Consultation on the Harmonized Classification and Labelling of Tea Tree Oil (TTO)

Considerations of the REACH Lead Registrant for TTO

We welcome the opportunity to comment into 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.

1. **Proposal to Classify TTO as a Category 2 Reprotoxin**

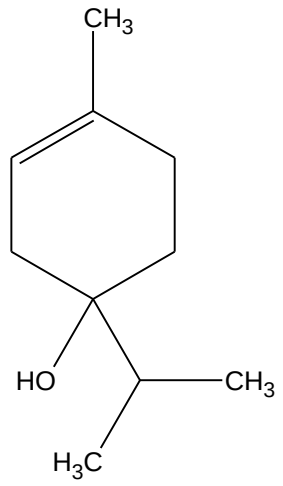
It has been proposed by the dossier submitter to classify TTO as a Category 2 reprotoxin based on observed male fertility effects observed in gavage studies on both rat and dog. In the conclusions of the STOT-RE classification proposal by the Dossier Submitter (DS) it states that:

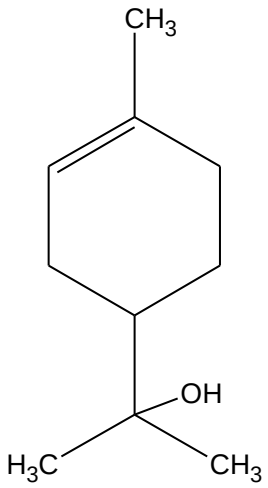
"Regarding all available repeated dose toxicity studies, it becomes clear that Tea Tree Oil has a detrimental effect on spermatogenesis. However, as extensively discussed under Point 10.10., it is most likely that these effects were due to the administration type (gavage vs. dietary). Effects were seen 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. The structural formulae of both substances are presented below:





Terpinen-4-ol ($C_{10}H_{18}O$) *α*-Terpineol ($C_{10}H_{18}O$)

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.

2. Proposal to Classify TTO as a Skin Sensitiser

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.