AROMA ZONE
EXPERT NATUREL EN SOINS & BEAUTÉ

# Comments submitted by Aroma-Zone, as part of the Consortium HE on the targeted consultation proposing a harmonized classification and labelling for Malaleuca alternifolia, ext, -essential oil; Tea tree oil

## General comments:

We 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.

As part of the Consortium HE, we call 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**.

Aroma-Zone's comments below relate to the following elements:

- Skin sensitization assessment
- Sexual function and fertility assessment
- Developmental toxicity assessment
- Endocrine disruption assessment

## Skin sensitization assessment

### Aroma-Zone's comments:

- 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**[1].

- **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 $K_{ow}$ 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**[2]. The LLNA protocol is particularly favorable for autoxidation[1].

[1] D.W. Roberts, T.W. Schultz, A.M. Api. Chemical applicability domain of the Local Lymph Node Assay (LLNA) for skin sensitisation potency. Regul. Toxicol. Pharmacol., 80 (2016), pp. 260-267 10.1016/j.yrtph.2016.07.018
[2] OECD: Annex 6: Analysis of LLNA reference data to conclude on predictivity of alternative methods for skin sensitization for lipophilic chemicals

- 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[3]. 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.**

## Sexual function and fertility

### Aroma-Zone's comments:

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**

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

**effects are not reported in humans and Tea Tree Oil is generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients[4].**

## Developmental toxicity assessment

**Aroma-Zone's comments:**

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**.

[4] FEMA GRAS assessment of natural flavor complexes: Lavender, Guaiac Coriander-derived and related flavoring ingredients, July 2020. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 145:111584. DOI:10.1016/j.fct.2020.111584

## Endocrine disruption assessment

### Aroma-Zone'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 ßhCG secretions, **could be described as addressing early/intermediate Key Events and a knowledge gap on female reproduction/fertility via placental function**[6].

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).

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[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.

[8] Fouyet, 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

- 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.**

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**About AROMA-ZONE :**

AROMA-ZONE is a French company created at the beginning of 2000, and inspired
by a family passion for essential oils and plants.
As a specialist in essential oils, plant extracts and natural cosmetic ingredients, Aroma-Zone brings the consumer the benefit of these concentrated natural ingredients and allows the consumer to create their own natural well-being and beauty products.

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