

Comments submitted by the Consortium HE on the dossier proposing a harmonised classification and labelling for Malaleuca alternifolia, ext, - essential oil; Tea tree oil

General comments:

The Consortium HE thanks the European Chemicals Agency for the opportunity to provide comments on the dossier proposing a harmonised 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 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 Consortium HE's comments below relate to the following elements:

- **Proposed harmonised classification for CLP Annex VI:**
 - Skin Sens. 1B, H317: May cause an allergic skin reaction.
 - Repr. 2, H361f: Suspected of damaging fertility.
- **Studies to evaluate the potential endocrine disruptive properties** in the framework of active substance renewal in plant protection products.

Skin sensitization assessment

Consortium HE's comments:

- **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.
- 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 K_{ow} substances. **Some substances** (such as limonene, linalool, citronellol) **are rated as sensitizers by LLNA, but are non-sensitizers in humans based on a weight of evidence analysis**¹.
- 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.**

¹ [OECD: Annex 6: Analysis of LLNA reference data to conclude on predictivity of alternative methods for skin sensitization for lipophilic chemicals](#)

As supportive information, the positive response in LLNA test of limonene (component of TTO) was submitted by applicant². 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.

Cf. Table 18: Summary table of animal studies on skin sensitisation – Pages 60 – 62 of the CLH report - Tea Tree Oil (TTO) Volume 1

Table 18: Summary table of animal studies on skin sensitisation

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

² Christensson, J.B., Johansson, S., Hagvall, L., Jonsson, C., Börje, A., Karlberg, A.T. (2008) Contact Dermatitis 59(6): 344- 352

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

Reproductive toxicity assessment

Consortium HE's comments:

- 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:

Data on Terpineol multiconstituent³ (α -Terpineol is a constituent of TTO 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 was given in the diet to male rats at the same dose of 750 mg/kg bw/d the sperm motility remained unaffected³. 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)⁴.

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.**

Cf. Table 34: Summary table of animal studies effects on sexual function and fertility –
Page 98 of the CLH report -Tea Tree Oil (TTO) Volume 1

³ <https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/22822/7/9/1>

⁴ Vandenberg et al.: Should oral gavage be abandoned in toxicity testing of endocrine disruptors? Environmental Health 2014 13:46.

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

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

Developmental toxicity assessment

Consortium HE's comments:

In the **prenatal developmental toxicity study (Anonymous 2018b)** performed according to OECD 414 and in GLP conditions (Cf. Table 51 below):

- At a dose of 75 mg/kg bw/d a significant increase in post implantation loss was observed. **Main developmental parameters such as number early resorptions, late resorptions, live fetuses, weight of fetuses, incidence of malformations and skeletal anomalies were not affected.**
- **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 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 below), as reported by the Rapporteur Member State.

We agree 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).

Cf. Table 51: Summary table of animal studies on adverse effects on development – Page 113 of the CLH report - Tea Tree Oil (TTO) Volume 1

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

- NB: We note that more detailed results are presented in Table 54 and not in table 52 as indicated in the summary table 51.

Cf. Table 54: Body weight gain, Food consumption and maternal data during the total gestation period (days 0- 29) in a developmental toxicity study in rabbits with Tea Tree Oil – Page 116 of the CLH report - Tea Tree Oil (TTO) Volume 1

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

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

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

Specific target organ toxicity-repeated exposure (STOT RE) assessment

Consortium HE's comments:

For 90-day studies in rats (Anonymous 2011 and 2016a), we note that in Table 23 below 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 (Cf. page 84 of the CLH report).

- Therefore, **the method of administration by gavage should be indicated instead of "feeding" in Table 23.**

Cf. Table 23: Summary table of animal studies on STOT RE – Page 76 of the CLH report - Tea Tree Oil (TTO) Volume 1

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

Cf. Pages 105 -107 of the CLH report - Tea Tree Oil (TTO) Volume 1

Anonymous (Anonymous 2011b) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats

This 90-day repeated dose toxicity study in rats with Tea Tree Oil was performed according to OECD TG 408 and in GLP conditions. Tea Tree Oil administered by gavage for 90 days (males) or 91 days (females) at doses of 30, 60 or 120 mg/kg bw/day did not induce significant changes in feed consumption, body weight, locomotor activity,

Cf. Pages 107 -108 of the CLH report - Tea Tree Oil (TTO) Volume 1

Anonymous (2016a) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats

The study performed in GLP conditions according to OECD TG 408, however with a major deviation, since only one dose was used, is considered reliable and results can be used for assessment of health hazard caused by TTO. This study is supplementary to the previous one (Anonymous 2011b). **Tea Tree Oil administered by gavage** for 90 days to female and male rats at dose of 60 mg/kg bw/dav did not induce significant changes in food consumption

Endocrine disruption assessment

Consortium HE's comments:

- **The recent Fouyet et al. (2022)⁵ 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 βhCG secretions, **could be described as addressing early/intermediate Key Events and a knowledge gap on female reproduction/fertility via placental function⁶.**

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^{7,8}.

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

⁵ 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>

⁶ Grignard E, de Jesus K and Hubert P (2022) Regulatory Testing for Endocrine Disruptors; Need for Validated Methods and Integrated Approaches. *Front. Toxicol.* 3:821736. doi: 10.3389/ftox.2021.821736

⁷ Fouyet, S.; Olivier, E.; Leproux, P.; Dutot, M.; Rat, P. Bisphenol A, Bisphenol F, and Bisphenol S: The Bad and the Ugly. Where Is the Good? *Life (Basel)* 2021, 11, 314. <https://doi.org/10.3390/life11040314>.

⁸Fouyet, S.; Olivier, E.; Leproux, P.; Dutot, M.; Rat, P. Pregnant Women and Endocrine Disruptors: Role of P2X7 Receptor and Mitochondrial Alterations in Placental Cell Disorders. *Cells* 2022, 11, 495. <https://doi.org/10.3390/cells11030495>

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.

About the Consortium HE:

The Essential Oils Consortium brings together 10 companies specialised in the marketing of products containing essential oils, representing more than 90% of the aromatherapy products market in France, the CIHEF (Interprofessional Committee of French Essential Oils) representing the producers, main buyers and distillers of French essential oils and the GEHEM (Group of exporters of essential oils from Madagascar).

The members of the Consortium HE have joined forces to address the three major challenges for the future of this sector: to collect and provide up-to-date scientific information on essential oils, to reinforce their proper and safe use by consumers and to promote a new regulation adapted to aromatherapy.

Contact: contact@consortium-he.org