Title

alpha-Terpinene: Developmental Data Review for CLP Purposes

Test Item

QRD 420 as it relates to QRD 460

Data Requirement(s) / Guideline(s)

OECD Guidelines for the testing of Chemicals: Test Guideline 414
US EPA OCSPP Guideline OPPTS 870.3700

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Summary
alpha-Terpinene does not meet the classification under CLP as Reproductive Toxicant, Category 2. This classification was proposed due to a significant reduction in the number of litters observed at the high dose level in a non-OECD guideline, non-GLP developmental toxicology study. This effect was only observed in dams with significant maternal toxicity with body weight loss (41.9% body weight gain of control animals) and secondary non-specific consequences on the foetuses (i.e. decreased fetal and litter weight and retarded ossifications).

The CLP classification criteria states:

> "Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.
>
> Such effects shall have been observed in the ***absence of other toxic effects***, or if occurring together with other toxic effects the adverse effect on reproduction is considered ***not to be a secondary non-specific consequence of the other toxic effects***."

Therefore, classification according to CLP, Repr. Tox. 2 and H361: suspected of damaging fertility or the unborn child, **is not warranted**.

Background
alpha-Terpinene (1-isopropyl-4-methylcyclohexa-1,3-diene) is a naturally occurring cyclic monoterpene produced in the secondary metabolism of plants like citrus, peppermint, thyme, basil, and papaya. It has been identified in numerous plant extracts and is present in several commonly used oils, including tea tree oil and may be used in fragrances for soap, detergents, creams/lotions and perfumes as part of natural oils. Due to its anti-oxidant activity, alpha-terpinene is suggested as an agent for maintaining the oxidative stability of different matrices such as food, cosmetics, medicaments, and plant protection products. alpha-Terpinene is listed in the Code of Federal Regulations Title 21 172.515 and EU regulation 872/2012 as a food additive permitted for direct addition to food for human consumption (CFR 2015; EC 2012).

alpha-Terpinene is a component of QRD 420, a terpenoid blend active substance. QRD 420 was patterned after the composition of the original plant extract active substance (Extract of *Chenopodium ambrosioides* near *ambrosioides*), which consisted of

approximately 67% of the three terpenes and 33% plant derived impurities. QRD 420 replaced the impurities with canola oil in order to maintain the same proportionality as the plant extract. QRD 420 is registered as an active substance in the United Stated. For the EU submission, canola oil was removed and QRD 460 was registered, which contains only the three terpenes in proportions equivalent to those found in the plant extract and QRD 420.

Experimental Data

There are two relevant studies which can be evaluated for reproductive toxicity:

1) A non-GLP, non-guideline study with 89% alpha-terpinene; Araujo et al., 1996
2) A GLP, OECD 414 guideline study with QRD 420, containing 38.8% alpha-terpinene; [redacted] 2009

In a study to assess developmental toxicity in rats, 89% alpha-terpinene was orally administered to pregnant Wistar rats at 0, 30, 60, 125 or 250 mg/kg bw/day in corn oil from gestational day (GD) 6-15 (Araujo et al., 1996). On GD21, caesarean sections were done and the numbers of implantation sites, living/dead foetuses, resorptions and corpora lutea were recorded. Foetuses were examined for visible malformations and weighed, and one-third of the foetuses of each litter were evaluated for visceral anomalies by micro sectioning technique. As summarized in Table 1, a significant reduction of the ratio of pregnant/sperm-positive females (as described by the authors) or litters was observed at 250 mg/kg bw/day, which was observed in the presence of ***clear maternal toxicity.*** However, whether the animals lost entire litters after implantation or were not pregnant to begin with cannot be determined with the data provided in the Araujo et al. publication. Providing daily body weights or body weights every 3 days (as in the OECD 414 guidelines) rather than after 5 days could have be useful for interpretation. Nonetheless, dams showed significantly reduced body weight gain minus uterus weight at study termination (23.7% and 58.1% decrease from control at 125 and 250 mg/kg, respectively). Reduced individual fetal and litter weights (250 mg/kg) and alterations in fetal ossification (125 and 250 mg/kg) were also observed and associated with maternal toxicity (and reviewed in [redacted], 2011). As noted in the CLH report, the NOAEL for maternal toxicity is 60 mg/kg bw/day.

In a study to assess developmental toxicity in rats with QRD 420, an OECD 414 study was conducted according to GLP and OECD requirements ([redacted] 2009). QRD contains 38.8% alpha-terpinene, in addition to 11.5% d-limonene and 15.5% p-cymene. In this study, female Sprague-Dawley rats were orally administered QRD 420 at 0, 60, 120 and 240 mg/kg bw/day from GD 6-19. Terminal evaluations were conducted on GD20. Reduced maternal weight gain was observed at 120 and 240 mg/kg and decreased fetal body weights and alterations in fetal ossification were observed at 240 mg/kg. The NOAEL for maternal toxicity was 60 mg/kg bw/day (Table 1, for detailed review of study, see [redacted], 2011).

Table 1[1]: Summary table of Developmental Studies with alpha-Terpinene and QRD420

Endpoint	α-Terpinene	QRD 420
Implantation Loss	Only 56% of mated females given 250 mg/kg/day were pregnant at termination due to whole-litter losses at the time of implantation.	No effect at highest dose of 240 mg/kg/day on implantation.
Maternal Body Weight	Severe effect (weight loss) following the start of treatment with 250 mg/kg/day. Reduced weight gain with 125 mg/kg/day; a more severe effect than that of 120 mg QRD 420/kg/day. NOAEL 60 mg/kg/day	Reduced maternal weight gain due to 240 mg/kg/day and to a lesser extent to 120 mg/kg/day NOAEL 60 mg/kg/day
Foetal Body Weight	Lower foetal body weight with 250 mg/kg/day - lower by 14.9 % following 10 daily doses as measured on day 21. No effect of 125 mg/kg/day.	Lower foetal body weight with 240 mg/kg/day - lower by 8.6% following 14 daily doses as measured on day 20. No effect of 120 mg/kg/day.
Foetal Anomalies	No teratogenicity	No teratogenicity
Foetal Skeletal Observations	Altered ossification of a small number of bones at 125 and 250 mg/kg/day.	Altered ossification of a small number of bones at 240 mg/kg/day.
NOAEL maternal toxicity	60 mg/kg/day	60 mg/kg/day
NOAEL developmental toxicity	125 mg/kg/day	120 mg/kg/day

[1]From [redacted], 2011

These data support the conclusion that there is a clear impact of high-dose alpha-terpinene exposure on maternal health, as indicated by significantly reduced body weight gain observed during gestation. In consideration of the classification and labelling criteria, the **role of maternal toxicity** should be considered when considering potential developmental toxicity as a basis for classification, following EU CLP regulation 1272/2008, Annex I: 3.7.2.4, Maternal toxicity.

Although 1272/2009 , Annex I:3.7.2.4 describes maternal toxicity as a complex issue regarding uncertainties, it is clear in both of the described studies that no adverse effects occurred to the development of the fetus in the absence of maternal toxicity. Similar observations (decreased fetal body weight and delayed ossifications) were observed *only in the presence of decreased maternal body weight*, in 2 different rat species, with 2 different alpha-terpinene concentrations. This is aligned with the assertion in Annex I: 3.7.2.4.2 which states that “maternal toxicity may, depending on severity, influence

development via non-specific secondary mechanisms……However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species." These results were in fact consistent and reproducible.

Food and Flavoring Uses of alpha-Terpinene

alpha-Terpinene is listed as a food additive and is permitted for direct addition to food consumed by humans, and therefore an extensive safety review has been done on alpha-terpinene by both the US Food and Drug Administration and by European Food Safety Authority, EFSA. Upon joint evaluation by FAO/WHO Expert Committee on Food Additives (JEFCA Chemical) here is no safety concern at current levels of alpha-terpinene intake when used as a flavouring agent (WHO, 2005).

JECFA summarized the absorption, distribution, metabolism and elimination mechanism of alpha-terpinene as "being lipophilic, the aliphatic and alicyclic hydrocarbons in this group are likely to cross biological membranes by passive diffusion. After oral and inhalation exposure, they are rapidly absorbed and distributed to body tissues, elimination from blood being triphasic, with a slow terminal phase." It was concluded that the flavouring agents in these groups (aliphatic and alicyclic hydrocarbons) are expected to be metabolized to innocuous products and the estimated daily per capita intake of alpha-terpinene is below the threshold of concern (maximum of 1800 ug/person per day, WHO, 2005).

By comparison, the dose level tested in the developmental toxicity was 5333 times higher (1800 ug/80 kg BW = 0.0225 mg; 120 mg/kg / 0.0225 mg= 5333) than the threshold of concern for alpha-terpinene. This further demonstrates the reproductive classification is not warranted, as no human would ever possibly consume, or be exposed to, alpha-terpinene at such concentrations. JECFA has recently reviewed aliphatic and aromatic hydrocarbons approved for flavourings for use in animal feeds, one of which was 89% pure alpha-terpinene (EFSA, 2015). The calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 1.0 mg/kg complete feed for pigs and poultry. Maternal body weight loss occurred in the developmental toxicity study at levels 80 and 120 times higher, respectively, than the calculated safe levels of 1.5 and 1.0 mg/kg in non-feed producing animals (EFSA, 2015).

Additional Considerations

In addition to the approval and safe use of alpha-terpinene in feed and flavourings, Terpenoid Blend QRD 460 meets the requirements for Annex IV listing. Substances included in Annex IV are exempted from registration for all their possible uses, irrespective of the tonnage in which they are manufactured or imported. Information is known about Annex IV substances that they are considered to cause minimum risk because of their intrinsic properties. Moreover, residues arising from use of the active

substance are indistinguishable from natural background levels or other sources substances for which MRLs are not required. Following application of product containing QRD 460, components are expected to quickly decline to natural background levels (EFSA, 2014).

Discussion in relation to classification and labelling – CLH/GHS
There is no evidence of alpha-terpinene causing adverse effects in humans on sexual function, fertility or development; therefore it is proposed that the available animal data are the appropriate basis for potential classification.

In consideration of the classification and labelling criteria (EU CLP regulation 1272/2008, 3.7); no adverse effects on sexual function, fertility, development of the conceptus, lactation or the development of the offspring **without exhibiting maternal toxicity** occurred (Table 2). It is considered that the weight of the evidence supports that there is sufficient information to conclude there is **no concern on which to base alpha-terpinene as a Category 2 reproductive toxicant**.

Conclusions on Classification and Labelling
alpha-Terpinene, an approved flavoring and food additive (EFSA, 2015; WHO, 2005), does not meet the criteria for Reproductive Toxicant, Category 2 according to Annex VI of Regulation (EC) No 1272/2008.

Table 2. Experimental Evidence for alpha-Terpinene to meet Developmental Toxicity Classifications

Categories	Criteria	Experimental Evidence	Conclusion
Category 1A	*Known human reproductive toxicant* The classification of a substance in Category 1A is largely based on evidence from humans.	None	Does not meet criteria for Category 1A
Category 1B	*Presumed human reproductive toxicant* The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.	None	Does not meet criteria for Category 1B
Category 2	*Suspected human reproductive toxicant* Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.	↓ Maternal BW gain Secondary, non-specific consequences of maternal toxicity: • ↓ litters • ↓ fetal weights • ↓ litter weights • Delayed ossification	Does not meet criteria for Category 2

References

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