Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

(*R*)-p-mentha-1,8-diene; d-limonene

EC Number: 227-813-5
CAS Number: 5989-27-5

CLH-O-0000001412-86-275/F

Adopted

15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON (*R*)-P-MENTHA-1,8-DIENE; D-LIMONENE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: (R)-p-mentha-1,8-diene; d-limonene
EC number: 227-813-5
CAS number: 5989-27-5
Dossier submitter: Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
18.06.2018	Denmark		MemberState	1
Comment received				
In Table 3 page 5, The proposed classification according to the CLP regulation: In the Table it is stated that the Proposed classification is Skin Sens. 1 H317 and that the current classification is Skin Sens. 1B H317. It is opposite. The proposed classification is Skin Sens. 1B H317 and the current classification is Skin Sens. 1 H317.				
Dossier Submitter's Response				
Thank you for pointing us towards this error in table 3. Indeed the current classification should be Skin Sens. 1 and the proposed classification should be Skin Sens. 1B.				
RAC's response				
Thank you for proposed correction.				

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	United States	Bayer AG	Company-Manufacturer	2
Comment received				
General comment on the review process of d-limonene as a separate substance rather than as part of Terpenoid Blend QRD 460. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comment letter on QRD 460 ECHA.pdf				

Dossier Submitter's Response
Thank you for your comments. A harmonised classification is, according to Title V of CLP, only possible for substances. It is not possible to propose a harmonised classification for a mixture of chemicals other than UVCBs. Notably, for non-CMR endpoints, it is possible to classify a mixture based on mixture-specific data (if available) rather than based on information with the individual components. Mixtures do have to be classified for CMR endpoints based on the individual components rather than information with the mixture itself. This information can be derived from the CLP guidance paragraph 1.1.6.2. and to some extend from the CLP regulation (EC 1272/2008) Title II, article 6, paragraph 2 and 3.
RAC's response
Thank you for remark. The response of the DS above is supported.

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	Germany		MemberState	3
Comment received				
In part B section 4.5.1.3 of the CLH-Report the dossier submitter states, that "[…] a higher potency for skin sensitisation may be present in d-limonene with a higher level of oxidisation products" Currently the dossier submitter proposes a classification as Skin Sens. 1B; H317 based on the LLNA results of "a highly purified form of d-limonene". The human data presented in section 4.5.1.2. of the dossier and the additional data sources indicated in the endpoint specific comment by the German CA may point to the classification of the oxidised Substance as Skin Sens. 1A. Pursuant to the agreed strategy in the CLH process in principle an Annex VI entry should deal with the substance as such and not with a specific marketed composition of a manufactured substance*. Especially as the ICI of an Annex VI entry does not reflect which (if any) impurities or additives have been considered§. Therefore the influence of the autoxidation products on the classification of the substance may need to be reflected in the Annex VI entry, if the evaluation of the extended data set indicates such. However, as the autoxidation products form over time, they cannot be regarded as impurities of the substance in the meaning of the REACH and CLP regulations and guidance, as impurities are only regarded as part of a substance if they are "derived from the process used [to manufacture or obtain the substance]". This is also elaborated in the Guidance for identification and naming of substances under REACH and CLP, Section 4.2. Moreover, substances which result from a chemical reaction that occurs incidental to exposure of another substance or article to environmental factors such as air, moisture, microbial organisms or sunlight should be exempted from registration#. That means that the oxidation products should be regarded as substances acc. to the substance definition under REACH and CLP. Therefore the German CA is of the opinion that autoxidation products are not part of the substance as described by the current SID and should in principle be disregarded for harmonised classification of the substance. However, to utilize the available data to the greatest extent and to maintain a high level of protection of human health different ways forward to implement the substance entry				

into Annex VI could be considered if the need to address the autoxidation products arises from the evaluation of the additional data.

1) Listing the substance in Annex VI as proposed by the DS but utilizing only the data on the non-oxidised substance (i.e classify as Skin Sens. 1B see our specific comments on Skin sensitisation below). In this proposal the oxidation products are not considered for the entry. And adding an additional entry for the autoxidation products (classified as Skin Sens. 1A, see specific comments below) and derive the classification of the actual marketed substance(s) by way of the mixture rules.

This would actually be the systematically most desirable approach; however as SID information on the autoxidation products in the report is scarce, formulating an appropriate entry may be difficult. In addition suppliers may fail to realize that such an additional entry relates to their substance.

2) Listing 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, see specific comments below) and amend the ICI 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.

This would actually be not entirely formally correct, as the oxidisation products are strictly speaking not regarded as impurities, however it would reflect the contribution of the peroxides to the classification.

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, see specific comments below) 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) or the oxidisation products (again relying on the summation method).

*)
https://echa.europa.eu/documents/10162/13626/clh_impurities_purity_en.pdf/cc0406ba-2e6c-4ee0-3082-2b2b3f123ee4
§) Pursuant to Annex VI Section 1.1.1.4 Paragraph 2 of Regulation (EG) 1272/2008 (CLP Regulation), regarding the international chemical Identification in Annex VI, "[i]mpurities, additives and minor components are normally not mentioned unless they contribute significantly to the classification of the substance."
#) Regulation (EC) 1907/2006, Annex V Number 1.

As stated in the CLH report "section 4.5.1.2 Human information" d-limonene forms unstable peroxides when exposed to air and light.

In the opinion of the German CA labelling of d-limonene with EUH019 "May form explosive peroxide" is justified.

Limonene is described as a peroxidisable compound in Bretherick's Handbook of Reactive Chemical Hazards just like Tetrahydronaphthalene (CAS-No. 119-64-2) which is labelled with EUH019 in Annex VI of the CLP Regulation.

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

Dossier Submitter's Response
Thank you for your comments. - First, it is agreed that the oxidized products formed after d-limonene is exposed to air may be classifiable as Skin Sens. 1A. See also our response to specific comment number 8 - It is also agreed CLP should deal with the substance itself rather than any impurities or substances that result from chemical reactions by incidental contact with e.g. air or water. We believe option one proposed will likely not yield a proper classification of d-limonene in practice because the amount of oxidised d-limonene over time is uncertain. However, it is probably the most correct option to follow. Option 2 is interesting, but has a similar problem in practice, the mixtures will likely be classified as Skin Sens. 1B because of the initial concentration of d-limonene and no oxidation products. Note D pursuant Annex VI Section 1.1.3.1. seems to be a good option and is supported by the DS (option 3). - With respect to the proposed labelling with EUH019, peroxides are generally regarded as explosive. However, d-limonene is not a peroxide and not all the oxidation products are necessarily peroxides. Indeed some of the oxidation products formed are peroxides such as the potent skin sensitiser Limonene-2-hydroperoxide. However, because the amount formed of such products after air-exposure is unclear and products in practice generally have small fractions of d-limonene, which may be additionally stabilized, it is not considered appropriate to label the product with EUH019.
RAC's response
Thank you for the considerations and proposal.

Date	Country	Organisation	Type of Organisation	Comment number
16.07.2018	United Kingdom		MemberState	4
Comment received				
Please can you clarify the Henry's Law constant value as s. 5.1.1 includes the value 1.30 x 10-3 Pa m3/mol and s. 5.2.2 the value 1.30 x 103 Pa m3/mol.				
Dossier Submitter's Response				
Thank you for pointing out this error. The Henry's Law constant in the DAR is reported as 1.3 x 10^3 Pa m^3/mol.				
RAC's response				
Thank you for the proposed correction.				

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	France		MemberState	5
Comment received				
Identity The IUPAC name should be indicated as (R)-1-methyl-4-(1-methylethenyl) cyclohexene Physical hazards Some studies and results are different from those indicated in the monograph 2012 of the active substance orange oil. Nevertheless, as these differences don't change the classification, no more data required				

Dossier Submitter's Response
Thank you for your comments. It appears several IUPAC names exist and either is correct. With respect to the physico-chemical properties, the DS assumes that MSCA France refers to EFSA (2013; http://www.efsa.europa.eu/en/efsajournal/doc/3090.pdf) It is indeed noticed that for some of the physico-chemical properties different values were presented by EFSA (2013) when compared to the literature sources as used by the DS. However, this does not affect the proposed classification.
RAC's response
Thank you for contribution.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
18.06.2018	Denmark		MemberState	6
Comment received				
According to the studies the positive reactions are > 500 µg/cm2 Therefore Skin Sens. 1B (H317) is warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for pointing out this information.				

Date	Country	Organisation	Type of Organisation	Comment number
17.07.2018	Finland		MemberState	7
Comment received				
The Netherlands CA proposes a modification of current entry in Annex VI, CLP Skin Sens. 1 (H317) to Skin Sens. 1B (H317). Classification into sub-categories is required when data are sufficient (CLP Annex I 3.4.2.2.1.1). CLH report for d-limonene refers to two studies of mouse LLNA, both conducted according to OECD 429 (with deviations). The reported EC3 values are 22% and 68.5%. According to CLP (Annex I, Table 3.4.4), a substance is classified for sub-category 1B, if LLNA EC3 value is >2 %. The Finnish CA considers that there is sufficient evidence for classification of (R)-p-mentha-1,8-diene; d-limonene to Skin Sens. 1B; H317.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your assessment.				

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	Germany		MemberState	8
Comment received				
The Dossier Submitter presented findings from two LLNA studies (EC3 values of 22% and 68.5%) in mice with purified d-Limonene supporting a classification as a moderate sensitiser in subcategory Skin Sens. 1B. A study with limitations in human volunteers showed no sensitisation reactions towards d-				

limonene (Grief 1967 in EPA 2009).

Further animal studies using d-limonene after prolonged air exposure or oxidized d-limonene are mentioned but not presented in detail. The conclusion thereof is that "air oxidation of d-limone is essential for its sensitising potential".

Thus, positive test reactions towards oxidised limonene, air exposed limonene or limonene hydroperoxides were reported in three studies using human patch test data from dermatitis patients (Christensson 2014, Brared Christensson 2014, Karlberg and Dooms-Goossens 1997). No assessment of these studies in regard to the CLP criteria was performed by the DS. Therefore, it is unclear whether these data fulfil the criteria for Skin Sens. 1A or 1B classification. To evaluate whether based on the human data a more severe classification (i.e. Skin Sens. 1A) would be applicable than based on the animal data (Skin Sens. 1B) please provide a more thorough evaluation of the human data and comparison to the CLP criteria (see Tables 3.2-3.4 of the Guidance on the CLP criteria).

Additionally, to complement the human data base, further studies should be considered for inclusion in the human data section (list not necessarily exhaustive):
Matura et al. (2002) Oxidized citrus oil (R-limonene): A frequent skin sensitizer in Europe, Contact Dermatitis, Vol. 47 (5), pp 709-714.
Matura et al. (2003) Patch testing with oxidized R- (+)- limonene and its hydroperoxide fraction, Contact Dermatitis, Vol. 49 (1), pp 15-21.
Matura et al. (2006) Not only oxidized R-(+)- but also S-(−)-limonene is a common cause of contact allergy in dermatitis patients in Europe, Contact Dermatitis, Vol. 55 (5), pp. 274-279.

Therefore, based on the two valid LLNA studies with purified d-Limonene showing moderate sensitising potential a classification as Skin Sens. 1B appears reasonable.

However, taking into account results with oxidized limonene derivatives in humans may impact this result (evaluation in relation to classification pending). For these reasons the DE CA proposes several ways forward in phrasing the actual Annex VI entry (see general comments above).

Dossier Submitter's Response

Thank you for your comments.
The human data with oxidised d-limonene products is briefly summarised and compared with the criteria as requested.

<u>The human data from the CLH report:</u>

- Limonene-1-hydroperoxide gave most reactions, with 2.4% in 763 dermatitis patients showing positive patch test reactions. Limonene-2-hydroperoxide and oxidized d-limonene (0.5%) gave 1.7% and 1.2% positive patch test reactions, respectively (Christensson et al. 2014).
- 5.2% (range 2.3-12.1%) of 2900 patients had a positive patch test reaction to oxidized d-limonene (Brared Christensson et al. 2014)
- Up to 12.5% of oxidised d-limonene (10 weeks 4h/day and stirred air exposed d-limonene) applied in patches to dermatitis patients gave in 0.9-1.6% reactions in Leuven and 1.9-5.1% reactions in Stockholm (Karlberg and Dooms-Goossens 1997)

- 0.4% of the patients in Leuven reacted to 0.5% d-limonene hydroperoxide while this was 2.4% in Stockholm and 3.2% when applied at a 1% concentration (Stockholm only).

Additional information put forward by Germany:

- Matura et al. (2002, 2003) performed patch testing in 2273 dermatitis patients from 4 clinics in Europe, Stockholm, Leuven, Lisbon and Seville. Different oxidised d-limonene products were applied including 10 weeks air-exposed d-limonene (4h/day, stirred). The frequency of positive patch test reactions to any of the 4 oxidized *R*-(+)-limonene patch test materials in Leuven and Stockholm was similar: 33 of 877 (3.8%) and 13 of 331 (3.9%) patients reacted, respectively. The frequency of test reactions in Lisbon (3/850, 0.3%) and in Seville (14/215, 6.5%) was significantly different. In a second phase of the study, patients who reacted to any of the limonene patch test materials in the first study were recruited for repeat testing. Of the 30 patients tested, 18 (60%) showed positive test reactions to oxidized *R*-(+)-limonene during the second test session. No reactions to pure *R*-(+)-limonene were observed. The oxidized mixture produced more positive reactions (84% of all reactions) than the limonene hydroperoxide fraction (59% of all reactions). Moreover, 41% of all reactions were observed only in response to the limonene mixture, and 16% of them were observed only in response to the hydroperoxide fraction.
- Matura et al. (2006) extended the above investigations to 6 European clinics of dermatology, where the oxidation mixture of both enantiomers of limonene (R and S) were tested in 2411 dermatitis patients. Altogether, 63 out of 2411 patients tested (2.6%) reacted to 1 or both the oxidized limonene preparations. Only 2.3% reacted to the oxidized R-limonene and 2.0% to the oxidized S-limonene. In 57% of the cases, simultaneous reactions were observed to both oxidation mixtures

Comparison with the CLP criteria:

Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT — induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

It is unclear if (a) is met based on the available information since no concentration in this unit is mentioned and calculation is not possible without information on the patch size and applied volume of the substance.
(b) may be fulfilled if 1-4% reactions in dermatitis patients is considered high as a response to 1-12.5% of oxidised d-limonene products (of which the lower end is mostly the most potent hydroperoxide).
In the same way, (c) may be fulfilled.

In the opinion of the DS, the weight of evidence from several human studies indicate that classification for oxidised d-limonene products as Skin Sens. 1A is warranted. However this does not mean d-limonene should be classified in this way since d-limonene itself is not considered allergenic as also mentioned in the papers by Matura et al.
In practice, it is unclear if sufficient oxidised d-limonene can be formed in a product containing some d-limonene that can possibly meet the criteria for Skin Sens. 1A.

Products may also be more stabilised with additives, therefore Note D may be a good addition which states that the label must include non-stabilized if that is the case (see also our response to comment nr 3). The animal data with d-limonene did produce reactions that fall in the criteria for skin sens. 1B. and the reactions are not close to the criteria of Skin sens. 1A which is an EC below 2% while the EC3 found were above 22%. These reactions may also be attributable to a small proportion of formed oxidation products, as d-limonene may indeed not be allergenic at all. It is the opinion of the DS this information should be used to classify the substance d-limonene as a proxy of the product d-limonene with some formation of oxidised d-limonene products. There is no indication the oxidised products will be formed to significant extend in practice that can produce reactions severe enough for Skin sens. 1A. Most human studies were performed with air-oxidised d-limonene after at least 10 weeks of air exposure (4h/day stirred). This is considered unrealistic for most situations. Overall, the DS is of the opinion Skin Sens. 1B is warranted for d-limonene as it likely represents the practical situation most. With respect to phrasing the actual Annex VI entry, please view also our response to comment 3.
RAC's response
Thank you for the proposals for an Annex VI entry and initiation of a more thourough analysis of human data.

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
18.06.2018	Denmark		MemberState	9
Comment received				
The proposed classification Asp. Tox. 1 H304 is warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for comment.				

Date	Country	Organisation	Type of Organisation	Comment number
17.07.2018	Finland		MemberState	10
Comment received				
(R)-p-mentha-1,8-diene; d-limonene is a hydrocarbon with a stereoisomer (S)-p-mentha-1,8-diene, S-(-)-limonene. Kinematic viscosity values for S-(-)-limonene (1.002 mm2/s) and d-limonene (0.9 - 1.1 mm2/s) are indicated in the CLH report. The values are calculated using a conversion between dynamic and kinematic viscosity according to CLP Annex I 3.10.1.6.2. According to the report, the values for dynamic viscosity are obtained from studies conducted similarly to OECD Guideline 114 measured at one temperature (25 oC). Moreover, the CLH report states that higher values for kinematic viscosity are not expected at 40 oC. According to the CLP (Annex I 3.10.2.) a substance is classified for aspiration toxicity in Category 1, if it has kinematic viscosity of 20.5 mm2/s or less at 40 oC. The Finnish CA considers that classification of (R)-p-mentha-1,8-diene; d-limonene in Asp. Tox 1; H304 is justified.				

Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you for comment.

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	Germany		MemberState	11
Comment received				
Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) is supported				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
17.07.2018	Finland		MemberState	12
Comment received				

FI CA supports the conclusion that d-limonene is rapidly degradable and potentially bioaccumulative. FI CA also supports the proposed environmental classification Aquatic Acute 1, H400 with M-factor of 1 but has no definitive conclusion about the proposal to modify long-term hazard classification of d-limonene from Aquatic Chronic 1, H400 to Aquatic Chronic 3, H412.

In the CLH proposal the long-term hazard classification is based on the lowest chronic toxicity value of 0.14 mg/L for algae. However, the only valid chronic toxicity study available for fish is Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages (OECD test guideline 212). In general this is considered as short-term test.

According to the OECD test guideline 212 points 3-4:
"3. This Guideline does not replace Guideline 210 but it would provide useful information in that it could (a) form a bridge between lethal and sublethal tests, (b) be used as a screening test for either a Full Early Life Stage test (Guideline 210) or for chronic toxicity and (c) be used for testing species where husbandry techniques are not sufficiently advanced to cover the period of change from endogenous to exogenous feeding.

4. It should be borne in mind that only tests incorporating all stages of the life-cycle of fish are generally liable to give an accurate estimate of the chronic toxicity of chemicals to fish, and that any reduced exposure with respect to life stages may reduce the sensitivity and thus underestimate the chronic toxicity. It is therefore expected that the embryo and sac-fry test would be less sensitive than the Full Early Life Stage test (Guideline 210), particularly with respect to chemicals with high lipophilicity (log Pow > 4) and chemicals with a specific mode of toxic action. However smaller differences in sensitivity between the two tests would be expected for chemicals with a non-specific, narcotic mode of action".

As stated in the CLH proposal OECD TG 212 is listed as a chronic test in REACH guidance (R.7.8.4.1). According to REACH guidance Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages is considerably shorter and less sensitive than FELS toxicity test (OECD

TG 210) and it offers an alternative method to the FELS toxicity test for substances with log Kow less than 4. In the CLH-dossier the experimentally determined reliable log Kow of 4.38 is reported for d-limonene, which is higher than recommended (log Kow<4) in the test guideline 212 and in the REACH guidance. Thus we propose that applicability of OECD TG 212 as a chronic test for classification purposes of high lipophilicity substance like d-limonene will be discussed further.
Dossier Submitter's Response
Thank you for your comments. The OECD 212 is not mentioned in the CLP guidance but it is named in Annex IX (section 9.1.6.2) of the REACH regulation as one of the test to fulfill the data requirments for long term toxicity testing on fish. The OECD guideline document for 212 states "It is therefore expected that the embryo and sac-fry test would be less sensitive than the Full Early Life Stage test (Guideline 210), particularly with respect to chemicals with high lipophilicity (log Pow > 4)". The OECD TG for 212 does not suggest that it is only for substances with logKow < 4. However it indicates that the endpoint from this test might underestimate the toxicity of substances with a low kow higher than 4. In our choice to use this study for the chronic clssification, we considered that d-limonene does not have a specific toxicity. As no other experimental results for long-term toxicity in fish are avaialble, the alternative is that in the chronic classification the surrogate method is applied for fish. An acute EC50 of 0.695 mg/L is presented for fish in the CLH report. The OECD 212 test under discussion reported a LC50 for survival of 0.41 mg/L. The latter value is lower than the EC50 of 0.702 mg/L (based on the inability of fish to maintain an upright position) selected in the CLH report and would be the key endpoint for acute aquatic toxicity to fish. On the basis of table 4.1.0 and 4.1.3 of the CLP guidance this leads to a classification as Aquatic Chronic 1 with an M-factor of 1. This is much more stringent than a classification on the basis of experimental endpoints for daphnids and algae and would determine the chronic classification.
RAC's response
RAC agrees with the DS comment to use the OECD TG 212 test result in this particular case although realising the limitations of the study. RAC agrees with the DS on the lowest acute toxicity value for fish being 0.41 mg/L.

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	France		MemberState	13
Comment received				
Environmental hazards FR agrees with the classification for environmental hazard and acute M-factor proposed in the CLH report.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON (*R*)-P-MENTHA-1,8-DIENE; D-LIMONENE

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	Belgium		MemberState	14

Comment received

BE CA thanks RIVM for this CLH report.

Based on the reported studies in the CLH report, BE CA supports the proposal of classification for acute toxicity: Acute Tox.1, H400. L(E)C50s for the 3 trophic levels are all < 1 mg/L. The most sensitive species is algae (Pseudokirchniriella subcapitata) with a 72hErC50 of 0.25 mg/L, warranting a M=1.

Concerning the chronic aquatic toxicity BE CA agrees with RIVM that the substance is rapidly degradable and that the criterion for bioaccumulation (based on the valid log Kow) is fulfilled.
However BE CA does not support the proposed classification of Aquatic Chronic 3, but is of the opinion that the substance rather warrant a classification with Aquatic Chronic 2 based on the more conservative NOECgrowth=0.059 mg/L for fish (Pimephales promelas).
The measured concentrations in this key study (OECD 212-FELS) were 0.059, 0.19, 0.37 and 0.67 mg/L.
We agree with RIVM that the NOEC depends strongly on the experimental study design and the number of doses and on the width of the inter-dose interval and that the EC10 values take into account the whole concentration-response curve and are therefore considered more appropriate.
BE CA questions however the appropriateness of the use of the calculated EC10 survival (=0.32 mg/L) based on the observed effects on mortality and effect on growth at a lower concentration.
Therefore we prefer the NOEC growth :
- Up to 0.37 mg/L no significant effect was seen on mortality and thus the NOEC for this endpoint was considered to be 0.37mg/L. 10% effect on mortality was calculated to be 0.32 mg/L, which is thus lower than the NOEC.
- Significant effects on growth were already observed at 0.19 mg/L, which seems thus a more sensitive endpoint than mortality. The available data did not allow the calculation of an EC10growth but it is mentioned that it will be in the range of 0.37 mg/L(<10% effect on growth rate) and 0.67 mg/L (>10% on growth rate).

Dossier Submitter's Response

Thank you for your comments.
The observed effects for mortality and effects at growth at lower concentrations than the EC10 for survival were lower than 10%. In our choice we considered the fact that the EC10s for the latter endpoints will be higher than the EC10 for survival. Therefore the EC10 for survival had our preference over the NOEC.

RAC's response

Noted. RAC agrees to use the EC_{10} for survival as the lowest value and agrees with the DS's explanation.

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Date	Country	Organisation	Type of Organisation	Comment number
20.07.2018	Germany		MemberState	15
Comment received				
We agree with the proposal of classification for environmental hazards as Aquatic acute 1 (H400), Aquatic chronic 3 (H412) and the acute M-factor of 1.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.07.2018	United Kingdom		MemberState	16
Comment received				
The CLH proposal considers d-limonene rapidly degradable on the basis of a non-GLP OECD TG 301 B study (King, 1992) with a Klimish score of 2. We think further information is required to assess the reliability of the study to determine if d-limonene can be considered rapidly degradable for hazard classification. Please can you present study information to support OECD TG 301 and CO2 evolution method validity criteria. In addition we note that while 60.6% degradation was observed on day 10, 58.8% degradation was observed on day 14. Please can you present degradation displayed graphically to determine if the 10-day window was met. QSAR predictions do not fully support d-limonene as rapidly degradable. Although it is unclear if these QSAR are fully valid on the basis of the presented data. It would be useful to present details of model fragments, analogues in the training set and full BIOWIN outputs to consider the QSARs further. If the above data cannot be validated, we feel the case for considering d-limonene as rapidly degradable for hazard classification may be insufficient. Therefore the default position of non-rapidly degradable should apply unless further information is available. Due to the presented log Kow value, the DS considers that d-limonene meets hazard classification bioaccumulation criteria in the absence of experimental BCF data. We are unclear why the acute toxicity to fish endpoint for hazard classification is a geometric mean of an EC50 and LC50 from the same study. While these are for the same test species but we note ECHA guidance* includes the option of a geometric mean when 4 or more data points are available. We note that this does not impact the classification. The CLH presents chronic toxicity to fish endpoints for d-limonene based on an OECD Test Guideline 212 (Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages). According to ECHA Guidance (section R.7.8.4)** this is not a chronic endpoint test and is considered an short-term toxicity endpoint. As an additional chronic toxicity endpoint to fish is not available, the DS should consider the surrogate approach for fish using available acute toxicity data. This would result in Aquatic Chronic 1 (M=1) as d-limonene is considered to meet the bioaccumulation criteria for hazard classification. The CLH briefly mentions that QSARs are available for the chronic toxicity to fish endpoint				

and that a QMRF is available for one of the model endpoints. As these data support Aquatic Chronic 1 (M=1) for a not rapidly degradable substance, it would useful to clarify it the QSARs are reliable.

Please can you confirm that OECD TG 201 study validity criteria were met for the Betat, 2013 study? During the study, test item losses were observed and while endpoints are based on mean measured concentrations it is noted that some treatments were below the limit of detection.

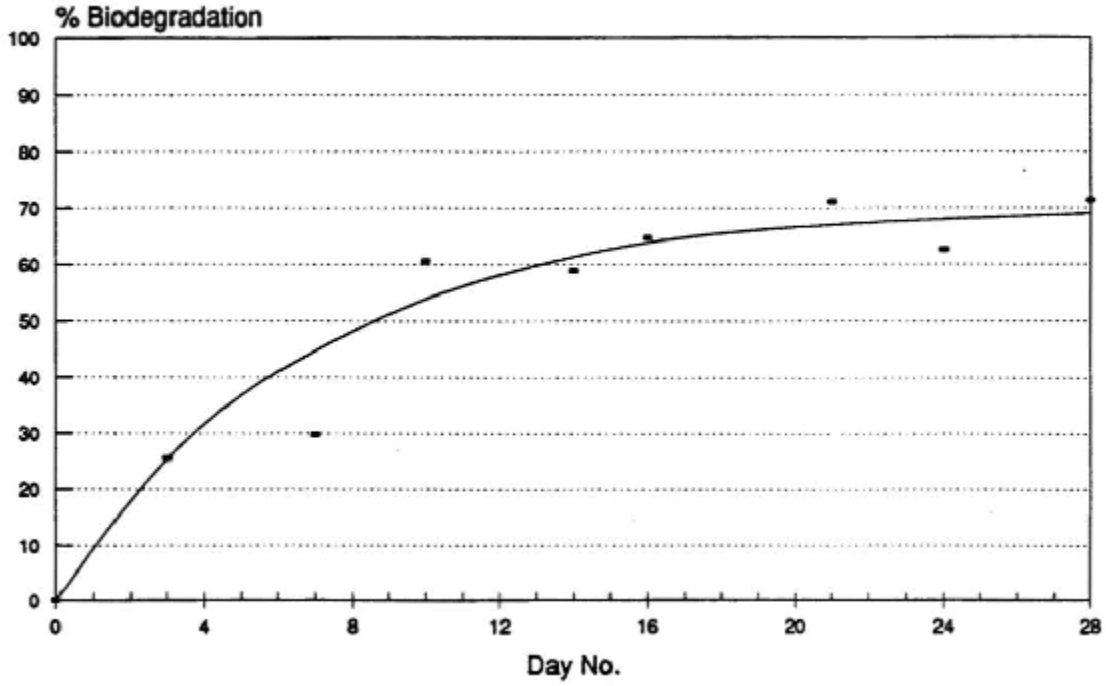
A second algal study using d-limonene (Seiero, 2015) is available with Reliability score 3 and 72 hour endpoints are not considered reliable. This appears to be due to test item losses over the 48-72 hour period. Please can you explain why the 72 hour results are not reliable as endpoints based on half the limit of detection at 72 hours have previously been employed where losses are observed and this approach is recommended in section I.4.1 of ECHA guidance*. It would also be useful to clarify if test guideline validation criteria are met. This is required to consider if the 72-h ErC10 of 0.09 mg/l (mm) is relevant for hazard classification resulting in a more stringent chronic classification. For example Aquatic Chronic 1 (M=1) if d-limonene is considered not rapidly degradable or Aquatic Chronic 2 if d-limonene is considered rapidly degradable.

*ECHA (2017) Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017
**ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance Version 4.0 June 2017

Dossier Submitter's Response

Thank you for your comments.

Biodegradation
The report of the King 1992 study contains limited data on the validity criteria of the test. The test was performed with secondary effluent from an unacclimatised activated sludge plant. Test were performed with a reference compound but details were not provided. Nevertheless, in total 7 different componds were tested showing levels of degradation variing from 2.9 to 85.3%. This indicates that the system had a proper ability of degradation. The degradation curve of d-limonene presented in the report is given below, please not that the 10 day criterium is geneally not based on the fitted curve.



The output of the BioWin 4.10 calculations for d-limonene are given below. The only relevant descriptor for d-limonene is molecular weight. This supports that the experimental data should be given preference over the QSAR data. Futhermore, in the REACH dossier for L-limonene (CAS: 5989-54-8), for biodegradation an OECD 301D study with L-limonene is presented where 85% degradation was established in 28 days with 76% degradation at day 14 (for the OECD 301D study a 14 day window is applied). Athough a full read-across justification is not provided, this support the results of the King study. Therefore, the DS is in the opinion that the substance should be considered as rapidly degradable.

```
SMILES : C(=CCC(C(=C)C)C1)(C1)C
CHEM   : Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-
MOL FOR: C10 H16
MOL WT : 136.24
-------------------------- BIOWIN v4.10 Results -------------------------
-

   Biowin1 (Linear Model Prediction)    :  Biodegrades Fast
   Biowin2 (Non-Linear Model Prediction):  Biodegrades Fast
   Biowin3 (Ultimate Biodegradation Timeframe):  Weeks
   Biowin4 (Primary  Biodegradation Timeframe):  Days-Weeks
   Biowin5 (MITI Linear Model Prediction)    :  Not Readily Degradable
   Biowin6 (MITI Non-Linear Model Prediction):  Not Readily Degradable
   Biowin7 (Anaerobic Model Prediction):  Does Not Biodegrade Fast
   Ready Biodegradability Prediction:  NO

------+-----+-----------------------------------------+---------+--------
-
 TYPE | NUM |       Biowin1 FRAGMENT DESCRIPTION      |  COEFF  |  VALUE
------+-----+-----------------------------------------+---------+--------
-
 MolWt|  *  |  Molecular Weight Parameter             |         | -0.0649
 Const|  *  |  Equation Constant                      |         |  0.7475
```

RESULT	Biowin1 (Linear Biodeg Probability)		0.6827

TYPE	NUM	Biowin2 FRAGMENT DESCRIPTION	COEFF	VALUE
MolWt	*	Molecular Weight Parameter		-1.9346

RESULT	Biowin2 (Non-Linear Biodeg Probability)		0.7454

A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast

TYPE	NUM	Biowin3 FRAGMENT DESCRIPTION	COEFF	VALUE
MolWt	*	Molecular Weight Parameter		-0.3011
Const	*	Equation Constant		3.1992

RESULT	Biowin3 (Survey Model - Ultimate Biodeg)		2.8981

TYPE	NUM	Biowin4 FRAGMENT DESCRIPTION	COEFF	VALUE
MolWt	*	Molecular Weight Parameter		-0.1966
Const	*	Equation Constant		3.8477

RESULT	Biowin4 (Survey Model - Primary Biodeg)		3.6512

Result Classification: 5.00 -> hours 4.00 -> days 3.00 -> weeks
(Primary & Ultimate) 2.00 -> months 1.00 -> longer

TYPE	NUM	Biowin5 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	2	Methyl [-CH3]	0.0004	0.0008
Frag	3	-CH2- [cyclic]	0.0197	0.0592

```
 Frag |  1  |  -CH -  [cyclic]                                |  0.0124 |  0.0124
 Frag |  3  |  -C=CH  [alkenyl hydrogen]                      |  0.0062 |  0.0186
 MolWt|  *  |  Molecular Weight Parameter                     |         | -0.4053
 Const|  *  |  Equation Constant                              |         |  0.7121
============+=================================================+=========+========
=
   RESULT   |  Biowin5 (MITI Linear Biodeg Probability)       |         |  0.3978
============+=================================================+=========+========
=


------+-----+-------------------------------------------+---------+--------
-
 TYPE | NUM |       Biowin6 FRAGMENT DESCRIPTION        |  COEFF  |  VALUE
------+-----+-------------------------------------------+---------+--------
-
 Frag |  2  |  Methyl  [-CH3]                           |  0.0194 |  0.0389
 Frag |  3  |  -CH2-  [cyclic]                          |  0.2365 |  0.7096
 Frag |  1  |  -CH -  [cyclic]                          | -0.1295 | -0.1295
 Frag |  3  |  -C=CH  [alkenyl hydrogen]                |  0.0285 |  0.0855
 MolWt|  *  |  Molecular Weight Parameter               |         | -3.9330
============+=================================================+=========+========
=
   RESULT   |Biowin6 (MITI Non-Linear Biodeg Probability)|         |  0.3312
============+=================================================+=========+========
=

 A Probability Greater Than or Equal to 0.5 indicates --> Readily Degradable
 A Probability Less Than 0.5 indicates --> NOT Readily Degradable


------+-----+-------------------------------------------+---------+--------
-
 TYPE | NUM |       Biowin7 FRAGMENT DESCRIPTION        |  COEFF  |  VALUE
------+-----+-------------------------------------------+---------+--------
-
 Frag |  2  |  Methyl  [-CH3]                           | -0.0796 | -0.1591
 Frag |  3  |  -CH2-  [cyclic]                          | -0.1200 | -0.3600
 Frag |  1  |  -CH -  [cyclic]                          |  0.0395 |  0.0395
 Frag |  3  |  -C=CH  [alkenyl hydrogen]                | -0.0735 | -0.2206
 Const|  *  |  Equation Constant                        |         |  0.8361
============+=================================================+=========+========
=
   RESULT   |   Biowin7 (Anaerobic Linear Biodeg Prob)  |         |  0.1358
============+=================================================+=========+========
=

 A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
 A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast

Ready Biodegradability Prediction: (YES or NO)
----------------------------------------------
 Criteria for the YES or NO prediction:  If the Biowin3 (ultimate survey
 model) result is "weeks" or faster (i.e. "days", "days to weeks", or
 "weeks" AND the Biowin5 (MITI linear model) probability is >= 0.5, then
 the prediction is YES (readily biodegradable).  If this condition is not
 satisfied, the prediction is NO (not readily biodegradable).  This method
 is based on application of Bayesian analysis to ready biodegradation data
```

(see Help). Biowin5 and 6 also predict ready biodegradability, but for degradation in the OECD301C test only; using data from the Chemicals Evaluation and Research Institute Japan (CERIJ) database.

Bioaccumulation
The DS agrees, as indicated in the CLH report, that on the basis of the current data should be concluded that the substance meets the bioacumulation criteria for the classification purposes.

Aquatic toxicity
-Please note that the endpoint selected for acute toxicity is the geometric mean of EC50 values from two different tests presented in one report. Both EC50 values are based on the inability of fish to maintain an upright position.
-Considering the use of the result of the OECD 212 test as a chronic endpoint, we have given our opinion on the use of this endpoint in reply to Comment number 12. There, we indicate that this test potentially underestimated the chronic toxicity of substances with a log Kow higher than 4. The use of the surrogate method as alternative would lead to a classification as chronic 1 with an M-factor of 1.
-The QSAR provided for chronic aquatic toxicity to fish has a domain between log water solubility (in log (mol/L)) of -5.56 to -0.32 and covers the the class of non-polar narcotic compounds. The training set consisted of data for six fish species and 26 chemicals. d-Limonene falls within the domain.
-In the Betat study, only for the lowest concentration the measured endpoint was below the LOD. This concentation was not included in the calculation of the EC50. In the concentrations were the concentration declined below LOD over 72 hours, the geometric mean was calculated with a concentrations of LOD/2 for t=72h. This approach is considered valid for the determination of the endpoints.
-Concerning the Seiero study, as indicated in the CLH report, the reason to consider the 72h endpoint invalid was not because of the calculation using the LODs but because the decline in the concentrations was only observed over the last 24 hours indicating uncetainties with the analysis.

RAC's response

RAC also evaluated the ready biodegradability study. Information needed to assess validity was missing in the study report. RAC, however, sees the test as reliable. Please see the ODD for details. RAC does not support taking geometric mean of the two fish test results available. RAC sees the OECD TG 212 as an acute test but agrees to use it for chronic classification in case of a substance with the narcotic mode of action and a log Kow not so much above 4. The validity criteria in Betat OECD TG 201 was fulfilled according to the study report. Concerning the Seierø study it was informed in the study report that the difference between the 48-hour and 72-hour endpoint values was expected to be due to the significant decrease in detectability of the test item in the period 48-72 hours and not to increased toxicity of the test substance with time. RAC supports the use of 48-hour results from this test.

PUBLIC ATTACHMENTS
1. Comment letter on QRD 460 ECHA.pdf [Please refer to comment No. 2]