Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

p-mentha-1,3-diene; 1-isopropyl-4-methylcyclohexa-1,3-diene; alpha-terpinene

EC Number: 202-795-1
CAS Number: 99-86-5

CLH-O-0000001412-86-274/F

Adopted

15 March 2019

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CLH-O-0000001412-86-274/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **p-mentha-1,3-diene; 1-isopropyl-4-methylcyclohexa-1,3-diene; alpha-terpinene**

EC Number: **202-795-1**

CAS Number: **99-86-5**

The proposal was submitted by **Netherlands** and received by RAC on **17 April 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **21 May 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **20 July 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	p-mentha-1,3-diene; 1-isopropyl-4-methylcyclohexa-1,3-diene; alpha-terpinene	202-795-1	99-86-5	Flam. Liq. 3 Repr. 2 Asp. Tox. 1 Skin Sens. 1A Aquatic Chronic 2	H226 H361 H304 H317 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H361 H304 H317 H411			
RAC opinion	TBD	p-mentha-1,3-diene; 1-isopropyl-4-methylcyclohexa-1,3-diene; alpha-terpinene	202-795-1	99-86-5	Flam. Liq. 3 Acute Tox. 4 Asp. Tox. 1 Skin Sens. 1 Aquatic Chronic 2	H226 H302 H304 H317 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H361 H304 H317 H411		oral: ATE = 1680 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	p-mentha-1,3-diene; 1-isopropyl-4-methylcyclohexa-1,3-diene; alpha-terpinene	202-795-1	99-86-5	Flam. Liq. 3 Acute Tox. 4 Asp. Tox. 1 Skin Sens. 1 Aquatic Chronic 2	H226 H302 H304 H317 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H361 H304 H317 H411		oral: ATE = 1680 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Alpha-terpinene is one of the ingredients of the active substance Terpenoid Blend QRD 460. The terpenoid blend, consisting of p-cymene, d-limonene and alpha-terpinene, was approved as an active substance (insecticide) for plant protection products under Regulation (EC) 1109/2009. Besides its use as a pesticide, it is widely used in consumer products (e.g. use in cleaning agents and as a solvent), personal care products (as a fragrance), and cosmetics. It is registered under REACH.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Alpha-terpinene is a colourless to pale yellow, oily liquid at 20 °C and 101.3 kPa (DAR for Terpenoid blend (QRD 460), 2013).

A summary of the relevant physico-chemical studies/statements submitted by the Dossier Submitter (DS) is provided below:

Method	Results	Reference
Explosive properties	Not explosive	DAR for Terpenoid blend (QRD 460), 2013
Self-ignition temperature	Not available	-
Oxidising properties	Not available	-
Flash point	47 °C	DAR for Terpenoid blend (QRD 460), 2013

Alpha-terpinene has a flash point of 47 °C which is higher than 23 °C but lower than 60 °C (Annex I, Table 2.6.1, CLP), therefore classification as Flam. Liq. 3; H226 according to regulation (EC) 1272/2008 (CLP Regulation) was proposed by the DS.

Comments received during public consultation

One Member State Competent Authority (MSCA) agreed with the proposed classification of alpha-terpinene.

A second MSCA noted that alpha-terpinene forms unstable peroxides when exposed to air (as stated in section 4.6.1.1 of CLH report). Furthermore, 1,5-p-menthadiene (an isomer of alpha-terpinene) is described as a 'peroxidisable compound' in Bretherick's Handbook of Reactive Chemical Hazards (Urben, 1999), as is tetrahydronaphthalene (CAS No. 119-64-2) which is labelled with EUH019 in Annex VI of the CLP Regulation. Therefore the MSCA is of the opinion that the labelling of alpha-terpinene with EUH019 "May form explosive peroxide" is justified.

The DS responded to the above comment as follows:

According to Woodward et al. (1953), a conversion of about 7-10 % tetrahydronaphthalene to its peroxide was achieved at 70 °C within 48 hours air exposure (blown through the liquid). It is unclear how this would reflect the oxidation state at normal temperatures or without catalysts, which would have been helpful to compare to the information available with alpha-terpinene.

In conclusion, the DS doubted whether there is sufficient information that alpha-terpinene will produce significant amounts of peroxide upon auto-oxidation under common circumstances that justifies labelling with EUH019.

Assessment and comparison with the classification criteria

With a flash point of 47°C, alpha-terpinene **fulfils the criteria as Flam. Liq. 3; H226: (Flammable liquid and vapour)**.

According to Annex I: 2.1.4.3 of the CLP Regulation a substance is not classified as explosive when there are no chemical groups associated with explosive properties present in the molecule, therefore **alpha-terpinene should not be classified as an explosive substance**.

According to Annex I: 2.13.4 of the CLP Regulation for organic substances the classification procedure for oxidising liquids class shall not apply if:

- the substance or mixture does not contain oxygen, fluorine or chlorine; or
- the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen. Therefore, alpha-terpinene should not be classified as an oxidising liquid.

According to Annex II: 1.1.5. of the CLP Regulation the supplemental hazard statement - EUH019 - 'May form explosive peroxides' is applicable "*for substances and mixtures which may form explosive peroxides during storage, such as diethyl ether, 1,4-dioxane"*.

As stated in section 4.6.1.1 of the CLH report, alpha-terpinene is expected to auto-oxidise upon air exposure to form allergenic compounds, as is the case with structurally related monoterpene pre-haptens (e.g. limonene). According to a literature study (Rudbäck *et al*., 2012) the substance degrades rapidly to form oxidation products (more details in the skin sensitisation section below).

Taking into account lack of clear data on formation of explosive peroxide during storage of alpha-terpinene, RAC is of the opinion that **labelling with EUH019 (May form explosive peroxides) is not justified**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Classification for acute toxicity (oral, dermal, inhalation) is not proposed for alpha-terpinene by the DS due to lack of data.

Comments received during public consultation

One company supported the proposal for no classification for acute dermal and inhalation toxicity of alpha-terpinene. Two companies noted that the LD_{50} = 1 680 mg/kg bw for alpha-terpinene, supported by a more detailed description of the acute oral toxicity study in the registration dossier, justified classification of alpha-terpinene as Acute Tox. 4; H302 Harmful if swallowed.

In their response, the DS acknowledged that in the registration dossier a study summary of an oral acute toxicity study in rats has been provided, considered reliable with restriction (Klimisch score of 2) and similar to OECD TG 401, from which an LD_{50} value of 1 680 mg/kg bw (95 % confidence interval 1 460-1 900 mg/kg bw) was calculated. No clinical findings and deaths occurred in the lowest tested dose group (1 050 mg/kg bw). Lethargy was observed on the day of dosing in rats dosed at 1 310, 1 640, 2 050 and 5 000 mg/kg bw. The rats dosed at 2 050 and 5 000 mg/kg bw exhibited loss of righting reflex and piloerection.

Based on these data presented in the registration dossier, although the original study report was not available, the DS concluded that alpha-terpinene should be classified as Acute. Tox. 4; H302.

Assessment and comparison with the classification criteria

Taking into account that the oral LD_{50} of alpha-terpinene of 1 680 mg/kg bw (95 % confidence interval 1 460-1 900 mg/kg bw) is within the range of 300-2 000 mg/kg bw, RAC concludes to **classify this substance as Acute Tox. 4 with the hazard statement H302: Harmful if swallowed**. The LD_{50} value **1 680 mg/kg bw is proposed as the acute toxicity estimate (ATE)** for the classification of alpha-terpinene in a mixture.

RAC agrees with the DS that **classification of alpha-terpinene for acute dermal and inhalation toxicity is not warranted due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

For the evaluation of skin sensitising properties of alpha-terpinene the DS provided three local lymph node assays (LLNAs) summarised in the two tables below.

Method	Results			Remarks	Reference
In vivo mouse local lymph node assay (LLNA) Female CBA/Ca mice (9 weeks old) Alpha-terpinene Purity: 90 %, purified by column chromatography on silica gel Test concentrations: 0, 1, 5, 10, 15, 25 % w/v alpha-terpinene in acetone/olive oil (4:1 v/v)	Dosing at: 0 (control) 1 5 10 15 25	3[H]thymidine incorporation (disintegrations per minute (dmp)/lymph node) 755 848 1 153 2 595 6 740 17 176	Stimulation Index (SI) - 1.1 1.5 3.4 8.9 23 EC3 = 8.9 % w/v	Klimisch score 2 (reliable with restrictions) Key study	Bergstrom et al., 2006

The effective concentration (EC) of purified alpha-terpinene required to induce a stimulation index (SI) of 3 (EC3) was 8.9 % w/v (Bergstrom *et al.*, 2006).

The skin sensitisation potency of air exposed alpha-terpinene was determined in the LLNA performed by Rudbäck *et al*., 2012. The pure substance was placed in an Erlenmeyer flask covered with aluminium foil at room temperature under a daylight lamp (12 hours/d) and stirred for 1 hour four times a day for 10 or 24 days. The results of this study indeed show that alpha-terpinene degrades rapidly to 53 % after 10 days and 21 % after 24 days. After 66 days, it could not be detected in the oxidation mixture any longer, while allylic epoxides and p-cymene were found as the major oxidation products.

With this knowledge, groups of mice (three females/dose) received 25 µL three or seven weeks oxidised alpha-terpinene on the dorsum of the ears daily for three consecutive days. Five days after the first treatment mice were injected with ^{3}[H]thymidine, five hours later the draining auricular lymph nodes were excised and measured for radioactivity and expressed as dmp/lymph node). The following EC3 values were afforded for air exposed alpha-terpinene: three weeks oxidised alpha-terpinene, 0.9 % w/v; seven weeks oxidised alpha-terpinene, 1.0 % w/v.

Method	**Results**			**Remarks**	**Reference**
In vivo LLNA Female CBA/Ca mice (around 8 weeks old) Alpha-terpinene Purity: >95 %, purified by column chromatography on silica gel Test concentrations: 0, 0.1, 1, 5, 10, 25 % w/v three weeks oxidized alpha-terpinene (content of pure alpha-terpinene ca. 20 %) in acetone/olive oil (4:1 v/v)	Dosing at: 0 (control) 0.1 1 5 10 25	3[H]thymidine incorporation (dmp/lymph node) 1 075 857 3 380 14 168 18 399 13 365	Stimulation Index (SI) - 0.8 3.2 13 17 12 EC3 = 0.9 % w/v	Klimisch score 2 (reliable with restrictions)	Rudbäck et al., 2012
In vivo LLNA Female CBA/Ca mice (around 8 weeks old) Purity: as indicated above Test concentrations: 0, 1, 5, 10, 15, 30 % seven weeks oxidized alpha-terpinene (content of pure alpha-terpinene ca. 2 %) in acetone/olive oil (4:1 v/v)	Dosing at: 0 (control) 1 5 10 15 30	3[H]thymidine incorporation (dmp/lymph node) 1 290 3 900 13 074 16 781 10 593 12 074	Stimulation Index (SI) - 3.0 10 13 8.2 9.4 EC3 = 1.0 % w/v	Klimisch score 2 (reliable with restrictions)	Rudbäck et al., 2012

Human data

Limited information is available on skin sensitisation in humans on alpha-terpinene, though allergic reactions to *Melaleuca alternifolia* oil (tea tree oil, TTO which contains alpha-terpinene; Larson and Jacob, 2012) are frequently reported (for a short review see de Groot and Schmidt (2015)). In a human study, patients sensitive to TTO were exposed to typical constituents and degradation products (due to oxidation) of TTO. All eleven patients reacted to alpha-terpinene. Moreover, degradation products of alpha-terpinene were found to be mainly p-cymene, ascaridol, isoascaridol, a ketoperoxide, and colourless crystals that likely were 1,2,4-trihydroxy methane (Hausen *et al*., 1999). The sensitising compounds formed by oxidation are considered responsible for the development of allergic contact dermatitis, emphasising the potency of auto-oxidised alpha-terpinene.

In the opinion of the DS, taking into account the EC3 value of 0.9 % for alpha-terpinene containing auto-oxidation products, classification as Skin Sens. 1A; H317 is warranted. The data were considered sufficient for sub-categorization, given that also lower concentrations (i.e. below 2 %) were tested and showed SI-values below 3.
Classification for skin sensitisation is supported by human data, though these are considered limited.
According to section 3.4.2.2.5 of the CLP-guidance, specific concentration limits for skin sensitisation should be set based on potency. An EC3 value of 0.9 % for alpha-terpinene containing auto-oxidation products corresponds (according to table 3.4.2-f) to a strong potency for which the generic concentration limit of 0.1 % applies. Setting an SCL is therefore not warranted.

Comments received during public consultation

One MSCA agreed with the classification for skin sensitisation proposed by the DS for alpha-terpinene.

One MSCA pointed out that the EC3 values show that the pure (unoxidised) alpha-terpinene (EC3 value = 8.9 %) is a moderate sensitiser, while two EC3 values (0.9 % and 1 %) of the oxidised alpha-terpinene show that these degradation products are strong sensitisers and fulfil the criteria for classification as skin sensitiser in category 1A. The MSCA noted that the auto-oxidation products that form over time cannot be regarded as impurities of the substance in the meaning of the REACH and CLP regulations. Thus, the oxidation products that form due to auto-oxidation of alpha-terpinene due to exposure to air should be regarded as separate substances according to the substance definition under REACH and CLP. The MSCA is of the opinion that the auto-oxidation products are not part of the substance as described by the current SID and should in principle be disregarded for the harmonised classification of alpha-terpinene. The MSCA further noted that chemical analysis demonstrates that alpha-terpinene degrades forming oxidation products upon exposure to air. The experimental data point towards very fast auto-oxidation of pure alpha-terpinene, however no information is available on the extent of auto-oxidation upon exposure to air of the commercial product. Additionally, it is not known whether auto-oxidation of alpha-terpinene marketed in the EU is limited by the presence of an additive (antioxidant). For these reasons the MSCA proposed alternative versions the Annex VI entry (please refer to the RCOM for details):

1) utilizing for classification only the data on the non-oxidised substance (i.e. classify as Skin Sens. 1B and add an additional entry in Annex VI for the autoxidation products (classified as Skin Sens. 1A) and derive the classification of the actual marketed substance(s) by way of the mixture rules,

2) classify the substance in Annex VI as proposed by the DS based on the data of the oxidised substance (i.e. classify as Skin Sens. 1A) and amend the International Chemical Identification with an appropriate minimum concentration of oxidation products pursuant to Annex VI Section 1.1.1.4 Paragraph 6 of the CLP Regulation, while optionally listing a second entry for the "ideal" (or potentially stabilised) substance,
3) listing the substance in Annex VI as proposed by the DS based on the data of the non-oxidised substance (i.e. classify as Skin Sens. 1B) and add nota D pursuant to Annex VI Section 1.1.3.1 of the CLP Regulation, while optionally listing a second entry for the "non-stabilised" substance (i.e. Skin Sens. 1A).

The DS commented on the options proposed by the MSCA arguing that the Skin Sens. 1A classification is preferred in their view.

The lead registrant, did not agree with classification as Skin Sens. 1A, but considered Skin Sens. 1B as more appropriate for the following reasons:

1) the substance revealed in a LLNA an EC3 of 8.9 %, thus being well above the trigger value of 2 % for sub-category 1A,
2) the EC3 values (0.9 and 1 %) obtained with artificially and to a high degree oxidised and decomposed material with 53 % and 21 % alpha-terpinene remaining, respectively, are not considered relevant for the classification and labelling of the substance, for which a specification ≥ 90 % is defined in the REACH dossier and would be guaranteed within the supply chain. The substance tested in this assay is not the substance registered under REACH and therefore those results are not representative for the substance placed on the market. In addition, the results with this mixture also show, that a massive oxidation is needed to obtain EC3 values below the trigger value of 2. The relevance of the sensitisation potential of products containing high concentrations of oxidation products was already assessed by the RAC for other substances. In 2015, the RAC concluded for linalool, that exposure to its oxidised form was not relevant considering its current use and classification was therefore based on the compound linalool only.

One company acknowledged that chemical analysis showed that alpha-terpinene degrades rapidly forming oxidation products upon exposure to air, but noted that there is no information on whether the test material as used in the skin sensitisation study can be considered representative for the compound marketed in the EU. The company also pointed to the RAC conclusion on linalool (2015), where classification was based on the compound, linalool, itself.

In the present case, based on one valid animal study (LLNA) with an appropriate sample of alpha-terpinene for which the EC3 was 8.9 % w/v, it could be concluded that the substance should be classified as Skin Sens. 1B; H317 according to the CLP Regulation.

In their response, the DS agreed that a high auto-oxidation level may be required to result in an EC3 value below 2 %. However, because of the rapid auto-oxidation of alpha-terpinene it is reasonable to assume such auto-oxidation levels will be reached also when using products with alpha-terpinene, and hence a classification as Skin Sens. 1A is warranted. The DS also argued that the case of linalool is not relevant for alpha-terpinene for various reasons (see the RCOM for details).

Assessment and comparison with the classification criteria

While classification for skin sensitisation is supported by some human data (mainly derived from tea tree oil exposures), these are considered limited.

Alpha-terpinene, with 90 % purity, induced in the LLNA increased incorporation of ^{3}[H]thymidine by cells of the auricular lymph nodes with an EC3 equal 8.9 % (Bergstrom *et al.*, 2006), therefore it meets the classification criteria for Category 1, since according to the CLP Regulation, a stimulation index of three or more is considered a positive response in the LLNA. At lower concentrations tested (1 % and 5 %) the stimulation index was below 3, thus, based on results only of that study, it may be concluded that classification in sub-category 1A, requiring a value of EC3 ≤ 2 %, is excluded and therefore alpha-terpinene is within classification criteria for category 1B.

However, as noted above, it has been also demonstrated that this substance degrades substantially due to auto-oxidation when exposed to air at room temperature.

Alpha-terpinene containing high level of its auto-oxidation products due to exposure to air, induced proliferation of cells in auricular lymph nodes with an EC3 equal to 0.9 % after three weeks aeration, and 1.0 % after 7 weeks aeration (Rudbäck *et al.*, 2012). The concentration of alpha-terpinene in the mixture of this substance with its degradation products used in this LLNA was not provided, but can be assumed to be below 50 % after three weeks aeration, and below 20 % after 7 weeks aeration. The ability of alpha-terpinene to auto-oxidise is an inherent property of this substance, therefore the degradation products may be relevant to the assessment of skin sensitisation. If the degradation products are included in the evaluation of the potency, the alpha-terpinene together with its degradation products produced within several weeks of aeration meets the classification criteria for sub-category 1A, since the EC3 value for such mixtures is ≤ 2 %. However, the relationship between this simulation of oxidation under experimental conditions and actual oxidation of alpha-terpinene under typical use conditions is unknown.

The marketed products of alpha-terpinene contain a declared purity > 80 % and unknown impurities, thus it corresponds with the alpha-terpinene used in the Bergstrom *et al.* (2006) LLNA study which met the criteria for Skin Sens. 1B. However, it is not possible to exclude the auto-oxidation of alpha-terpinene during storage or potential use leading to increased skin sensitising potency, thus a higher potency of, e.g. older batches of alpha-terpinene, or depots of alpha-terpinene on the skin cannot be excluded. Therefore, in line the Guidance on the Application of the CLP Criteria (Version 5.0, July 2017), when the criteria in the table 3.4.4 for classification to subcategory 1B are fulfilled, but classification for subcategory 1A cannot be excluded, the substance should be classified as Category 1 without sub-categorisation.

Taking these arguments into account, particularly the inherent ability of alpha-terpinene to produce oxidation products (which have a high skin-sensitising potential) when exposed to air at room temperature, and considering the uncertainties as to whether it would oxidise to the same extent under natural conditions (as opposed to simulated conditions), in the opinion of RAC **alpha-terpinene warrants classification as Skin Sens. 1, H317: May cause an allergic skin reaction (without sub-categorisation)**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH report includes only a negative Ames test, where alpha-terpinene was incubated with *S. typhimurium* strains TA97a, TA98, TA100, or TA1535 (Gomes-Carneiro *et al*., 2005). The DS concluded that no classification for mutagenicity is warranted due to the lack of observed mutagenicity *in vitro* and absence of data *in vivo*.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees that **no classification for mutagenicity is warranted due to the lack of data**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

For the assessment of reproductive toxicity, the DS provided results of one study (Araujo *et al.*, 1996) which had not been performed according to internationally recognised guidelines or under GLP conditions.

In the study, at the highest dose (250 mg/kg bw/d), there was a higher incidence of sperm positive dams with no implantation sites, which was considered by the DS to be due to total litter loss at the beginning of pregnancy, thus warranting classification as Repr. 2; H361 (without specifying whether the classification was for fertility or developmental toxicity). This effect was observed in 12 out of 27 females (44.4 %) in the presence of moderate maternal toxicity, but only in 4 out of 28 control females (14.3 %).

Signs of delayed ossification were also observed at doses of 60 mg/kg bw/d or more (Araujo *et al*., 1996) but in the presence of maternal toxicity, thereby they were considered by the DS to be secondary, non-specific consequences of the maternal toxicity, and hence not relevant for classification.

Comments received during public consultation

One MSCA agreed with the proposed classification, but no justification was provided.

One MSCA considered that the teratogenicity study is not appropriate for assessing the toxic effects on fertility, because the substance was administered after fertilisation, i.e. GD 6-15. For assessment of fertility, a one-generation, two-generation and/or extended one-generation study is required. As no conclusion on sexual function and fertility effects is possible, no classification for this differentiation is supported. However, the MSCA supported the proposed classification as Repr. 2 for developmental effects; H361d (Suspected of damaging the unborn child).

In their response, the DS noted that the effects observed with alpha-terpinene may be attributable to either development or fertility (i.e. implantation) impairment, therefore the DS retained its proposal for classification as H361, without the d or f differentiations. The DS also acknowledged that these effects could not be considered as affecting the ability to become pregnant, as exposure did not begin before the start of gestation.

Six companies disagreed with the proposed classification and proposed no classification for reproductive toxicity, providing the justifications summarised below:

One company pointed out that administration of the compound did not begin until gestation day (GD) 6, when implantation occurs in rats. Therefore, had the animals that were sperm positive actually been pregnant, they would have left evidence in the form of implantation scars on the uterine wall. The fact that in the Araujo *et al.* (1996) study no evidence of implantation was found despite using the (more sensitive) Salewski technique may be due to the sperm-positive animals

not being pregnant at all, an effect that is attributable to animal husbandry, not the test compound. It may also be possible that animals were incapable of pregnancy because they were cohabited during the wrong phase of the oestrus cycle (which appeared to not have been checked in the study) and/or for a too short time (2 hours). While females are generally not receptive to copulation when they are not in oestrus, this is not a rigid rule of animal behaviour, and a female in close quarters (i.e., a small cage) with a male may not be impervious to copulation. In summary, there is no evidence that alpha-terpinene caused whole litter loss, and therefore the proposal for classifying it as a reproductive toxicant is inappropriate.

Other companies raised doubts on the interpretation by the DS of the findings reported in the available literature study, due to the study's substantial limitations (i.e. not a GLP study, no historical control data provided) and lack in important information. In the high dose group, where the claimed reproductive toxicity effects were noted, a significant reduction in body weight was observed (GD 6-11 day: -17.8 g) *vs.* body weight gain (+13.6 g) in controls, which, according to the study authors, may be due to systemic toxicity (and not to reduced feed consumption). No classification relevant findings were noted at 125 mg/kg bw/d, at which dose clear signs of systemic toxicity were also evident (reduction in body weight gain). In addition, QSAR modelling (DEREK) does not indicate any alerts with regard to reproductive toxicity.

In their responses, the DS pointed out that the mating conditions were identical in the control and all treatment groups, but only in the 250 mg/kg bw/d group was the ratio of non-pregnant/sperm positive females increased, therefore the effect was considered treatment related. The DS also considered that reduced body weight gain is of minor importance compared to total body weight loss at GD20, which was < 10 % and therefore unlikely to have impacted on the developing foetuses. Overall, the DS did not believe there was overt general maternal toxicity that may have had a significant and observable effect on the developing offspring.

In the DS's opinion, the limited ossification could be attributed to the treatment, even though it may not be considered adverse or of toxicological concern, and the proposed classification is based on the lower ratio of pregnant *vs.* sperm positive females.

Assessment and comparison with the classification criteria

In the study of Araujo *et al.* (1996) female Wistar rats (28, 15, 20, 26 and 27 per group) were orally dosed (via gavage) respectively with 0, 30, 60, 125 or 250 mg alpha-terpinene/kg bw/d in corn oil from GD 6-15. Mating was performed by transferring two females to the cage of one male for 2 hours per day. Copulation was confirmed by the presence of sperm in the vaginal smear; the day on which spermatozoa were found was designated as day 0 of pregnancy. The study was not performed according to OECD TG 414, although the design of study roughly corresponds to the OECD TG 414, and was not GLP-compliant, with no individual data available. It is also not known whether mated (sperm-positive females) were assigned in an unbiased manner to the control and treatment groups, as required by OECD TG 414. In fact, no information is provided on the procedure used for randomising distribution of sperm-positive females to different experimental groups. In addition, no historical control data were provided to assess the variability of the examined parameters.

Maternal toxicity

Maternal toxicity of alpha-terpinene at a dose of 250 mg/kg bw/d was observed during the first 6 days of exposure (GD 6-11), with reductions in maternal body weight (mean reduction in body weight by 17.8 g). Maternal body weight gain of females dosed with 250 mg/kg bw/d during GD 6-15 amounted to 1.4 g, while it was 30.7 g in control animals. The maternal body weight gain minus uterus weight during GD 0-21 was reduced by 58 % in the 250 mg/kg group and by 23.7 %

in the 125 mg/kg bw/d in comparison with control animals. Alpha-terpinene at doses of 30 and 60 mg/kg bw/d did not affect maternal body weight gain.

No clinical signs of maternal toxicity were reported, and at caesarean section no gross pathological alterations were found in maternal organs of any group.

Fertility

The only parameter related to fertility which was affected in the study of Araujo *et al.* (1996) was an increased to 44 % of sperm positive females without any implantation site in the 250 mg/kg bw/d group, in comparison with 14 % sperm positive females without any implantation in the control animals. The lowest proportion of sperm positive, but not pregnant females, amounting to 4 %, was observed in 125 mg alpha-terpinene/kg group, indicating that a reduction in proportion of sperm positive females without any implantation was not dose-related. The reduction of sperm positive females without any implantation only at the top dose, without dose-response relationship, cannot be taken reliably as treatment-related since the procedure to assign sperm positive females to treatment groups was not reported to be randomised, and the fertility of males used for insemination was not ascertained. It is not known whether females inseminated by the same male were evenly distributed across the groups (however, the variability of this parameter is not known because historical control data were not provided). All the other parameters, which could indicate alteration of fertility, such as the number of corpora lutea/dam, the number of visible implantation sites/litter, the number of live foetuses/ litter, were not altered by alpha-terpinene at any dose. In addition, the prenatal developmental toxicity study is not appropriate and not recommended by the OECD or Council Regulation (EC) No 440/2008 for the assessment of fertility effects.

Overall, RAC is of the opinion that alpha-terpinene does **not warrant classification for adverse effects on sexual function and fertility due to lack of data**.

Developmental toxicity

Neither the number of live foetuses nor the number of resorptions per pregnant female were affected by alpha-terpinene at any dose. Foetal weight (individual and mean of litters) was statistically significantly reduced only at 250 mg/kg bw/d (by 15 %), while in other groups it was the same or even slightly higher than in the control group.

No noticeable adverse effects were revealed by external examination, except for a higher incidence in kinky tail in the group exposed to 30 mg/kg bw/d (which was not observed in higher dose groups). No increase in visceral malformations was observed in any treated group.

The number and percentage of foetuses with delayed ossification was increased in animals exposed at 60, 125 and 250 mg/kg bw/d. This retardation of ossification and a small reduction in foetal body weight, only at 250 mg/kg bw/d, are considered as minor developmental changes, therefore they are not considered as significant adverse effects warranting classification for developmental toxicity.

Taking the above considerations into account RAC is of the opinion that alpha-terpinene **does not warrant classification for adverse effects on development of the offspring**.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Alpha-terpinene has a kinematic viscosity below 7 mm^2/s at 20 °C (method not known, purity not provided; Vigon, 2015), which might indicate the potential for aspiration toxicity.

The DS proposed to classified alpha-terpinene for aspiration toxicity as Asp. Tox 1; H304 (May be fatal if swallowed and enters airways).

Comments received during public consultation

One MSCA and one company agreed with the DS's proposal for classification as Asp. Tox 1; H304.

Assessment and comparison with the classification criteria

A substance is classified in category 1 for aspiration toxicity:

- based on reliable and good quality human evidence or,
- if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40 °C.

Given that alpha-terpinene is a hydrocarbon and has a kinematic viscosity < 7 mm^2/s at 20 °C and its expected kinematic viscosity at 40 °C would be lower than the viscosity at 20 °C, thus lower than 20.5 mm^2/s, despite the limitations of this data, RAC concludes that alpha-terpinene should be classified as **Asp. Tox 1; H304 – May be fatal if swallowed and enters airways**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance is currently not listed in Annex VI of the CLP Regulation. The Dossier Submitter (DS) proposed to classify p-mentha-1,3-diene (alpha-terpinene) as Aquatic Acute 1, M = 1. As acute toxicity data on algae were lacking, the read-across approach to toxicity of d-limonene in algae was seen by the DS as a justifiable realistic worst-case scenario. Furthermore, the DS considered alpha-terpinene having a high potential for bioaccumulation and considered the substance rapidly degradable, also based on read-across from d-limonene.
The DS presented a rationale for read-across and a comparison of key physical-chemical properties of alpha-terpinene and d-limonene. They also gave information on the structural similarities and on similar behaviour in the environment. The read-across approach was supported with ECOSAR v1.11 QSAR estimations. During Public Consultation, the DS was made aware of the new REACH Registration Dossier published just before PC began, which contained new toxicity data. Consequently, the DS changed their proposal. No classification for Aquatic Acute hazard was proposed based on data on the substance itself. For Chronic Aquatic hazards, the new proposal was Aquatic Chronic 2 based on the surrogate method with alpha-terpinene data.

Degradation

No experimental data on the stability of alpha-terpinene was available. Alpha-terpinene was not expected to undergo hydrolysis since it lacks functional groups that hydrolyse under

environmental conditions. The Henry's law constant was 2.59 × 10^{-3} Pa m^3/mol and from this and level III fugacity modelling, alpha-terpinene was expected to partition from water and soil to air. In air, it degraded rapidly (DT_{100}=20.8 hours) by interaction with hydroxyl and nitrate radicals. Alpha-terpinene was not expected to be affected by photolytic degradation.

Experimental data on biodegradation of alpha-terpinene was not available for the DS. Based on read-across from d-limonene and assuming that similar structure and physical properties would result in similar fate in the environment for alpha-terpinene, they considered alpha-terpinene to be rapidly degradable as proposed for d-limonene (based on 71.4 % degradation in 28 days in an OECD TG 301B). However, it was made known in the Public Consultation (PC) that there was a new REACH Registration Dossier available (published just before PC) on alpha-terpinene including a ready biodegradability study. The study was performed according to OECD TG 301F (Ready Biodegradability: Manometric Respirometry Test) following GLP. The test material was 94.1 % alpha-terpinene. The duration of the test was 70 days and the initial test concentration was 15.2 mg/L. The test showed that alpha-terpinene underwent 40 % biodegradation after 28 days under the test conditions, which was below the 60 % required in the guidelines to consider this substance as readily biodegradable. As a consequence of the newly presented data in the REACH dossier, the DS considered the study reliable and relevant for classification and labelling. This is in contrast to the conclusion derived from read-across to d-limonene. However, substance data is preferred to the read-across with d-limonene and was therefore used for the classification.

In an aquatic non-standard simulation study similar to OECD TG 309 presented in the DAR, alpha-terpinene volatilized from the natural water test systems rapidly with a DT_{50} of 4.1 and DT_{90}s of 13.7 hours. The trapping solution did not show the presence of the test substance or any degradation products. Degradants in the water were also not detected. Thus, rapid escape (fugacity via volatility) appeared to be the predominant pathway for alpha-terpinene in natural water.

In conclusion, after considering the additional data received during PC indicating 40 % biodegradation after 28 days under OECD TG 301F, the DS considers alpha-terpinene to be not rapidly degradable for classification purposes.

Bioaccumulation

An experimentally determined log K_{ow} of 5.09 was reported in the DAR for QRD 460 but this value was considered unreliable by the DS. Preference was given to the value of 4.25 determined with an estimation method based on the OECD TG 117 HPLC method. The standards chosen were especially selected for terpenoids and p-cymene, which has a comparable structure to alpha-terpinene, was also included in the set of standards. According to the REACH Registration Dossier, the surface tension of alpha-terpinene was 70.5 mN/m indicating that the substance is not surface active.

No experimental data on bioaccumulation (BCF) was available.

As the Log k_{ow} is above the CLP cut off of 4, the DS considered alpha-terpinene to have a high potential for bioaccumulation. This is supported by an estimated BCF (QSAR BCFBAF v3.01) of 625 L/kg (based on Log k_{ow} 4.25).

Aquatic toxicity

Initially, the Dossier Submitter (DS) proposed to use read-across from d-limonene for endpoints where data on alpha-terpinene were missing. However, it was made known during the Public Consultation (PC) that there is a new REACH Registration Dossier available for alpha-terpinene including aquatic toxicity studies for *Daphnia* and algae. The new data is included in the following

Table and the studies are summarised below along with the studies presented by the DS in the CLH report, as well as QSAR estimations performed by ECOSAR v1.11 based on Log k_{ow} 4.25.

***Table**. Reliable aquatic toxicity data on alpha-terpinene*

Test method and reference	**Test species**	**Result mg/L**	**QSARs for alpha-terpinene**
87 % alpha-terpinene Short-term fish toxicity ASTM E729 method, flow-through [3] Anonymous (1990b)	*Pimephales promelas*	96 h LC_{50}: 3.15 96 h EC_{50}: 1.48[1] based on measured average concentrations	LC_{50}*: 1.07 mg/L (freshwater fish); 1.36 mg/L (saltwater fish) (ECOSAR v.1.11)
alpha-terpinene			NOEC(*: 0.094 (freshwater fish) (ECOSAR v.1.11)
Invertebrates			
87 % alpha-terpinene Short-term invertebrate toxicity according to ASTM E729 method, GLP not reported, flow-through[3] Anonymous (1990b)	*Daphnia magna*	48 h LC_{50}: 1.85[1] 48 h EC_{50}: 1.85[1] based on mean measured concentrations	LC_{50}*: 0.75 mg/L, daphnids; LC_{50}*: 0.22 mg/L, saltwater mysids (ECOSAR v.1.11)
92.7 % alpha-terpinene [2] OECD TG 202 (*Daphnia* sp. Acute Immobilisation Test), GLP, semistatic (renewal after 24 hours) 2018	*Daphnia magna*	48 h EC_{50}: 1.7[3,4] (mobility) based on mean measured concentrations	
alpha-terpinene	*Daphnia magna*		Chronic NOEC*: 0.092 mg/L, daphnids (ECOSAR v.1.11)
Algae/Aquatic plants			
94.1 % alpha-terpinene [2] OECD TG 201 (Alga, Growth Inhibition Test), GLP, static 2018	*Pseudokirchneriella subcapitata*	72 h NOEC: 3.7 (cell number, yield) [4] mean measured concentrations no effects	LC_{50}*: 1.31 mg/L; NOEC*: 0.39 mg/L (ECOSAR v.1.11)

[1] Geometric mean of the NOEC and LOEC since at the LOEC 100 % effect was observed
[2] REACH Registration Dossier
[3] The beakers were covered with loose fitting glass panes
[4] vehicle DMF used
* neutral organics, based on log *K*ow 4.25

Acute Aquatic toxicity

The only reliable aquatic acute fish study was a 96-hour flow-through study with *Pimephales promelas.* The test volume was replaced 50.4 times a day and the fresh test medium was generated directly before addition from a continuously generated near saturated solution. The test concentrations were analysed every 24 hours and the toxicity endpoints were based on the average test concentrations of alpha-terpinene ranging from 1.05 to 4.82 mg/L. The reported LC_{50} and EC_{50} values were 3.15 mg/L and 1.48 mg/L, respectively. The EC_{50} was based on the geometric mean of the NOEC and LOEC since at the LOEC 100 % effect was observed.

There were two reliable studies available for *Daphnia*. The study cited in the CLH Report is a 48-hour flow-through study with *Daphnia magna*. The test volume was replaced 50.4 times a day and the fresh test medium was generated directly before addition from a continuously generated near saturated solution. The test concentrations were analysed every 24 hours and the toxicity endpoints were based on the average test concentrations of alpha-terpinene ranging from 1.36 to 5.89 mg/L. The reported LC_{50} and EC_{50} were both 1.85 mg/L. These endpoints were calculated as the geometric mean of the NOEC and LOEC since at the LOEC 100 % effect was observed.

The study from the REACH Registration Dossier was an OECD TG 202 (*Daphnia* sp. Acute Immobilisation Test) study following GLP. The test substance is 94.1 % alpha-terpinene. Dimethylformamide (DMF) was used as a vehicle. The study was run with a dilution water control and solvent control together with nominal concentrations of 0.0625, 1.25, 2.5, 5.0 and 10 mg/L (mean measured concentrations equivalent to 0, 0.21, 0.39, 0.97, 1.7, 5.3 mg/L, respectively). The test was performed under semi-static conditions, test solutions were renewed after 24 hours. No significant effects on mortality or immobilisation were observed in the bottom three doses. A 50 % effect was observed at a mean measured concentration of 1.7 mg/L. There was uncertainty in the analytical measurement at the top dose at 24 hours and therefore the results at the top dose were omitted from statistical analysis. As such, an EC_{50} of 1.7 mg/L (based on geometric mean measured concentrations) was reported for this study. In the response to PC comments, the DS noted that this result confirmed the data presented in the CLH report.

The only reliable algae test available is from the REACH Registration Dossier. The test was performed according to the OECD TG 201 (Alga, Growth Inhibition Test) following GLP with *Pseudokirchneriella subcapitata*. The test substance was 94.1 % alpha-terpinene. Dimethylformamide (DMF) was used as a vehicle. There was no significant effect on the yield at any concentration when compared to the control or solvent control. Measured concentrations in the test solutions were 50-107 % of the nominal at 0 hours but all dropped to below the limit of detection by 72 hours. As such, the geometric mean measured test concentrations were 0, 0.16, 0.47, 1.1, 2.7 and 3.7 mg/L (equivalent to nominal concentrations, 0, 0.625, 1.25, 2.5, 5.0 and 10 mg/L, respectively). Therefore, a 72-hour NOEC of 3.7 mg/L and a LOEC of >3.7 mg/L (based on geometric mean measured concentrations) were reported for this study. In the response to the PC comments, the DS noted that this new data shows that the EC_{50} for alpha-terpinene would be higher than 1 mg/L.

The DS concluded that with the new data available the read-across from d-limonene for acute algae toxicity becomes obsolete. Since the available endpoints for aquatic acute were all higher than 1 mg/L, no classification for Aquatic Acute toxicity was required.

Chronic Aquatic toxicity

No long-term experimental data for fish or invertebrates were available. For algae, there was a 72-hour NOEC of 3.7 mg/L available from the OECD TG 201 test described under acute toxicity. The DS originally proposed to use read-across from d-limonene for fish and *Daphnia* as described on the CLH report. As chronic data are not available for fish and invertebrates, the surrogate approach should be applied for alpha-terpinene for fish (LC_{50} of 1.48 mg/L) and *Daphnia* (EC_{50} 1.70 mg/L). This would lead to Aquatic Chronic 2 classification, as would the read-across from the d-limonene *Daphnia* data originally proposed in the CLH Report.

In conclusion, the DS proposed classification as Aquatic Chronic 2 based on the surrogate approach using an alpha-terpinene fish EC_{50} of 1.48 mg/L and considering that alpha-terpinene is not rapidly degradable and has a high potential for bioaccumulation.

Comments received during public consultation

Comments were received from three MSCAs and three companies. Two MSs supported the initial classification proposal made by the Dossier Submitter (DS). One MS wanted more data on the QSAR model used for alpha-terpinene. They also pointed out that they had made comments in the PC of d-limonene concerning the reliability of the biodegradation, algae, and chronic toxicity studies which might change the conclusion of the d-limonene classification and thus change the classification of alpha-terpinene based on read-across. They supported the conclusion on bioaccumulation.

Three companies brought up that there is a REACH Registration dossier available on the ECHA website (first published 4.5.2018) containing experimental data on biodegradation and on aquatic toxicity for *Daphnia* and algae. The data indicated that alpha-terpinene should not be classified for aquatic acute hazard, based on substance data. No read-across from d-limonene was necessary. For aquatic chronic toxicity, the companies agreed with the proposed Aquatic Chronic 3 classification, based on read-across from d-limonene. One company did not agree with the conclusion based on the ready biodegradation test presented in the REACH Registration dossier.

The DS considered the ready biodegradability study in the REACH Registration dossier reliable and indicated that alpha-terpinene should be considered not rapidly degradable. Read-across from d-limonene was no longer necessary. They also considered the *Daphnia* and algae test studies presented in the REACH Registration dossier reliable and sufficient for classification although there were limitations in the algae test and agreed that the classification proposal should be revised as described above.

Assessment and comparison with the classification criteria

Alpha-terpinene was expected to be hydrolytically stable and unlikely to be affected by photolytic degradation. In a ready biodegradation OECD TG 301F study brought to the Dossier Submitter's attention during the Public Consultation, alpha-terpinene underwent 40 % biodegradation after 28 days. This new information was not available in the CLH Report open for the Public Consultation but was publicly available in the REACH Registration Dossier and evaluated by the DS and RAC to be reliable and relevant for classification. In an aquatic simulation study, alpha-terpinene volatilized from test systems rapidly showing that volatility appeared to be the predominant pathway for loss of alpha-terpinene in natural water. Based on the ready biodegradation study result, RAC concludes that alpha-terpinene is not rapidly degradable for classification purposes. Read-across from d-limonene and the QSAR estimations presented in the CLH report are no longer needed.

RAC supports the decision of the DS to give use the log K_{ow} value of 4.25 for assessing bioaccumulation potential. Since no experimental data on bioaccumulation are available, RAC concludes that alpha-terpinene has a high potential to bioaccumulate based on this value.

Taking into account the new information from the REACH Registration dossier, there are alpha-terpinene acute toxicity data available for fish and *Daphnia* and a chronic NOEC for algae. The 96 h EC_{50} for fish is 1.48 mg/L, the lowest 48-hour EC_{50} for *Daphnia* is 1.7 mg/L and, whilst uncertain, the algae data indicates that the acute EC_{50} would not be ≤ 1 mg/L. The toxicity values for fish and *Daphnia* are >1 mg/L, which is the cut off for aquatic acute classification. In the absence of reliable substance data indicating toxicity ≤ 1 mg/L, **RAC concludes that aquatic acute classification is not warranted for alpha-terpinene**.

There is only chronic toxicity data available for algae. Considering the new data on algae and the removal of the read-across from d-limonene, RAC considers that the use of the surrogate approach using acute substance toxicity data is appropriate following CLP table 4.1.0(b)(iii). The acute EC_{50} values 1.48 mg/L for fish and 1.7 mg/L for *Daphnia*, combined with alpha-terpinene

being considered not rapidly degradable and having a potential to bioaccumulate, both lead to Aquatic Chronic 2 classification. The chronic NOEC for algae of 3.7 mg/L would result in no chronic classification CLP table 4.1.0(b)(i). Consequently, RAC concludes that alpha-terpinene warrants classification as Aquatic Chronic 2.

Overall, RAC agrees with the DS's proposal as amended after Public Consultation and agrees to **classify alpha-terpinene as Aquatic Chronic 2; H411.**

Additional references

Martan (1970), Oxidation of Tetralin, alpha tetralol and alpha-tetralone. Dependence of alcohol to ketone ratio on conversion. Tetrahedron, 26 (5), pp 3815-3827.

P. G. Urben (Ed.): Bretherick's Handbook of Reactive Chemical Hazards, 6th ed., Elsevier 1999, No 3338.

Woodward et al., (1953), Low temperature Auto-oxidation of Hydrocarbons. The Kinetics of Tetralin Oxidation, J. Am. Chem. Soc. 75 (24), pp 6189-6195.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).