at higher doses compared to gavage studies, but that is no reason for no classification. Furthermore, the argument that there is no residue present on treated crops is an exposure argument, not relevant for hazard classification.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	16
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 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 α-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 α-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 response to comment no. 1 (above) and DS's answer after consultation in January 2023.

RAC's response

Please, see response at comment 15.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	<confidential>	Company-Manufacturer	17

Comment received

☐ 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 clearly negative fully valid Guinea Pig Maximisation Test (GPMT), OECD 406/GLP (Anonymous 2015e) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were observed in any of the twenty test animals evaluated 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control and treatment group.
☐ 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 autoxidation1.
☐ 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.
☐ 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. 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.
In view of the very clear negative results obtained in the GPMTs, it is concluded that Tea Tree Oil does not meet the criteria for classification as a skin sensitizer.

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

Agreed with the DS response.

- Irritation was not present in two of the four LLNA tests.
- Agreed that the GPMT was negative, however, it could be noted that the positive control did not give a very strong result.
- The conclusion from the OECD report (2021) is: "*The analysis presented here does not indicate that the LLNA is wrong at higher LogP – but different lines of evidence indicate that the false-positive rate of the LLNA is higher for lipophilic chemicals. This could explain the observed apparent lower sensitivity of ca. 10-15% calculated for the defined approaches in this physicochemical range. Thus, we consider it proven that there is an uncertainty for the LLNA positive in vivo reference data at high LogP."* It is not concluded that the LLNA is not suitable, only that the false-positive rate is higher.
  Further, with regards to autoxidation, the CLP guidance notes (3.4.2.2.2.): "*It should be noted that in some cases a substance may autooxidise in contact with air or decompose to a more hazardous form. This may warrant classification of the parent substance even though it in itself is not or is less hazardous. A case-by-case evaluation should be done considering available hazard information on humans or animals and/or the rate and extent of autoxidation or decomposition."*
- Noted. Classification is based on all available data.
- Noted. In the RAC CLH opinion on d-limonene it was concluded that d-limonene warrants classification as Skin Sens. 1B; H317.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	HYTECK AROMA-ZONE	Company-Manufacturer	18

Comment received

* 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 clearly negative fully valid Guinea Pig Maximisation Test (GPMT), OECD 406/GLP (Anonymous 2015e) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were observed in any of the twenty test animals evaluated 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control and treatment group.

* 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.

* 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.

* 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. 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.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment AROMAZONE - Comments on the targeted consultation proposing a harmonized classification and labelling for Tea tree oil.pdf

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

Please, see response to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2023	France	Laboratoire Puressentiel	Company-Downstream user	19

Comment received

- 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 clearly negative fully valid Guinea Pig Maximisation Test (GPMT), OECD 406/GLP (Anonymous 2015e) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were observed in any of the twenty test animals evaluated 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control and treatment group.

- 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 autoxidation1.

- 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.

- 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. 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.

In view of the very clear negative results obtained in the GPMTs, it is concluded that Tea Tree Oil does not meet the criteria for classification as a skin sensitizer.

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

Please, see response to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2023	Germany	Stockton Europe Ltd.		20
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				
Please, see response to comment 17.				

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	COSMED - CONSORTIUM HE	Industry or trade association	21

Comment received

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 clearly negative fully valid Guinea Pig Maximisation Test (GPMT), OECD 406/GLP (Anonymous 2015e) conducted in accordance with the Magnusson and Kligmann method is also available. No positive reactions were observed in any of the twenty test animals evaluated 24 and 48 hours after removal of the test patches with 100% TTO (undiluted) in the control and treatment group.

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 autoxidation1.

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.

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. 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.

In view of the very clear negative results obtained in the GPMTs, it is concluded that Tea Tree Oil does not meet the criteria for classification as a skin sensitizer.

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
Please, see response to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Germany	<confidential>	Company-Importer	22
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.				
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
Please, see response to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	Ireland	Pure Australian Tea Tree Oil Limited	Company-Manufacturer	23

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.

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
Please, see response to comment 17.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2023	France	HYTECK AROMA-ZONE	Company-Manufacturer	24

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 in gavage studies. However, we note that these effects have not been confirmed by TTO administration via diet (which is a relevant route of exposure for humans). Such effects are not reported in humans and Tea Tree Oil is generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients .

Developmental toxicity assessment:
In the prenatal developmental toxicity study in rabbits (Anonymous 2018b) performed according to OECD 414 and in GLP conditions :
* 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 to 75 mg/kg bw/d , as reported by the Rapporteur Member State.

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.
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 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. ECHA note – An attachment was submitted with the comment above. Refer to public attachment AROMAZONE - Comments on the targeted consultation proposing a harmonized classification and labelling for Tea tree oil.pdf
Dossier Submitter’s Response
Thank you for comment. Please see response to comment no. 1 (above)
RAC’s response
Please, see response to comment 11.

PUBLIC ATTACHMENTS
1. Response to the Proposed Classification of TTO_Final.pdf [Please refer to comment No. 9, 16, 23]
2. Essential Oils Consortium.docx [Please refer to comment No. 2]
3. AROMAZONE - Comments on the targeted consultation proposing a harmonized classification and labelling for Tea tree oil.pdf [Please refer to comment No. 18, 24]
4. 2023.04.14 EO Consortium - Comments harmonized C and L for Tea tree oil_Final version.pdf [Please refer to comment No. 4]