CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance 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

Index Number: --

Contact details for dossier submitter: RIVM, The Netherlands

Bureau REACH
National Institute for Public Health and the Environment
Bilthoven, The Netherlands
bureau-reach@rivm.nl

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CONTENTS

Part A.

In addition to the contents of this CLH report, a confidential annex has been made containing the full references of studies using vertebrate animals or human studies that are not publicly accessible via the open literature.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***p-mentha-1,3-diene; alpha-terpinene; 1-isopropyl-4-methylcyclohexa-1,3-diene***
EC number:	***202-795-1***
CAS number:	***99-86-5***
Annex VI Index number:	*--*
Degree of purity:	***> 80%***
Impurities:	***unknown***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Flam. Liq. 3 (H226: Flammable liquid and vapour) Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) Skin Sens. 1A (H317: May cause an allergic skin reaction) Repr. Tox. 2 (H361: suspected of damaging fertility or the unborn child) Aquatic Acute 1 (H400: Very toxic to aquatic life), M=1 Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Flam. Liq. 3 (H226: Flammable liquid and vapour) Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) Skin Sens. 1A (H317: May cause an allergic skin reaction)

	Repr. Tox. 2 (H361: suspected of damaging fertility or the unborn child) Aquatic Acute 1 (H400: Very toxic to aquatic life), M=1 Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives			None	Data lacking
2.2.	Flammable gases			None	Hazard class not applicable
2.3.	Flammable aerosols			None	Data lacking
2.4.	Oxidising gases			None	Hazard class not applicable
2.5.	Gases under pressure			None	Hazard class not applicable
2.6.	Flammable liquids	Flam. Liq 3 (H226)		None	
2.7.	Flammable solids			None	Hazard class not applicable
2.8.	Self-reactive substances and mixtures			None	Data lacking
2.9.	Pyrophoric liquids			None	Data lacking
2.10.	Pyrophoric solids			None	Hazard class not applicable
2.11.	Self-heating substances and mixtures			None	Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases			None	Data lacking
2.13.	Oxidising liquids			None	Data lacking
2.14.	Oxidising solids			None	Hazard class not applicable
2.15.	Organic peroxides			None	Data lacking
2.16.	Substance and mixtures corrosive to metals			None	Data lacking
3.1.	Acute toxicity - oral			None	Inconclusive
	Acute toxicity - dermal			None	Data lacking
	Acute toxicity - inhalation			None	Data lacking
3.2.	Skin corrosion / irritation			None	Data lacking
3.3.	Serious eye damage / eye irritation			None	Data lacking
3.4.	Respiratory sensitisation			None	Data lacking
3.4.	Skin sensitisation	Skin Sens. 1A (H317)		None	
3.5.	Germ cell mutagenicity			None	Inconclusive
3.6.	Carcinogenicity			None	Data lacking
3.7.	Reproductive toxicity	Repr. Tox. 2		None	

		(H361)			
3.8.	Specific target organ toxicity –single exposure			None	Data lacking
3.9.	Specific target organ toxicity – repeated exposure			None	Data lacking
3.10.	Aspiration hazard	Asp. Tox. 1 (H304)		None	
4.1.	Hazardous to the aquatic environment	Aquatic acute 1 (H400) Aquatic Chronic 3 (H412)	M = 1	None	
5.1.	Hazardous to the ozone layer			None	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, conclusive but not sufficient for classification or hazard class not applicable

Labelling:

Signal word: Danger
Hazard statements:
H226: Flammable liquid and vapour.
H317: May cause an allergic skin reaction.
H304: May be fatal if swallowed and enters airways.
H361: Suspected of damaging fertility or the unborn child
H410: Very toxic to aquatic life with long lasting effects.

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

alpha-Terpinene has not previously been assessed for harmonized classification by RAC or TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

alpha-Terpinene is one of the ingredients of the active substance terpenoid blend QRD460. The terpenoid blend, consisting of p-cymene, d-limonene and alpha-terpinene, is accepted as an active substance for plant protection products. However, as the insecticide terpenoid blend QRD-460 is a mixture, harmonised classification is not possible. Therefore, a CLH proposal of the three substances p-cymene, d-limonene and alpha-terpinene will be submitted separately.

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

Data on alpha-terpinene were collected from the DAR of terpenoid blend QRD460, and publically available data through a search using several databases including e-chemportal, PubMed, and ToxNet. Alpha-Terpinene is currently not registered (2017-05-12).
The presence of alpha-terpinene in the Danish QSAR database (http://qsar.food.dtu.dk/) and the annex III inventory (https://echa.europa.eu/information-on-chemicals/annex-iii-inventory) has been checked. No indications that the substance can be classified in additional hazard classes were found.

Flammability
alpha-Terpinene has a flash of point of 47°C. Therefore, classification as Flam. Liq. 3 (H226: Flammable liquid and vapour) according to Annex I, Table 2.6.1 (Label elements for flammable liquids) in Guidance to Regulation (EC) 1272/2008 on CLP is warranted.

Skin sensitisation
alpha-Terpinene has an EC3 of 0.9 % w/v. Therefore classification as Skin Sens. 1A (H317: May cause an allergic skin reaction) according to Table 3.4.3 and 3.4.4 in Guidance to Regulation (EC) 1272/2008 on CLP is warranted.

Aspiration toxicity
alpha-Terpinene has a kinematic viscosity of <7 mm^2/s at 20°C. Therefore, classification as Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) according to Table 3.10.1 (Hazard category for aspiration toxicity) Regulation (EC) 1272/2008 is warranted.

Reproductive toxicity

A significant reduction in pregnant females was observed in the presence of body weight loss in a developmental study. It is unclear whether this is an effect on fertility or development. Therefore Repr. Tox. 2 and H361 without specification is warranted.

Aquatic toxicity
On the basis of read-across data from d-limonene, the substance is considered rapidly biodegradable. Experimental endpoints on acute aquatic toxicity of alpha-terpinene range from 1.5 to 3.2 mg/L for algae and fish, no further experimental endpoints are available for alpha-terpinene. Read-across data from d-limonene gives an acute value for algae of 0.15 mg/L. On the basis of the latter endpoint, classifications as Aquatic Acute 1 (H400) with an M factor of 1 is warranted. For chronic toxicity, no experimental toxicity endpoints are available for alpha-terpinene. Read across was performed with d-limonene. The relevant chronic endpoints for fish, daphnia and algae range from 0.14 to 0.32 mg/L. On the basis of these endpoints, classifications as Aquatic Chronic 3 (H412) is warranted. These classifications are proposed according to Annex I, Table 4.1.0 (Classification categories for hazardous to the aquatic environment) and Table 4.1.3 (Multiplying factors for highly toxic components of mixtures) in Regulation (EC) 1272/2008.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

There is no current harmonised classification and labelling for alpha-terpinene according to Annex VI of CLP regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification		Labelling				
Hazard Class and Category Code	Hazard Statement Code	Hazard Statement Code	Pictograms, Signal Word Code(s)	Number of notifiers	Total number of notifiers	Percent (%)
Flam. Liq. 3		H226		1203	1203	100
Acute Tox. 4	H302	H302		1202	1203	99,9
	H332	H332		78	1203	6,5
Aquatic Chronic 2	H411	H411	GHS07 GHS02 GHS09 GHS08 Dgr	1185	1203	98,5
Asp. Tox. 1		H304		1160	1203	96,4
Eye Irrit. 2	H319	H319		63	1203	5,2
Skin Irrit. 2	H315	H315		63	1203	5,2
STOT SE 3	H335 (lungs)	H335		43	1203	3,6
Skin Sens. 1	H317	H317		22	1203	1,8
Repr. 1B	H360	H360		19	1203	1,6
Repr. 2	H361			18	1203	1,5

d.d. February 5, 2016

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

alpha-Terpinene has currently no harmonized classification according to Annex VI of the CLP-regulation.

alpha-Terpinene is a cyclic monoterpene and is one of the ingredients of the active substance terpenoid blend QRD460. The terpenoid blend, consisting of p-cymene, d-limonene and alpha-terpinene, is accepted as an active substance for plant protection products under Regulation (EC) No. 1107/2009. Due to its anti-oxidant activity, alpha-terpinene causes disruption of respiration resulting in insect death. As QRD-460 is a mixture, harmonised classification of the terpenoid blend is not possible. Therefore, CHL proposals for the ingredients p-cymene, d-limonene and alpha-terpinene will be submitted.

Given that alpha-terpinene is part of an active substance under Regulation (EC) No 1107/2009 (plant protection products), classification at Community Level is necessary. The formal justification is therefore a requirement for harmonised classification by another legislation or process

Part B.

SCIENTIFIC EVALUATION OF THE DATA

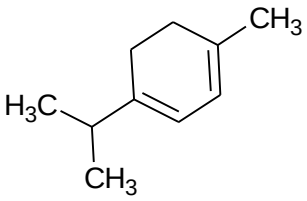
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	202-795-1
EC name:	p-mentha-1,3-diene
Other names:	alpha-terpinene; 1-isopropyl-4-methylcyclohexa-1,3-diene
CAS number (EC inventory):	99-86-5
CAS number:	99-86-5
CAS name:	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)
IUPAC name:	1-methyl-4-propan-2-ylcyclohexa-1,3-diene
CLP Annex VI Index number:	none
Molecular formula:	$C_{10}H_{16}$
Molecular weight range:	136.24

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
alpha-Terpinene	>80%		

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
unknown			

Current Annex VI entry:

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
unknown				

Current Annex VI entry:

1.2.1 Composition of test material

The composition of the test material concerns alpha-terpinene with unknown purity unless otherwise specified in the study summaries.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	colourless to pale yellow, oily liquid	DAR (2013)	
Melting/freezing point	<-20°C	DAR (2013)	
Boiling point	173°C	DAR (2013)	
Relative density	0.840	DAR (2013)	determined at 25°C
Vapour pressure	106.66 Pa	DAR (2013)	at 20°C
Surface tension	not available	DAR (2013)	
Water solubility	5.63 mg/L	DAR (2013)	at 25°C
Partition coefficient n-octanol/water	17782 (log = 4.25)	Griffin et al. (1999)	Determined with HPLC-method as described in the OECD 117 test guideline. Nine compounds (including p-Cymene) of known log Kow (ranging from 1.1 to 4.1) and of similar chemical structure to that of terpenoids were used as standards in the determination of log Kow values. HPLC analysis of samples and standards was carried out with a C18 column and diode array detector. The HPLC method is generally not preferred over experimental determination of log Kow values. However 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. Thus, this study is considered reduced reliable since it is an estimation method. The data are assigned a Klimisch score of 2, and will be used for classification.
	123707 (log = 5.09)	DAR (2013)	Measured: OPPTS 830.7570, OECD 117 Purity not provided Comment DAR: Acceptable. Despite the GLP claim, it is unclear if the testing site has been GLP inspected. Study complies with GLP standards

			therefore no new data required. Dependency on pH is not expected. The Dossier submitter reassessed the original study report: Method used is not OPPTS 830.7570 (= estimation by HPLC). The study was conducted in triplicate by dispersing pure *alpha-terpinene* (purity not reported) in water. Equal volume of *n*-octanol was added, followed by vigorous shaking. The *n*-octanol and water phases were then allowed to separate and were assayed by GC/MS. Therefore, this is a shake-flask study (OPPTS 830.7550; OECD 107). Shortcomings are: temperature, pH and test concentration were not reported. One ratio (1:1 v/v) was tested instead of required three ratios (2:1, 1:1 and 1:2 v/v). Water and *n*-octanol were not pre-saturated. Recovery was not reported. Above all, the shake-flask method can only be used to determine log P_{ow} values in the range -2 to 4. Thus, this study is considered unreliable. The data are assigned a Klimisch score of 3, and will not be used for classification.
Flash point	47°C	DAR (2013)	
Flammability	not available		
Explosive properties	not explosive	DAR (2013)	
Self-ignition temperature	not available		
Oxidising properties	not available		
Granulometry	not applicable		
Stability in organic solvents and identity of relevant degradation products	not available		
Dissociation constant	Not applicable for alpha-terpinene	DAR (2013)	
Kinematic viscosity	<7 mm^2/s	Vigon (2015)	at 20°C
Henry's law constant	2.59 x 10^3 Pa m^3/mol	DAR (2013)	calculated from vapour pressure and water solubility

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

alpha-Terpinene is one of the ingredients of the active substance terpenoid blend QRD460. The terpenoid blend, consisting of p-cymene, d-limonene and alpha-terpinene, is accepted as an active substance for plant protection products. In addition, alpha-terpinene is found in the essential oils of several plants and in public use since the 1950s in fragrances for soap, detergent, creams/lotions and perfume.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point	alpha-Terpinene is highly flammable (flash point 47°C)		DAR (2013)

3.1 Physical and Chemical Properties

3.1.1 Summary and discussion of physical chemical properties

alpha-Terpinene is a flammable substance (flash point 47°C) and is without explosive or oxidising properties.

3.1.2 Comparison with criteria

The CLP-criteria for flammable liquids are:
Category 1: Flash point < 23°C and initial boiling point ≤ 35°C
Category 2: Flash point < 23°C and initial boiling point > 35°C
Category 3: Flash point ≥ 23°C and ≤ 60 °C. (For the purpose of this Regulation gas oils, diesel and light heating oils having a flash point between > 55°C and ≤ 75°C may be regarded as Category 3)

alpha-Terpinene fulfils the criteria for flammability (category 3) according to Annex I: 2.6.2.1 of the CLP Regulation.

3.1.3 Conclusions on classification and labelling

Classification of alpha-terpinene for flammability as Flam. Liq.3 (H226: Flammable liquid and vapour) is required.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Information on the toxicokinetics of alpha-terpinene is general lacking although it is assumed that alpha-terpinene will be absorbed and metabolised in essentially the same manner as d-limonene and p-cymene (DAR 2013). As alpha-terpinene is a lipophilic alicyclic hydrocarbon, it likely crosses biological membranes via passive diffusion. Post absorption, via alpha-terpinene is oxidised into polar oxygenated metabolites via cytochrome P450 enzymes. Subsequently, by cleavage of the double bond and conjugation with glucuronic acid alpha-terpinene is excreted in the urine (WHO 2005). In mice, metabolic activation in the skin resulting in prohaptens that may evoke allergic reactions were observed (Anonymous 2006). However, it should also be noted that the volatility and autoxidation (Anonymous 2012) reduce the availability for dermal absorption.

4.1.2 Human information

No information available.

4.1.3 Summary and discussion on toxicokinetics

Information on the toxicokinetics of alpha-terpinene is limited. See further section 4.1.1.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Rat Oral	LD_{50} 1680 mg/kg bw/day	4 (not assignable) supporting study experimental results	(Anonymous 1973); EFSA (2015); (JECFA 2004); Sigma-Aldrich (2012) (IPCS 2006)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Various MSDS sheets have reported an oral LD_{50} in rats of 1680 mg/kg bw/day. This study is also included in a JECFA evaluation (IPCS 2006), and also in a recent report of EFSA's CEF panel the study is mentioned (EFSA 2015). However, the original study where this LD_{50} was based on (Anonymous 1973) was, though a request was made, not available to the Dossier Submitter.

4.2.1.2 Acute toxicity: inhalation

No relevant information available.

4.2.1.3 Acute toxicity: dermal

No relevant information available.

4.2.1.4 Acute toxicity: other routes

No relevant information available.

4.2.2 Human information

No relevant information available.

4.2.3 Summary and discussion of acute toxicity

Various MSDS sheets have reported an oral LD_{50} in rats of 1680 mg/kg bw/day, but the study where this LD_{50} was based on was, though a request was made, not available to the Dossier Submitter. Therefore, the reliability of the LD_{50} could not be evaluated. No other data are available for the oral route.

For the dermal and inhalation routes, no data on acute toxicity are available.

4.2.4 Comparison with criteria

Although the reported oral LD_{50} of 1680 mg/kg bw/day would indicate that classification for acute toxicity category 4 (i.e. ≤2000 mg/kg bw/day but >300 mg/kg bw/day) would be warranted, no classification is proposed for the oral route because the LD_{50} value could not be verified since the relevant study report was not available to the Dossier Submitter.

4.2.5 Conclusions on classification and labelling

Classification for acute toxicity (oral, dermal, inhalation) is not proposed for alpha-terpinene.

4.3 Specific target organ toxicity – single exposure (STOT SE)

With respect to acute toxicity, only a reported oral LD_{50} value is available (see section 4.2). The study report was not available to the Dossier Submitter and no information is available on the non-lethal effects observed in this study. No relevant information available.

4.3.1 Conclusions on classification and labelling

Classification of alpha-terpinene for STOT SE is not proposed because data is lacking.

4.4 Irritation

4.4.1 Skin irritation

Not considered in this report.

4.4.2 Eye irritation

Not considered in this report

4.4.3 Respiratory tract irritation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.5 Corrosivity

Not considered in this report.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 11: Summary table of relevant skin sensitisation studies

Method	Results			Remarks	Reference
In vivo mouse local lymph node assay (LLNA) Female CBA/Ca mice (9 weeks old) 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)	alpha-terpinene (% w/v) 0 (control) 1 5 10 15 25	^{3}[H]thymidine incorporation (disintegrations per minute (dmp)/lymph node) 755 848 1153 2595 6740 17176	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	Anonymous (2006)
In vivo LLNA Female CBA/Ca mice (around 8 weeks old) 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)	alpha-terpinene (% w/v) (air-exposed for 3 weeks to induce formation of oxidation products) 0 (control) 0.1 1 5 10 25	^{3}[H]thymidine incorporation (dmp/lymph node) 1075 857 3380 14168 18399 13365	Stimulation Index (SI) - 0.8 3.2 13 17 12 EC3 = 0.9% w/v	Klimisch score 2 (reliable with restrictions)	Anonymous (2012)
In vivo LLNA Female CBA/Ca mice (around 8 weeks old) Purity: >95%, purified by column chromatography on silica gel 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)	alpha-terpinene (% w/v) (air-exposed for 7 weeks to induce formation of oxidation products) 0 (control) 1 5 10 15 30	^{3}[H]thymidine incorporation (dmp/lymph node) 1290 3900 13074 16781 10593 12074	Stimulation Index (SI) - 3.0 10 13 8.2 9.4 EC3 = 1.0% w/v	Klimisch score 2 (reliable with restrictions)	Anonymous (2012)

4.6.1.1 Non-human information

In the local lymph node assay (LLNA) performed in CBA/Ca strain mice by Anonymous (2006), groups of mice (four females/dose) were applied with 25 µL of alpha-terpinene at concentrations of 0 (vehicle control), 1, 5, 10, 15, or 25% w/v in acetone/olive oil (4:1 v/v) to the dorsal surface of each ear for three consecutive days. On day 5, all mice were injected i.v. with ^{3}[H]thymidine, and after five hours the draining (auricular) lymph nodes were excised and measured for radioactivity expressed as number of disintegrations per minute per lymph node (dpm/lymph node), see Table 11. The estimated concentration (EC) required to induce a stimulation index (SI) of 3 (EC3) was 8.9% w/v (Table 11).

A similar study was performed by Anonymous (2012), the LLNA was used to determine the sensitization potency of air exposed alpha-terpinene as alpha-terpinene is expected to autoxidize upon air exposure to form allergenic compounds comparable to structural related monoterpene prehaptens (e.g. limonene) (Anonymous 1992, 1994). The substance was placed in an Erlenmeyer flask covered with aluminium foil at room temperature under a daylight lamp (12 hours/day) stirred for 1 hour four times a day for 10 or 24 days. The results of the study of Anonymous (2012) indeed show that alpha-terpinene degrades rapidly to 53% after 10 days and 21% after 24 days. After 66 days alpha-terpinene could not be detected in the oxidation mixture any more. Following chemical analysis, allylic epoxides, *p*-cymene and hydrogen peroxide are the major oxidation products. With this knowledge, groups of mice (three females/dose) received 25 µL three or seven weeks oxidized alpha-terpinene on the dorsum of the ears daily for three consecutive days. Five days after the first treatment mice were injected i.v. with ^{3}[H]thymidine, five hours later the draining auricular lymph nodes were excised and measured for radioactivity and expressed as dmp/lymph node (see Table 11). The following EC3 values were afforded for air exposed alpha-terpinene: three weeks oxidized alpha-terpinene, 0.9% w/v; seven weeks oxidized alpha-terpinene, 1.0% w/v.

Based on a comparison of the EC3-values, compared to the pure compound, autoxidized alpha-terpinene has increased sensitization potency.

4.6.1.2 Human information

Limited information is available on skins sensitisation in humans on alpha-terpinene, though allergic reactions to *Melaleuca alternifolia* oil (tea tree oil, TTO, contains alpha-terpinene (Larson and Jacob 2012)) are frequently reported (for a short review see 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. TTO kept in open and closed bottles or other containers undergoes photo-oxidation within a few days to several months making it hard for consumers to avoid exposure. All eleven patients reacted on alpha-terpinene. Moreover, degradation products of alpha-terpinene were found to be mainly p-cymene, ascaridol, isoascaridol, a ketoperoxide, and colorless crystals that likely were 1,2,4-trihydroxy methane (Anonymous 1999). The sensitizing compounds formed by oxidation are considered responsible for the development of allergic contact dermatitis, emphasizing the potency of autoxidized alpha-terpinene.

4.6.1.3 Summary and discussion of skin sensitisation

Three LLNA studies investigating the sensitizing potential of alpha-terpinene in mice reported EC3 values of 8.9, 0.9 and 1% w/v (alpha-terpinene, three weeks air-exposed oxidized alpha-terpinene, and seven weeks air-exposed oxidized alpha-terpinene, respectively).

Limited information is available on skin sensitisation in humans of alpha-terpinene, though serious allergic reactions were also observed in human (case)studies upon exposure to tea tree oil (containing alpha-terpinene).

Chemical analysis showed that alpha-terpinene degrades rapidly forming oxidation products upon exposure to air (Anonymous 2012). The question is whether the test material as used in the skin sensitisation study of Anonymous (2012)(i.e. air exposed alpha-terpinene) can be considered representative for the compound marketed in the EU. The available experimental data point towards very fast autoxidation of pure alpha-terpinene. Although it can be questioned whether the experimental conditions as applied in the study of Anonymous (2012)for the preparation of oxidized alpha-terpinene fully represent the expected conditions of use and storage of products containing alpha-terpinene in the market, no information is available on the extent of autoxidation upon exposure to air of the commercial product. In addition, it is not known whether autoxidation of alpha-terpinene marketed in the EU is limited by the presence of an additive (anti-oxidant). Therefore, it is assumed that alpha-terpinene marketed in the EU is subject to autoxidation upon exposure to air.
Clearly, autoxidation occurs rapidly in air exposed alpha-terpinene and in UVCB substances containing alpha-terpinene. As autoxidizing increases the sensitization potency *in vivo* and oxidized compounds seem to be responsible for skin sensitisation in humans., the values obtained from studies using air-exposed oxidized alpha-terpinene (Anonymous 2012)will be included in the final evaluation of classification and labelling of alpha-terpinene. In addition, exposure via consumer products to oxidized compounds is likely.

4.6.1.4 Comparison with criteria

Substances shall be classified as skin sensitizers in accordance with the following criteria:

- If there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or
- If there a positive results of an appropriate animal test (LLNA-test: EC3-value ≤2% (category 1A) or EC3-value >2% (category 1B))

Given that the results of the LLNA showed an EC3-value of 0.9% for alpha-terpinene containing autoxidation products, classification as Skin Sens. 1A (H317) is warranted. The data are considered sufficient for sub-categorization, given that also lower concentrations (i.e. below 2%) were tested showing 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 autoxidation products corresponds (according to table 3.4.2-f) to a strong potency for which the generic concentration limit of 0.1% applies. Setting of an SCL is therefore not warranted.

4.6.1.5 Conclusions on classification and labelling

Classification of alpha-terpinene for skin sensitisation as Skin Sens. 1A (H317: May cause an allergic skin reaction) is warranted.

4.6.2 Respiratory sensitisation

Not considered in this report

4.7 Repeated dose toxicity

4.8 Not considered in this reportSpecific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not considered in this report

4.9 Germ cell mutagenicity (Mutagenicity)

Table 12: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Ames test (plate incorporation method) *S. typhimurium* TA97a; TA98; TA100; TA1535 Test concentration: up to 5000 µg/plate (+/- S9-mix) Positive control included	No evidence of mutagenicity for alpha-terpinene (+/- S9-mix)	Klimisch score 2 (reliable with restrictions) Key study	Gomes-Carneiro et al. (2005)

4.9.1 Non-human information

4.9.1.1 In vitro data

No evidence of mutagenicity was observed in Ames assays when alpha-terpinene was incubated with *S. typhimurium* strains TA97a, TA98, TA100, or TA1535 (Gomes-Carneiro et al. 2005).

4.9.1.2 In vivo data

No relevant information available.

4.9.2 Human information

No relevant information available.

4.9.3 Other relevant information

No relevant information available.

4.9.4 Summary and discussion of mutagenicity

No classification for mutagenicity is warranted due to the lack of observed mutagenicity *in vitro* and absence of data *in vivo*.

4.9.5 Comparison with criteria

Considering that no positive response was observed in the available *in vitro* data and due to a lack on *in vivo* data, no classification is required for alpha-terpinene.

4.9.6 Conclusions on classification and labelling

Classification of alpha-terpinene for mutagenicity is not warranted.

4.10 Carcinogenicity

Not considered in this report

4.11 Toxicity for reproduction

Table 13: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Teratogenicity study female Wistar rats Exposure on gestational day (GD) 6-15, purity alpha-terpinene 89% 0 mg/kg bw/day (*n*=24) 30 mg/kg bw/day (*n*=14) 60 mg/kg bw/day (*n*=18) 125 mg/kg bw/day (*n*=25) 250 mg/kg bw/day (*n*=15) Caesarean sections on GD21	Examination foetuses for (visceral) malformations and weight. Maternal no observed adverse effect level (NOAEL) 60 mg/kg bw/day Developmental NOAEL 125 mg/kg bw/day (Dossier Submitter); 30 mg/kg bw/day (study authors),	Klimisch score 2 (reliable with restrictions)	Anonymous (1996)

4.11.1 Effects on fertility

No relevant information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The embryo toxicity of alpha-terpinene is reported in one study (Anonymous 1996) (also reviewed in DAR (2013)). Female Wistar rats were orally dosed (via gavage) with 0, 30, 60, 125 or 250 mg/kg bw/day in corn oil from gestational day (GD) 6-15. On GD21, caesarean sections were done and the number of implantation sites, living/dead foetuses, resorptions and corpora lutea were recorded. Foetuses were examined for extremely visible malformations and weighed, and one-third of the foetuses of each litter were evaluated for visceral anomalies by micro sectioning technique.

As shown in Table 14, no significant difference in pregnancy weight gain between the control and the groups treated with 30 and 60 mg alpha-terpinene/kg bw/day were observed, but reductions during the treatment period (days 6-15) were observed in the two highest doses tested (125 and 250 mg/kg bw/day). A decrease in weight gain during whole pregnancy (days 0-21) was observed with 250 mg alpha-terpinene/kg bw/day. A statistical significant reduction in total pregnancy weight gain minus gravid uterus weight was also found at 125 and 250 mg alpha-

terpinene /kg bw/day (see Table 14). The ratio of pregnant/sperm-positive treated dam did not differ significantly from that of the control group in rats treated with doses up to 125 mg alpha-terpinene/kg bw/day but it was reduced at 250 mg alpha-terpinene/kg bw/day.

Neither the number of corpora lutea graviditatis/dam nor the number of visible implantation sites/litter was altered by alpha-terpinene (Table 15). Also, the number of resorptions/litter and the ratio of resorptions/implantation sites and the number of live foetus/litter were not affected.

A decrease in foetal body weight (Table 15) as well as an increase in the proportion of foetuses showing signs of delayed ossification (Table 16) may indicate that 250 mg alpha-terpinene/kg bw/day retards embryofoetal development. In addition, a dose-dependent increase in the frequency of foetuses showing signs of retarded ossification was noted at doses higher than 30 mg alpha-terpinene/kg bw/day (Table 16).

As shown in Table 17, no noticeable adverse effects were revealed by external examination, except for a higher frequency in kinky tail in the group exposed to 30 mg alpha-terpinene/kg bw/day (which was not observed at higher dose groups).

The reduction in foetal weight as presented in Table 18 is in the highest dose accompanied by a decrease in the absolute weights of heart, liver, lungs and thymus while the kidneys were heavier compared to controls.

A dose-related increase in the number of foetuses showing one or more abnormalities was found with higher doses (see Table 19). The overall increase in the occurrence of skeletal anomalies seem to result from higher incidences of os squamosum irregularly shaped, os supraoccipitale incompletely ossified, shorter ribs, extra cervical ribs, sternum dislocation and os processus deltoid irregularly shaped.

The maternal no observed adverse effect level (NOAEL) is based on the reduced weight gain in the two highest dose levels and was established to be 60 mg/kg bw/day. The developmental NOAEL was reported by Araujo et al. (1996) to be 30 mg/kg bw/day (see further 4.11.4), based on developmental effects which include signs of delayed ossification (poorly ossified and not ossified bones as well as irregular spongy bones), and a higher incidence of minor skeletal alterations at 60 mg/kg bw/day.

Table 14: Maternal weight gain of rats treated orally with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)				
Treatment	0	30	60	125	250
Treated females	28	15	20[b]	26	27
Pregnant females	24	14	18	25	15
Pregnant/sperm positive females (%)	86	93	90	96	56*
Maternal weight (g)					
Day 0	227±20	230±22	229±11	227±18	240±23
Day 21	348±29	347±39	357±23	341±28	324±29*
Gravid uterus weight (g)	71.8±18.1	72.8±23.1	77.0±19.9	76.3±11.0	63.0±18.7
Maternal weight gain (g)					
Days 0-6	27.5±8.2	30.8±8.4	31.1±9.0	29.1±7.8	27.3±7.6
Days 6-11	13.6±5.7	16.9±5.7	11.8±5.8	6.3±7.1*	-17.8±12.9*
Days 6-15	30.7±21.9	35.7±9.4	29.2±6.8	21.0±9.1*	1.4±9.7*
Days 15-21	63.0±11.4	64.5±15.2	67.9±12.0	63.9±17.6	55.1±19.3
Days 0-21	121.2±21.9	131.1±23.2	128.3±17.4	114.1±22.1	83.7±27.1*

Days 0-21 (minus uterus weight)	49.4±15.6	58.3±11.5	51.2±14.4	37.7±19.0*	20.7±13.7*

[a]One pregnant female delivered on day 20.
[b]Percentage of pregnant females was analysed by the chi-square test. All other parameters were analysed by one-way analysis of variance and Student's *t*-test. Values are mean ± SD.
*$p < 0.05$ v. controls.

Table 15: Parameters assessed at caesarean section of rats treated orally with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)				
	0	30	60	125	250
Corpa lutea	12.5±3.0	12.9±2.1	12.6±2.5	12.9±1.8	12.1±2.9
Implantation sites					
Total	306	179	232	327	189
Per litter	12.6±3.2	12.8±3.8	12.9±3.1	13.2±1.9	12.6±2.4
Resorptions					
Total	37	27	15	27	24
Resorptions/implantations (%)	12.1	15.1	6.5	8.2	12.7
Live foetuses					
Total	275	158	218	299	165
Foetuses/implantations (%)	88	85	94	92	87
Per litter	11.5±3.1	11.2±3.9	12.1±3.1	11.9±1.9	11.0±3.3
Foetal weight (g)					
Individual	4.7±0.3	4.8±0.4*	4.8±0.4*	4.7±0.4	4.1±0.5*
Litter	4.7±0.2	4.9±0.3	4.8±0.3	4.7±0.4	4.0±0.4*
Sex ratio (M/F)	139/130	70/82	115/102	160/140	85/80

[a]Proportions were analysed by the chi-square test. All other parameters were analysed by one-way analysis of variance and Student's *t*-test. Values are mean ± SD.
*$p < 0.05$ v. controls.

Table 16: Signs of delayed ossification in foetuses of rats treated with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)				
	0	30	60	125	250
Foetuses examined	189	109	151	207	114
Foetuses with signs of delayed ossification (%)	11.1	14.7	53.0*	73.4*	88.6*
Foetuses (%) with retarded ossification in					
Skull bones	0.5	4.6*	2.6	2.9*	16.6*
Vertebral column	1.6	0.9	22.5*	34.8*	21.0*
Sternum	11.6	5.5*	45.0*	70.0*	87.7*
Ribs	0	0	6.0*	13.5*	6.1*
Forelimbs	1.6	1.8	13.2*	9.2*	9.6*
Hindlimbs	4.8	9.2	37.7*	37.2*	47.4*

Signs of delayed ossification: not ossified (whole bone not stained); poorly ossified (whole bone is poorly ossified); and irregular spongy bones.
[a]Data were analysed by the chi-square test.
*$p < 0.05$ v. controls.

Table 17: Externally visible and visceral anomalies in foetuses of rats treated orally with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)

Treatment	0	30	60	125	250
External examination (no. of foetuses)	275	158	218	299	165
Foetuses with anomalies (%)					
Haematoma	10 (3.6)	7 (4.4)	7 (3.2)	16 (5.3)	13 (7.9)
Tail					
Bent end	1 (0.4)	0	2 (0.9)	6 (2.0)	4 (2.4)
Kinky	3 (1.1)	10 (6.3)*	3 (1.4)	6 (2.0)	5 (3.0)
Pale	0	0	1 (0.4)	0	0
Oedema	1 (0.4)	0	0	0	0
Irregular positioning of forepaws	0	1 (0.6)	0	4 (1.3)	0
Irregular positioning of hindpaws	2 (0.7)	2 (1.3)	1 (0.4)	3 (1.0)	2 (1.2)
Visceral examination (no. of foetuses)	86	49	67	92	51
Foetuses with anomalies (%)					
Spleen (ectopic)	1 (1.2)	0	0	0	0
Heart (smaller)	0	1 (2.0)	0	0	0
Liver (smaller)	1 (1.2)	0	0	0	0
Adrenal gland (smaller)	0	0	1 (1.5)	0	0
Testes (ectopic)	3 (3.5)	0	1 (1.5)	1 (1.1)	0
Ureter (thicker)	0	0	0	0	1 (2.0)

[a]Proportions were analysed by the chi-square test or, alternatively, by Fischer's exact test.
*$p < 0.05$ v. controls.

Table 18: Foetal organ weight in rats treated orally with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)				
Treatment	0	30	60	125	250
Foetuses examined	86	49	67	92	51
Foetal body weight (g)	4.9±0.5	5.3±0.5	5.3±0.4	5.2±0.5	4.2±0.5*
Foetal organ weights (mg)					
Spleen	4.9±1.5	4.0±1.8	4.8±1.6	4.7±1.8	4.7±1.4
Heart	29.1±5.0	29.9±5.0	28.7±4.0	29.2±5.0	26.5±5.0*
Liver	370.0±66.0	372.0±48.0	375.0±39.0	362±80.0	335.0±64.0
Kidneys					
Right	10.8±2.0	11.1±1.7	12.2±1.7*	12.1±2.4*	11.8±2.0*
Left	10.4±2.2	10.4±1.6	11.4±1.7*	11.1±2.0*	12.0±2.0*
Lung	143.0±18.0	139.0±12.0	142.0±14.0	138.0±15.0*	131.0±24.0*
Thymus	7.6±1.1	7.4±1.5	8.0±1.4	7.5±1.6	5.3±1.7*

[a]Data were analysed by one-way analysis of variance and Student's *t*-test. Values are mean ± SD.
*$p < 0.05$ v. controls.

Table 19: Skeletal anomalies in foetuses of rats treated orally with alpha-terpinene on days 6-15 of pregnancy[a]

	alpha-terpinene (mg/kg bw/day)				
	0	30	60	125	250
Foetuses examined	189	109	151	207	114
Foetuses with skeletal anomalies (%)	19.6	27.5	33.1	61.3	89.5
Foetuses (%) showing anomalies in:					
Skull	5.3	8.2	16.5*	34.8*	63.1*
Os basisphenoid Bifurcated	0	0.9	0.7	0	0
Os basoccipitale Irregular	0	0	0	0	1.7

shape					
Os squamosum Irregular shape	4.8	6.4	13.2*	24.6*	35.1*
Os frontale Distance too large	0	0.9	0	0	0.9
Os interparietale Bone hole	0	0	0	0	0.9
Os palatinum Bone hole	0	0	1.3	1.4	0.9
Os parietale Distance too large	0	0.9	0	1.0	0.9
Os suproccipitale					
Discontinuous	0	0	0	0	0.9
Gap	0	0	0	0.5	0.9
Incomplete ossification	0.5	0.9	2.6	12.6*	36.0*
Os tympanicum Discontinuous	0	0	0	0	0.9
Vertebral column	0	0.9	0	0	2.6
Atlas					
Thicker	0	0	0	0	1.7
Cervical vertebra					
Irregular shape	0	0	0	0	0.9
Fused	0	0	0	0	0.9
Thoracic vertebra					
Fused with rib	0	0	0	0	0.9
Two ossification centra	0	0.9	0	0	0.9
Ribs	6.9	10.1	8.6	20.3*	53.5*
Shorter	5.8	6.4	6.0	19.8*	50.0*
Extra					
Cervical	0.5	0.9	1.3	1.0	7.0*
Lumbar	0.5	2.7	1.3	1.0	0.9
Sternum	5.8	5.5	3.3	8.2	11.4*
Dislocated	5.8	5.5	3.3	8.2	11.4*
Forelimbs	2.6	5.5	6.6	17.9*	6.1
Irregular position	0.5	0.9	0	2.9	0
Os processus deltoid					
Bone hole	1.6	0	1.3	1.9	3.5
Irregular shape	0.5	4.6	6.6	14.5	2.6

[a]Data were analysed by the chi-square test.
*$p < 0.05$ v. controls.

4.11.2.2 Human information

No relevant information available.

4.11.3 Other relevant information

No relevant information available.

4.11.4 Summary and discussion of reproductive toxicity

A significant reduction of the ratio of pregnant/sperm-positive females was observed at 250 mg/kg bw/day (Table 14). The absence of a reduction in implantations per pregnant female shows that this is caused by whole litter loss. This effect was observed in the presence of clear maternal toxicity namely body weight loss day 6-11, reduced body weight gain day 6-15 and day 0-21 and reduced body weight gain minus uterus weight. According to Anonymous (1996), the observation that only

whole litter loss was observed and the overt maternal toxicity suggests that the whole litter peri-implantation loss are maternally mediated.

For a few areas of the foetal skeleton there was some indication of a dose-related effect on ossification (Table 16). However, the number of ossification centres affected in relation to the number examined was very small, even at the highest dose level tested indicating that the effect of treatment on the developing foetus was minimal (see Table 19).

For the 250 mg/kg bw/day group, the reduction in mean foetal weight (Table 15) would also contribute to an alteration in the rate of ossification. However, there is no reduction in mean foetal weight at 125 mg/kg bw/day. The publication lacks key information which could be used to better understand the reasons for the apparent increase in occurrence of these altered areas of ossification. It is possible that a few litters of lower weight foetuses are contributing to the elevated incidences, but unfortunately no litter based information is reported. In addition, there is no information provided for the range of normal variation in frequency of occurrence in control foetuses (i.e. historical control data). Despite the omission of these aids to understanding, it can be argued that there is an effect of treatment on foetal ossification at 125 and 250 mg/kg bw/day but given the number of ossification centres affected this effect is minimal and also, is transient in nature. In the absence of an effect of 125 mg/kg bw/day on foetal weight it could be reasonably argued that the changes in ossification are too minimal to be considered indicative of developmental toxicity per se, i.e., they are of no toxicological significance. However, additional data is needed to confirm the (absence of) observed effects.

For the 60 mg/kg bw/day group, only one area of the foetal skeleton is seen to be less well ossified in comparison with the controls. Although apparently dose-related, in isolation this single finding should not be considered to represent developmental toxicity due to 60 mg/kg bw/day. It is reasonable to describe this dose as a NOAEL for developmental toxicity. However, again, the publication lacks information for better understanding the altered/delayed ossification.

In the results and discussion of the study, the effect on embryofoetal development is incorrectly described as adverse, the changes seen do not have an effect on the foetus, they only represent an alteration in the timing of ossification, a transient process in itself, and affect only very few of the many ossification centres. Furthermore, 60 mg/kg bw/day should not be considered as an effect level for developmental toxicity on the basis of the appearance of one ossification centre only.

Although the study lacks some key information, clear maternal and developmental toxicity is observed. Contrary to the conclusions of Anonymous (1996)(NOAEL for developmental toxicity of 30 mg/kg bw/day) the conclusion from this study should probably be that 60 mg/kg bw/day is a NOAEL for maternal toxicity and that 125 mg/kg bw/day is a NOAEL for developmental toxicity in the rat. Nevertheless, the study shows some evidence for developmental toxicity.

It is noted that the DAR of terpenoid blend presents an OECD TG414 rat oral developmental toxicity study. The test compound in this study is a mixture containing 38.8% alpha-terpinene, in addition to 11.5% d-limonene and 15.5% p-cymene. In this OECD 414 rat developmental toxicity study, a reduction of the ratio of pregnant/sperm-positive females (such as noticed in the Anonymous (1996)-study with alpha-terpinene) was however not observed at dose levels up to and including the highest dose tested (i.e. 240 mg/kg bw/d of the terpenoid blend, corresponding to 93.1 mg/kg bw/d of alpha-terpinene). It is noted that this dose level of 93.1 mg alpha-terpinene/kg bw/d is a factor 2.4 below the effective dose level of 250 mg alpha-terpinene/kg bw/d (or, 223 mg alpha-terpinene/kg bw/d when corrected for 89% purity) as applied in the study of Anonymous (1996). It is considered that the positive results of the study of Anonymous (1996)are not conflicting with the negative results ofthe OECD TG414 study mentioned in the DAR.

4.11.5 Comparison with criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- Category 1A: Known human reproductive toxicant. The classification of a substance in this Category 1A is largely based on evidence from humans.
- Category 1B: Presumed human reproductive toxicant. The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate

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

Developmental and fertility effects:

In the available rat developmental toxicity study, a significant reduction of the ratio of pregnant/ sperm-positive females was observed at 250 mg/kg bw/day. The absence of a reduction in implantations per pregnant female suggests that this is probably caused by whole litter loss. This effect was observed in the presence of clear maternal toxicity namely body weight loss day 6-11 (7%), reduced body weight gain day 6-15 and day 0-21 and reduced body weight gain minus uterus weight. It is unclear whether the decrease in pregnant females is secondary to the maternal toxicity. However, a feed restriction study in rats showed that a body weight loss of approximately 10% from day 6-9 and 5% from day 9-12 does not result in a reduction in pregnant females (Anonymous 2005). However, as there are no repeated dose toxicity studies which could indicate more specific effects, it cannot be excluded that alpha-terpinene also induces other maternal effects that were not determined in this developmental study. Therefore, it cannot be excluded that the observed maternal reproductive effects are secondary to general maternal toxicity.

In conclusion, given that 1) the observed effects (i.e. a reduction in the ratio of pregnant/sperm-positive females) can be considered relevant to human, and 2) the effects on reproduction were observed together with other general effects, the adverse effects on reproduction is considered not

to be a secondary non-specific consequence of the other toxic effects, it is considered that there is some evidence of adverse effects on the reproduction. This effect warrants classification in category 2. As it is unclear whether this effect is on the ability to get pregnant, on implantation or on development, it is unclear whether H361f or H361d would be appropriate. Therefore, H361 without further specification of the effect is proposed.

In addition, effects related to retarded ossification are observed upon exposure to alpha-terpinene. This was considered toxicologically relevant at the high dose of 250 mg/kg bw/day at which also reduced foetal body weight was observed. These effects points towards a retarded embryofoetal development. However, these effects were observed in the presence of maternal toxicity. Maternal toxicity included reduced bw gain. It is considered likely that retarded ossification and reduced foetal bw are related to the observed maternal toxicity. Therefore, these effects are considered to be a secondary non-specific consequence of the maternal toxicity. The observed effects on ossification are considered not relevant for classification.

4.11.6 Conclusions on classification and labelling

Classification of alpha-terpinene for reproductive toxicity in category 2 with H361 (Suspected of damaging fertility or the unborn child) is required.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No relevant information available.

4.12.1.2 Immunotoxicity

No relevant information available.

4.12.1.3 Specific investigations: other studies

alpha-Terpinene has a kinematic viscosity of <7 mm^2/s at 20°C (see section 1.3, Table 8), which might indicate the potential for aspiration toxicity.

4.12.1.4 Human information

No relevant information available.

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

Aspiration Toxicity Hazard Category 1 (Asp. Tox. 1) (H304: May be fatal if swallowed and enters airways) is warranted for liquid substances and preparations because of their low viscosity. Low viscosity leads to flow and low surface tension leads to spread of a liquid through the respiratory tract. Aspiration toxicity hazard category 1 (Asp. Tox. 1) is warranted if the substance is a hydrocarbon and has a kinematic viscosity ν of 20.5 mm^2/s or less, measured at 40°C (Regulation (EC) No 1272/2008, section 3.10.2). Given that alpha-terpinene is a hydrocarbon and has a kinematic viscosity of <7 mm^2/s (at 20°C), classification of alpha-terpinene for category 1 Asp. Tox (H304) is warranted.

4.12.4 Conclusions on classification and labelling

Classification of alpha-terpinene for aspiration toxicity as Asp. Tox 1 (H304: May be fatal if swallowed and enters airways) is required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of Terpeniod blend which contains alpha-terpinene were assessed in the Draft Assessment Report, addenda and Proposed Decision of the Netherlands prepared in the context of the possible approval Terpenoid blend QRD 460 under Reg. (EC) 1107/2009. The DAR is publicly available via the EFSA web site (http://dar.efsa.europa.eu/dar-web/provision).

Where available endpoints for alpha-terpinene are taken over from the DAR, however since the DAR is for the Terpenoid blend containing more substances, only little data on alpha-terpinene as single compound is available in the DAR. Additional data is searched for in public literature and databases. Endpoints from databases were only used for classification purposes when original test reports could be assessed for their reliability. When available QSARs have been used to complement the dataset and for aquatic toxicity and biodegradation a read-across with the substance d-limonene is performed. A justification for this read-across is given in Section 5.1. When reliable experimental endpoints are available, QSAR endpoints are only used for informational purposes.

5.1 Rational / Justification Read-across

For alpha-terpinene, acute toxicity data are available for fish and daphnia and no chronic toxicity data are available. Available experimental data on biodegradation only considers evaporation rather than biodegradation. Classification and labelling dossier for d-limonene is also being prepared and data is available. alpha-Terpinene and d-limonene are structurally similar showing trends in physical chemical properties, fate and transport and ecotoxicity toxicological properties. For classification purposes, we propose to use data from d-limonene and read-across to alpha-terpinene for the following endpoints: acute algae, chronic toxicity and biodegradation.

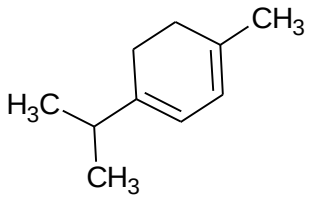
5.1.1 Physical-chemical properties

alpha-Terpinene is a liquid with a melting point of < -20°C, a boiling point of 173°C and a vapour pressure of 107 Pa. The octanol-water partition coefficient (log Kow) is 4.25 and the water solubility is 5.63 mg/L. D-limonene is a liquid with a melting point of -73.65°C, a boiling point range of 175-178 °C and a vapour pressure of 200 Pa. The octanol-water partition coefficient (log Kow) is 4.38 and the water solubility is 12.3 mg/L.

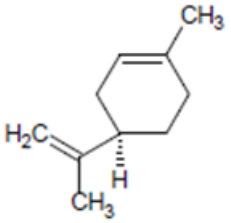
5.1.2 Structure

alpha-Terpinene and d-limonene belong to a class of monoterpenes that consist of two isoprene units and have the molecular formula $C_{10}H_{16}$. Monoterpenes maybe linear (acyclic) or contain rings. Biochemical modifications such as oxidation or rearrangement produce the related monoterpenoids. The most common ring size in monoterpenes is a six-membered ring.

Both substances are six-membered monocyclic monoterpenes with molecular formula $C_{10}H_{16}$. They differ in the position of the carbon-carbon double bonds. For alpha-terpinene, two carbon-carbon double bonds are positioned in a six membered ring. For d-limonene, one carbon-carbon double bond is positioned in a six membered ring and the other double bond is situated at the alkene –end of the structure.



alpha-Terpinene



d-Limonene

5.1.3 Similar behaviour in the environment

alpha-Terpinene and d-limonene do not contain any functional groups that are susceptible to hydrolysis under environmental conditions. Therefore, hydrolysis of the substances are not expected in aquatic environments. The substances are considered to be highly volatile and will dissipate from water rapidly. Based on the Henry's law constant, alpha-terpinene and d-limonene are expected to partition from water and soil to air. Rapid escape (fugacity via volatility) appears to be the predominant cause of dissipation for both, alpha-terpinene and d-limonene, in natural waters. For both substances, no degradation products in water were detected. Soil degradation studies show that the fate of alpha-terpinene and d-limonene in soil is of limited relevance as it volatilises and evaporates rapidly into the air compartment.

5.1.4 Biodegradation

Experimental data on biodegradation of alpha-terpinene is not available. Biodegradation estimations as presented in Section 5.2.2.1 performed with the BIOWIN v4.10 QSAR are comparable to those of d-limonene: fast degradation according to the BIOWIN 1 and BIOWIN 2 biodegradation models; ultimate biodegradation according to BIOWIN 3 within weeks; initial steps of biodegradation (BIOWIN 4) within days to weeks; BIOWIN 5 and 6 considered d-limonene to be not readily biodegradable; and no quick biodegradation under anaerobic conditions (BIOWIN 7). Experimental data on biodegradation for d-limonene is available (King 1992). D-Limonene is considered rapidly biodegradable, after 28 days biodegradation was 71.4%. The biodegradation measured data will be used in the read-across approach. Based on the close structural similarity for both substances, common biodegradation properties and pathways are expected.

5.1.5 Ecotoxicity

alpha-Terpinene and d-limonene are considered to act by narcosis as their sole mode of toxic action in aquatic organisms (ECOSAR v1.11). Acute experimental effect concentration (EC50s) for alpha-terpinene for fish and invertebrates are 1.48 and 1.85 mg/L respectively (see Table 29). For d-limonene these values are 0.42 mg/L (geometric mean of four endpoints) and 0.70 mg/L for fish and invertebrates respectively (Table 30). This shows a difference in the acute ecotoxicity between the

two substances of at most a factor 4.4. This suggests that d-limonene is acutely lightly more toxic to aquatic organisms than alpha-terpinene but this difference is limited and as such the read-across of ecotoxicity data from d-limonene for the classification of alpha-terpinene would represent a reasonable worst-case for ecotoxicity. This observation is also supported by the QSAR results presented in Table 29 and Table 31 below.

5.1.6 Conclusion

Ecotoxicity data for d-limonene are deemed reliable and read-across from d-limonene (acute algae and chronic fish, invertebrates and algae data) for classification of alpha-terpinene is a justifiable realistic worst-case scenario. Available aquatic toxicity data for both substances are comparable, which is also supported by QSAR results. For biodegradation, the available measured data for d-limonene will be used in the read-across approach. Similar physico-chemical properties and comparable fate in the environment are an additional argument.

5.2 Degradation

Table 20: Summary of relevant information on degradation of alpha-terpinene

Method	Results	Remarks	Reference
Hydrolysis	alpha-terpinene does not contain any functional groups that are susceptible to hydrolysis.	Statement in the DAR	DAR (2013)
Half-life in air	QSAR estimations: hydroxyl radicals: 29.1 min. ozone: 1.7 min.	Reaction with nitrate radicals is also reported as "may be important" AOPWIN in EPI Suite 4.11	US-EPA (2012)
Ready biodegradability	not readily biodegradable	BIOWIN in EPI Suite 4.11	US-EPA (2012)

Table 21: Summary of relevant information on degradation of d-limonene

Method	Results	Remarks	Reference
Hydrolysis	d-limonene does not contain any functional groups that are susceptible to hydrolysis under environmental conditions.	Statement in the DAR	DAR (2013)
Half-life in air	QSAR estimations: hydroxyl radicals: 53 min. ozone: 37.3 min. nitrate radicals: 0.9-9 min.	AOPWIN in EPI Suite 4.11	US-EPA (2012)
Ready biodegradability	readily biodegradable	71.4% over 28 days	King (1992); Author not disseminated (2010)

5.2.1 Stability

No experimental data is available. Alpha-terpinene is not expected to undergo hydrolysis since it lacks functional groups that hydrolyse under environmental conditions (DAR 2013). However, the Henry's law constant is determined to be 2.59 x 10^{-3} Pa m^3/mol and from this and level III fugacity modelling, alpha-terpinene is expected to partition from water and soil to air. In air it will be degraded rapidly (the DT100 was determined to be 20.8 hours) by interaction with hydroxyl and nitrate radicals (see section 5.2.3) (DAR 2013). alpha-Terpinene is not expected to be affected by photolytic degradation (DAR 2013).

5.2.2 Biodegradation

5.2.2.1 Biodegradation estimation

The BIOWIN v4.10 QSAR contained within EPI Suite™ version 4.11 consists of six models. alpha-Terpinene is predicted to biodegrade fast using linear (BIOWIN 1) and non-linear (BIOWIN 2) biodegradation models. Ultimate biodegradation, i.e., conversion of alpha-terpinene to carbon dioxide (BIOWIN 3), is predicted to occur within weeks while initial steps of biodegradation (BIOWIN 4) are predicted to occur within days to weeks. In two of the models, BIOWIN 5 and 6, representing MITI testing, alpha-terpinene was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. alpha-Terpinene is not predicted to biodegrade quickly under anaerobic conditions (BIOWIN 7) (DAR 2013). Thus, even though BIOWIN 3 estimates ultimate biodegradation within “weeks”, as BIOWIN 5 indicates that alpha-terpinene will not be readily biodegradable, the overall conclusion is that alpha-terpinene is estimated not to be readily biodegradable.

5.2.2.2 Screening tests

No information available for alpha-terpinene.

Read across with analogue d-limonene
For the dossier of d-limonene, a study on ready biodegradability was submitted by the registrant. Additionally, 4 studies were obtained from the public registration information on the ECHA dissemination website (https://echa.europa.eu/web/guest/information-on-chemicals/ registered-substances; date of access 27-9-2016). Information on these studies is provided below.

Reference	:	King (1992)	study type		OECD 310
year of execution	:	1992	incubation time	:	28 days
GLP statement	:	No	nominal concentration	:	10 mg/L
Guideline	:	OECD 301B with adaptations	Temperature	:	20-23°C
test substance	:	*d-limonene*	Degradability	:	71.4% based on CO_2
Purity	:	95%	Metabolites		not reported
test system	:	sealed vessel	Acceptability	:	**acceptable with restrictions**

The study was performed according to OECD guideline 301B with adaptations for volatile substances (sealed vessel). The test method as adapted is in line with the latest adopted OECD test guideline 310 (Ready Biodegradability - CO2 in sealed vessels (Headspace Test)). The study tested

the degradation of d-limonene with a purity of 95% by an inoculum from an unacclimated sludge plant in a sealed vessel. The test was performed at 20-23°C and the pH of the medium was adjusted to 6.5. Samples were taken at day 3, 7, 10, 14, 16, 21, 24 and 28 when the concentration of inorganic CO_2 is determined in the headspace and medium with an inorganic carbon analyser. The amount of inorganic carbon was related to that produced in a control to determine the extent of degradation. Details on the control are not reported, neither are details on a reference substance given. The percentage of degradation based on the CO_2 development is given in the table below.

Table 22: Degradation of d-limonene

Day	Percentage biodegradation of d-limonene
3	25.5
7	29.8
10	60.6
14	58.8
16	64.7
21	71.1
24	62.6
28	71.4

After 28 days the biodegradation was 71.4% (95% confidence interval 68.3 - 74.5%). On this basis, it is concluded that d-limonene is readily biodegradable fulfilling the 10d window criterion because after 10 days, 60.6% degradation was achieved. The study can be considered reliable with restriction as for example details on the controls are not provided. The data are assigned a Klimisch score of 2, and are used for classification purposes.

In addition to the study above, data were obtained from the public registration information on the ECHA dissemination website (https://echa.europa.eu/web/guest/information-on-chemicals/ registered-substances; date of access 27-9-2016). Four studies were available on this website, these are discussed below.

ECHA dissemination site key study (Author not disseminated 2010)
The key study in the REACH dossier is an OECD guideline 301D Closed Bottle test performed with non-adapted activated sludge from a domestic sewage treatment plant from 2010. The biodegradation was assessed by the determination of the oxygen consumption. After 28 days the O_2 consumption was 5.3 mg/L related to a reduction in concentration of 80%. For the reference compound the O_2 consumption related to 82% was 4.5 mg/L and this was achieved at day 14. Details on the biodegradation are given in Table 23.

Table 23: Oxygen consumption (mg/L) and the percentages biodegradation of the test substance, dipentene (BOD/ThOD) and sodium acetate (BOD/ThOD) in the Closed Bottle test.

Time (days)	Oxygen consumption (mg/L)		Biodegradation (%)	
	Test substance	Acetate	Test substance	Acetate
0	0.0	0.0	0	0
7	2.7	4.1	41	76
14	4.7	4.5	71	83
21	5.0		76	
28	5.3		80	

Although the test seems reliable, the details on the ECHA website give insufficient details on the substance actually tested. Under the heading test material no information is given, only in the heading of a result table and in the applicant summary was it mentioned that actually dipentene was tested. According to the database on the ECHA website, three different reaction masses are registered under the name dipentene (EC numbers: 205-341-0; 907-808-0 and 939-009-8). Although all three different mixtures contain d-limonene the actual content of the d-limonene is not specified. The dossier submitter was informed by the registrant that the dipentene tested consisted of 48.4% d-limonene; 20.6% β-phellandrene; 9.8% α-terpinene; 5.8% γ-terpinene and 4.5% terpinolene (personal communication, September 2016). A rationale could be given that these structures have a structural resemblance and will be similarly biodegradable but taken the complexity of the mixture consisting of five different components, the actual extent of the biodegradation of d-limonene is not known. Therefore this study is only used as supporting information on the biodegradability of d-limonene.

ECHA dissemination site supporting study "002" (Author not disseminated 1980)
In the REACH dossier, the reliability of this study from 1980 was reported as "not assignable". Indeed the information in the REACH dossier was too limited to assess this study for the purpose of the current report and the results will not be used for classification purposes.

ECHA dissemination site supporting study "003" (Author not disseminated 1997)
In the REACH dossier this study is described as a ready biodegradation study soil-slurry biodegradation assay with an inoculum originating from a forest soil. The inoculum was described as 20% (w/v) soil-slurry. Volumetric biodegradation rate and soil-normalised biodegradation rate were determined to be 0.38 mg/L/h and 1.9 µg/g/h. The details in the REACH dossier are too limited to actually assess the reliability of the study. For example, the test protocol is poorly described and a test guideline is not mentioned. Additionally, the registrant has indicated that this study is not in line with the standard test methods for ready biodegradability (personal communication, September 2016). Given the above mentioned, the results of this study are not used in this report for classification purposes.

ECHA dissemination site supporting study "004" (Author not disseminated 1996)
In the REACH dossier this study is described as a ready biodegradation study with enriched cultures from a forest soil. A degradation rate for cultures unadapted to the test material was reported of 0.044 mg/L/h with a lag period of 180 hours. The details in the REACH dossier are too limited to actually assess the reliability of the study. For example, the test protocol is poorly described and a test guideline is not mentioned. Additionally, the registrant has indicated that this study is not in line with the standard test methods for ready biodegradability (personal communication, September 2016). Given the above mentioned, the results of this study are not used in this report for classification purposes.

5.2.2.3 Simulation tests

In the DAR two studies have been assessed that have addressed the fate and behaviour of Terpenoid Blend QRD 460 by testing the three terpene constituents, i.e. alpha-terpinene, *p*-cymene and d-limonene, individually in separate test vessels. The relevant sections of the DAR summaries that report on **alpha-terpinene** as a single compound are provided below.

Aquatic simulation study *DAR reference STUDY IIA, 7.8.3/001*

reference	:	Moser (2011)	study type	:	non-standard study with natural lake water similar to OECD 309
year of execution	:	2011	incubation time	:	48 hours
GLP statement	:	Yes	nominal concentration	:	1 mg/L
guideline	:	None	Temperature	:	18.1-21°C
test substance	:	d-limonene, *p*-cymene, alpha-terpinene	DT50	:	4.1 hours (for alpha-terpinene)
purity	:	92.6% (alpha-terpinene; lot # 812097)	Metabolites		not detected
test system	:	Filtered (0.45µ) lake water	Acceptability	:	**acceptable**

This study is not a water sediment study, rather a study in natural waters that is similar to OECD 309. Degradation of alpha-terpinene, *p*-cymene and d-limonene, QRD 460, was studied in natural lake water (Lake Constance, Horn, CH, see details below). **The test substances were tested individually** to provide information on the degradability and the formation of degradation products of each compound, if possible. Test vessels (20mL borosilicate glass tubes with Teflon-lined screw cap) were covered with aluminium foil to exclude light and incubated at 20 ± 2 ºC. The test was performed in a flow-through system with air slowly passing. Stock solutions of the three test items were filled into test vessels equipped with traps containing iso-octane to collect volatile test item or possible degradation products. Samples for alpha-terpinene were taken at application and after 1, 3, 6, 24 and 48 hours and analysed immediately. Their respective trapping solutions were also analysed.

Application solutions were prepared with a concentration of 1.01 mg a.i./L for alpha-terpinene (0.946 mg a.i./L for d-limonene and 0.993 mg a.i./L for *p*-cymene). The test substances were tested individually by adding 20 ml of test solution to a test vessel.

Duplicate samples were analysed at each test interval. The entire water sample was extracted with n-hexane containing an internal standard. The n-hexane phase was then analysed by GC-FID. The trapping solution was analysed by GC-FID without any further treatment. Method validation revealed mean recoveries for alpha-terpinene of 56.7% (low concentration) and 54.1% (10x concentration), respectively. Recovery of the three terpenes was low which is attributed to the high volatility. The repeatability of the test was good and high accuracy and precision were achieved.

The purity of the supplied test items was also tested using analytical standards.

A GC-MS method was applied for further characterisation to identify possible degradation products.

The disappearance time DT_{50} and DT_{90} was calculated using the GC-FID results and are based on the percentage a.i. found at t=0 h. Calculation were performed using SFO kinetics using FOCUS kinetics spreadsheet for 2 replicates. The RSS was minimized by adjusting M0 and k values.

Only the results for alpha-terpinene are shown and discussed below.

The purity of alpha-terpinene was determined to be 94.6%, which is slightly higher from the value reported with the test item.

Water Quality*:* Different batches of lake water were analysed. Characterisation of the lake water at the time of sampling yielded the following: pH of 7.86-8.28; dissolved oxygen of 6.73-9.13 mg O_2/L; TOC of 2.25-9.17 mg C/L; conductivity of 275-300 µS/cm; hardness of 142-164 mg $CaCO_3$/L; and alkalinity of 105-128 mg $CaCO_3$/L.

Test results: For alpha-terpinene the extracted concentration at t=0 was 0.472 and 0.465 mg a.i./L resp., which correspond to a recovery of 46.9 and 46.3% of the initial concentration of 1.01 mg a.i./L, similar to recoveries in the method validation. The concentration alpha-terpinene in the extracts decreased continuously to below the LOQ of 0.0657 mg a.i./L. alpha-Terpinene was not found in the trapping solution above the LOQ of 0.197 mg a.i./L. Detailed results for alpha-terpinene are given in Table 24.

GC-MS measurements of representative samples did not result in detection of degradation products of the test items.

Table 24: Concentration of alpha-terpinene in extracts and trapping solutions (DAR 2013)

Time hour	Concentration in the extract [mg a.i./L	Mean recovery [%]	Concentration test item used for DT_{50} [mg/L][a]	Concentration in trapping solutions [mg a.i./L]
0	0.472		0.491	-
0	0.465	81.7	0.485	-
1	0.246		0.256	<LOQ
1	0.27	45.8	0.282	<LOQ
3	0.231		0.241	<LOQ
3	0.226	44.4	0.235	<LOQ
6	0.209		0.217	<LOQ
6	0.182	40.1	0.189	<LOQ
24	<LOQ		<LOQ	<LOQ
24	<LOQ	24.0	<LOQ	<LOQ
48	<LOQ		<LOQ	<LOQ
48	<LOQ	0	<LOQ	<LOQ

[a] The recovered concentration was calculated using the mg a.i./L divided by the purity of the test item, which was 92.6% for alpha-terpinene

Note: <LOQ was defined to be 0 for further calculations

LOQ Limit of Quantification. Determined as 0.0657 mg a.i./L in extract and 0.197 mg a.i./L in trapping solution

Degradation rate: In Figure 1, the results of the kinetic fit using the FOCUS Kinetics spreadsheet are presented.

Figure 1: SFO degradation plot and error level Chi^2 test of alpha-terpinene (DAR 2013)

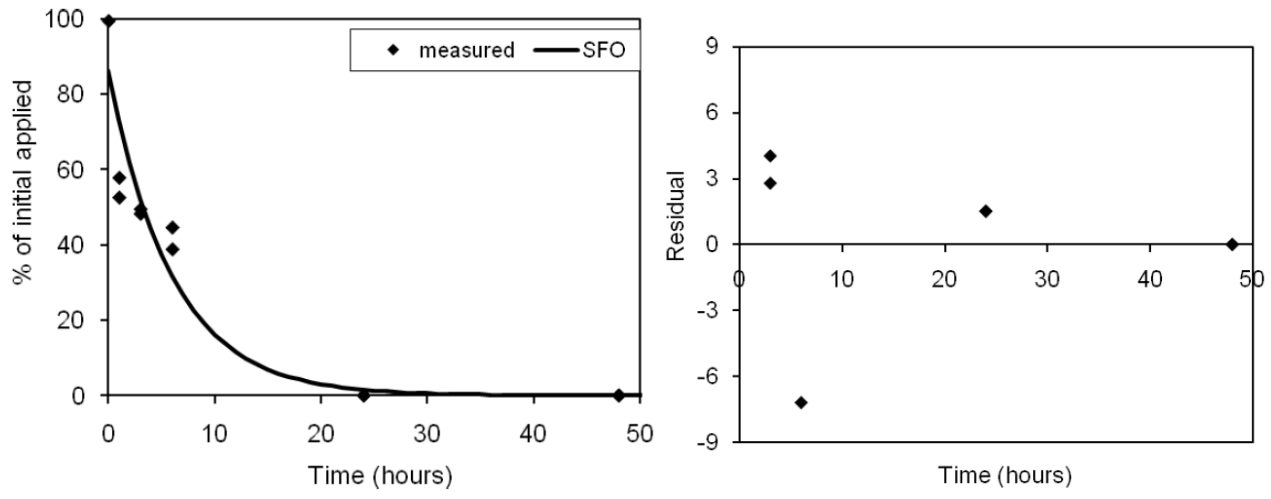


Table 25: Summary of DT50 and DT90 values, SFO parameters and chi2 test (DAR 2013)

	DT_{50} [hours]	**DT_{90} [hours]**	**M0 (fitted)**	**K (fitted)**	**Error level Chi^2 test**
alpha-terpinene	4.1	13.7	86.39	0.168	19.8

Conclusion: **alpha-terpinene volatilized from the natural water test systems rapidly with a DT50 of 4.1 and DT90s of 13.7 hours**. The trapping solution did not show the presence of the test substance or any degradates. Degradates in the water were also not detected. Thus, **rapid escape (fugacity via volatility) appears to be the predominant pathway for alpha-terpinene in natural water**.

RMS comments: The study was performed with non-radio labelled test material and therefore, no mass balance can be given. No metabolisation products were detected by GC-MS analyses, neither in the extracts of the aquatic systems not in the trapping solutions. The author arguments the test items volatilised from the water, however, only the test with *p*-cymene showed an increase in concentration of the a.i. in the trapping solution. The chi2 error level of the SFO fit to the data for alpha-terpinene is a little bit above 15, however, the visual fit of the data is good. The distribution of residuals is less optimal again. No t-test was performed.

Degradation in soil *DAR reference STUDY IIA, 7.2.1/01*

Reference	:	Moser (2010)	study type	:	aerobic soil degradation according to OECD 307
year of execution	:	2010	incubation time	:	up to 4 d
GLP statement	:	Yes	nominal concentration	:	-
guideline	:	OECD 307 (2002)	temperature	:	20°C
test substance	:	d-limonene, *p*-cymene, alpha-terpinene	DT50	:	< 24 h
purity	:	92.6% (alpha-terpinene; lot # 812097).	metabolites	:	not applicable
soils	:	Sandy loam	acceptability	:	**acceptable**

The **aerobic soil degradation** of **alpha-terpinene**, *p*-cymene and d-limonene was studied in one representative sandy loam soil. The test soil was field collected in Sevelen (Switzerland), sieved (2 mm) and stored refrigerated until 5 days before use and then acclimatised to test temperature. Test vessels (500 ml) containing 100 g (dry weight) soil were pre-incubated under aerobic conditions for four days prior to application. **The three test substances were applied individually** to achieve final nominal concentrations of approximately 1.82 mg/kg alpha-terpinene, 0.68 mg/kg *p*-cymene and 0.55 mg/kg d-limonene, this reflects the relative proportion of each terpene in the active substance QRD 460. A continuous flow-through test system was used at a temperature of 20 ± 2ºC in the dark at 50% of MWHC. Aerobic conditions were maintained by continuously bubbling moistened air through the water layer. Each replicate was equipped with a trap containing iso-octane as trapping solution to collect volatile test item or possible degradation products. Samples were analysed after 0 and 7 hours, and 1, 2 and 3 days after application. The trap of the respective sample was analysed too.

Duplicate samples for each test item were analysed at each sampling interval. The soil was extracted with acetonitrile. The acetonitrile fraction was further extracted by liquid/liquid extraction with hexane. The hexane was concentrated and then analysed by GC. The trapping solution was analysed by GC without any further treatment. The analytical method was subject to validation as part of the study. The LOQ was 0.4 mg a.i./kg soil for alpha-terpinene.

Only the results for alpha-terpinene are shown and discussed below.

Table 26: Concentration of alpha-terpinene, in soil extracts and trapping solutions.

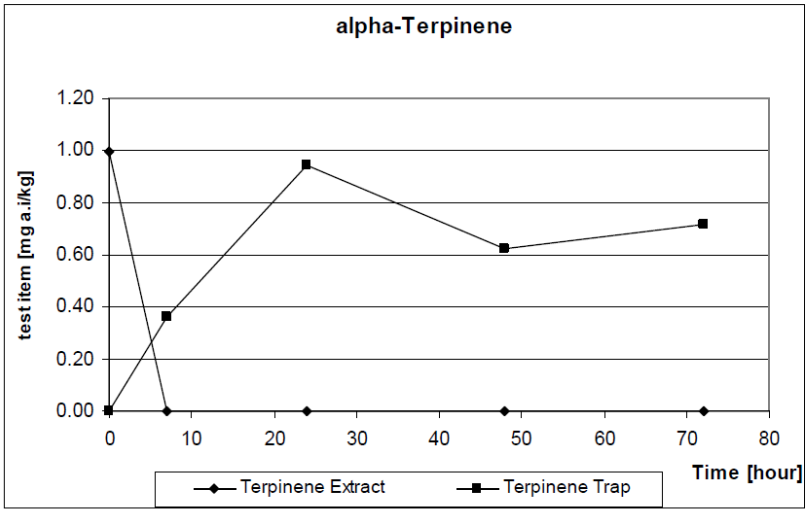
Sample	**Sample time [hours]**	**Concentration [mg a.i./kg]**	**Metabolite (p-Cymene) [mg/kg]**
Soil extract	0	0.79 1.20	0.055 0.06
	7	0.03[a] 0.05[a]	0.09[a] 0.04[a]
	12	n.d. <LOQ	n.d. <LOQ
	24	n.d. n.d.	n.d. n.d.
	36	n.d. n.d.	n.d. n.d.
		Concentration [mg a.i./L]	**mg/L**
Trap	7	<LOQ[b] 0.58	0 0.1
	12	0.79 1.09	0.09 0.16
	24	0.77 0.47	0.1 0
	36	0.65 0.78	0.07 0.15

n.d. not detectable
LOQ = 0.4 mg a.i./kg
[a] The values are lower than the defined LOQ (0.4mg a.i./kg). Within the validation, the lower concentration of 0.04mg a.i./kg could not be confirmed to be repeatable. Therefore, if used, these values may be uncertain.
[b] LOQ = 0.192 mg a.i./L. (concentration of lowest analytical standard)

The soil extract at T0, i.e. directly after application showed less alpha-terpinene than originally applied. In total, 0.79 and 1.20 mg a.i./kg were found in the soil extracts. A minor amount of p-cymene was detected as degradation product. The level of p-cymene did not exceed 0.06 mg a.i./kg in both replicates of T0. Seven hours after application, the level of alpha-terpinene in soil extract was already below the LOQ of 0.4 mg a.i./kg. The level on day 1 was again <LOQ. From day 2 onwards, no residue was detectable.

For all three test items levels of volatile test item and/or degradation products increased from 7 hours to one day after application. Thereafter amounts decreased. **The test item and their degradation products disappeared from the soil into the trapping solution. Due to the continuous aeration, the test items were pushed out of the trapping solution with ongoing time**. The study was performed with **non-radio labelled test material**. Therefore, no mass balance can be given.

Figure 2: Distribution of alpha-terpinene in soil extract and trapping solution (DAR 2013)



It was concluded that alpha-terpinene disappears rapidly from the soil into the trapping solution by evaporation. The DT_{50} was calculated to be <24 hours. The DT_{90} which was actually also the DT_{100} was <48 hours.

RMS comment: This study confirms the assumptions made based on the physical chemical properties of the terpenoid blend QRD 460 and the fugacity models conclusions that the fate of the terpenoid blend (alpha-terpinene, p-cymene and d-limonene) QRD 460 in soil is of limited relevance as it volatilises and evaporates rapidly into the air compartment. **No kinetics of degradation could be calculated as the substances dissipated within 24 hours**.

5.2.3 Summary and discussion of degradation

alpha-Terpinene is expected be hydrolytically stable and unlikely to be affected by photolytic degradation.

The available water and soil degradation studies with alpha-terpinene show rapid DT50 values. The aquatic simulation study was not a water sediment study, rather a study in natural water. The water was continuously aerated, and the non-radiolabelled alpha-terpinene was not detected in the trapping solution. No degradation products were detected. The DT50 was calculated to be 4.1 hours but the disappearance was considered to be caused by evaporation rather than biodegradation. The aerobic soil simulation study also used non-radiolabelled alpha-terpinene, and evaporation to the trapping solution was shown as the predominant disappearance route. Therefore, these studies cannot be used to assess the biodegradability of alpha-terpinene.

The BIOWIN QSAR predicts that alpha-terpinene is not readily biodegradable. However, when all BIOWIN models are taken into account it appears that the predictions are unequivocal, i.e. BIOWIN 1, 2, 3 and 4 indicate rapid biodegradation, while BIOWIN 5, 6 and 7 predict that alpha-terpinene will not biodegrade rapidly.

The study of King (King 1992) provided by the registrant has shown that d-limonene is readily biodegradable, after 28 days biodegradation was 71.4%. This is supported by the results from the key study in the REACH dossier for d-limonene. In this case, the experimental data are preferred over the calculated QSAR data.

Considering the available read-across data for d-limonene, alpha-terpinene is considered to be rapidly degradable for the purpose of classification.

5.3 Environmental distribution

5.3.1 Adsorption/Desorption

No experimental studies on the sorption behaviour of alpha-terpinene in soil are available. In the DAR a Koc value of 4877 L/kg, calculated with KOCWIN in EPIsuite, is used in PEC calculations. The height of this value indicates that it should sorb relatively strong to soil and sediment.

5.3.2 Volatilisation

alpha-Terpinene has a vapour pressure of 107 Pa at 20 °C and the Henry's law constant was estimated to be 2.6 x 10^3 Pa x m^3/mol. The substance is considered to be highly volatile and will dissipate from water rapidly.

5.3.3 Distribution modelling

Fugacity model output as presented in the DAR is given in the table below.

Table 27: Fugacity model outputs for alpha-terpinene presented in the DAR (DAR 2013)

Compartment	Mass Amount (%)	Half Life (hours)	Reaction (%)	Advection (%)
Air	0.0211	0.00311	97.6	0.00438
Water	9.06	360	0.362	0.188
Soil	90.6	720	1.81	0
Sediment	0.353	3240	0.00157	0.000147

In the DAR, it is stated that the main environmental compartment receiving alpha-terpinene was air which also degraded alpha-terpinene much, much faster than the soil, sediment and water compartments. It was also remarked that the environmental compartment distribution in Level III is based on reaching steady state conditions and not equilibrium in a closed system. Therefore, alpha-terpinene entering the air will quickly degrade. Thus at steady state, very little alpha-terpinene will be in the air because degradation in air is so rapid. Full degradation in the total system was predicted to be 20.8 hours.

5.4 Aquatic Bioaccumulation

Table 28: Summary of relevant information on aquatic bioaccumulation of alpha-terpinene

Method	Results	Remarks	Reference

QSAR BCFBAF v3.01	296 L/kg	regression based method, log Kow = 4.25	US-EPA (2012)
QSAR BCFBAF v3.01	625 L/kg	Arnot-Gobas method, log Kow = 4.25	US-EPA (2012)

5.4.1 Aquatic bioaccumulation

In the DAR, an experimentally determined log K_{ow} of 5.09 is reported but this value is considered unreliable by the dossier submitter. Therefore preference is given to the value of 4.25 (Table 8). It is also stated in the DAR, that in general for terpenes (including alpha-terpinene) because of their high volatility and low water solubility, the residence time of terpenes in water is too low for accumulation by fish or other aquatic organisms. Also no accumulation in soil is expected since the dissipation time in soil is lower than 24 hours. Furthermore, it is stated that naturally occurring substances like terpenes will not have a propensity to bioaccumulate or bioconcentrate in aquatic organisms. These arguments could be used as supporting information but according to the guidance, in absence of experimentally determined bioconcentration data, conclusions on bioaccumulation should be based on the experimentally determined log Kow.

5.4.1.1 Bioaccumulation estimation

QSAR calculations can be performed with BCFBAF v3.01 in EPI Suite (US-EPA 2012). On the basis of the log Kow of 4.25, BCFs of 296 L/kg and 625 L/kg are estimated with the regression based method and Arnot-Gobas method respectively.

5.4.1.2 Measured bioaccumulation data

No information is available on test for bioaccumulation. Also for alpha-terpinene no information is available on metabolism. In the DAR, it is however estimated that in mammals alpha-terpinene like the other substances in terpenoid blend is readily metabolised to materials which are rapidly excreted within 48 hours.

5.4.2 Summary and discussion of aquatic bioaccumulation

According to the guidance (section 4.1.3.2.3.3), the log Kow of 4.25 being higher than 4, indicate that the substance has a high potential for bioaccumulation. This conclusion is supported by the estimated BCF of 625 L/kg that is higher than 500 L/kg.

5.5 Aquatic toxicity

Because of the limited data set on aquatic toxicity of alpha-terpinene, a read-across with the source substance d-limonene has been performed. The justification for this read-across is given in Section 5.1. Read across is only performed when data are missing nevertheless all ecotoxicity data for d-limonene are presented in the following sections because they are supportive information for the read-across rational. The read-across key studies will be indicated in the Tables below and in the text.

Table 29: Summary of information on aquatic toxicity for alpha-terpinene

Method	Results (mg/L)	Remarks	Reference
Experimental endpoints			
Fish			
Short-term fish toxicity according to ASTM E729 method; GLP not reported.	96 h LC50 = 3.15 96 h EC50 = 1.48	*Pimephales promelas* purity 87%; flow-through based on mean measured concentrations Ri=2	
Invertebrates			
Short-term invertebrate toxicity according to ASTM E729 method; GLP not reported.	48 h LC50 = 1.85 48 h EC50 = 1.85	*Daphnia magna* purity 87%; flow-through based on mean measured concentrations Ri=2	Anonymous (1990b)
Short-term invertebrate toxicity method and GLP not reported.	EC50 = 8.45	*Daphnia magna* exposure concentrations exceed water solubility; based on nominal concentrations Ri=3	Park et al. (2011)
Algae/Aquatic plants			
Aquatic toxicity study to algae Method and GLP not reported.	24 h NOEC <0.2 24 h NOEC <2.66	*Pseudokichneriella subcapitata* Ri =3	

Method	Results (mg/L)	Remarks	Reference
QSAR calculated endpoints			
QSAR - fish toxicity	96 h LC50 = 1.07 30 d NOEC = 0.094	ECOSAR v1.11 neutral organics based on log Kow of 4.25	US-EPA (2012)
QSAR - invertebrate toxicity	96 h LC50 = 0.22 (mysid) 48 h LC50 = 0.75 (daphnid) 16 d NOEC = 0.092 (daphnid)	ECOSAR v1.11 neutral organics based on log Kow of 4.25	US-EPA (2012)
QSAR - algae toxicity	LC50 = 1.31 NOEC = 0.39	ECOSAR v1.11 neutral organics based on log Kow of 4.25	US-EPA (2012)

Table 30: Summary of experimental endpoints on aquatic toxicity from the read across analogue, d-limonene

Method	Results (mg/L)	Remarks	Reference
Experimental endpoints			
<u>Fish</u>			
Short-term fish toxicity according to ASTM E729 method; GLP not reported.	96 h LC50 = 0.702 (test 1) 96 h EC50 = 0. 702 (test 1) 96 h LC50 = 0.720 (test 2) 96 h EC50 = 0.688 (test 2)	*Pimephales promelas* purity 99%; flow-through geometric mean of the endpoints from two tests based on mean measured concentrations endpoint not used for read across to alpha-terpinene Ri=2	
Chronic toxicity to fish according to OECD test guideline 212 GLP reported	NOEC growth = 0.059 (EC10 between 0.37 and 0.67 mg/L) NOEC hatching = 0.37 NOEC behaviour = 0.19 EC10 survival = 0.32 NOEC survival = 0.37	*Pimephales promelas* endpoints based on mean measured concentration; EC10 for growth could not be statistically determined. Ri=2 **Key study used for read-across to alpha-terpinene**	

Method	Results (mg/L)	Remarks	Reference
Invertebrates			
Short-term invertebrate toxicity according to OECD test guidance 202 GLP reported	48 h EC50 = 0.307 (mobility)	*Daphnia magna* endpoint based on mean measured concentration endpoint not used for read across to alpha-terpinene Ri=1	Betat (2013b)
Short-term invertebrate toxicity according to OECD test guidance 202	48 h EC50 = 0.456 (mobility)	*Daphnia magna* endpoint based on mean measured concentration endpoint not used for read across to alpha-terpinene Ri=1	Delpit (2014)
Short-term invertebrate toxicity according to OECD test guidance 202	48 h EC50 = 0.51 (mobility)	*Daphnia magna* endpoint based on mean measured concentration endpoint not used for read across to alpha-terpinene Ri=1	Bjørnestad (2013)
Short-term invertebrate toxicity method and GLP not reported.	EC50 = 7.85	*Daphnia magna* exposure concentrations exceed water solubility; based on nominal concentrations Ri=3	Park et al. (2011)
Short-term invertebrate toxicity according to ASTM E729 method; GLP not reported.	48 h LC50 = 0.924 (mortality) 48 h LC50 = 0.577 (mortality) 48 h EC50 = 0.42 (mobility)	*Daphnia magna* purity 87%; flow-through based on mean measured concentrations; the LC50 is the geometric mean of the endpoints from two tests endpoint not used for read across to alpha-terpinene Ri=2	
Short-term invertebrate toxicity method and GLP not reported	48 h EC50 = 69.6	*Daphnia magna* endpoint based on nominal concentrations, Ri=3	May Passino and Smith (1987)

Method	Results (mg/L)	Remarks	Reference
Short-term invertebrate toxicity according to OECD test guidance 202, GLP reported	48 h EC50 = 0.36	*Daphnia magna* endpoint based on nominal concentrations, Ri=3	Author not disseminated (2007)
Chronic invertebrate toxicity according to OECD test guideline 211	21 day EC10 = 0.153	*Daphnia magna* renewal test, endpoint based on mean measured concentration Ri=1 **Key study used for read-across to alpha-terpinene**	Kamper (2016a, 2016b)
Algae/Aquatic Plants			
Aquatic toxicity to algae according to OECD guideline 201	72 h ErC50 = 0.32 72 h ErC10 = 0.174	*P. subcapitata* endpoint based on mean measured concentration Ri=2 **Study used for read-across to alpha-terpinene**	Betat (2013a)
Aquatic toxicity to algae according to OECD guideline 201	48 h ErC50 = 0.25 48 h ErC10 = 0.14 72 h ErC50 = 0.15 72 h ErC10 = 0.09	*P. subcapitata* endpoint based on mean measured concentration Endpoints for 48 h: Ri=2 Endpoints for 72 hours: Ri=3 **Study used for read-across to alpha-terpinene**	Seierø (2015)
Aquatic toxicity study to algae Method and GLP not reported.	24 h NOEC <0.05 24 h NOEC <1.5	*Pseudokichneriella subcapitata* Ri =3	Anonymous (1990b); LMC ASIS (2014)

Table 31: Summary of QSAR calculated endpoints on aquatic toxicity from d-limonene. This is information is provided for informational and supportive purposes.

Method	Results (mg/L)	Remarks	Reference
QSAR calculated endpoints			
QSAR - fish toxicity	96 h LC50 = 0.459 28 day NOEC = 0.080	iSafeRat® Holistic HA-QSAR and iSafeRat® HA-QSAR for chronic aquatic toxicity	KREATiS (2015a) KREATiS (2015b)
	96 h LC50 = 0.845 30 day NOEC = 0.073	ECOSAR v1.11 neutral organics based on log Kow of 4.38	US-EPA (2012)
QSAR - invertebrate toxicity	48 h EC50 = 0.62 21 day NOEC = 0.05	iSafeRat® Holistic HA-QSAR and iSafeRat® HA-QSAR for chronic aquatic toxicity	KREATiS (2015c) KREATiS (2015e)
	96 h LC50 = 0.154 (mysid) 48 h LC50 = 0.577 (daphnid) 16 d NOEC = 0.074 (daphnid)	ECOSAR v1.11 neutral organics based on log Kow of 4.38	US-EPA (2012)
QSAR - algae toxicity	72 h EC50 = 0.50	iSafeRat® Holistic HA-QSAR	KREATiS (2015d)
	LC50 = 1.07 NOEC = 0.32	ECOSAR v1.11 neutral organics based on log Kow of 4.38	US-EPA (2012)

5.5.1 Fish

5.5.1.1 Short-term toxicity to fish

Experimental EC50 values for *Pimephales promelas* are available in the OECD toolbox (LMC ASIS 2014) and PAN database (Kegley et al. 2014). These values range from 1.48 to 3.15 mg/L. Considering the high volatility of the substance the original test reports should be assessed in order to ensure that the toxicity endpoints are based on the actual exposure concentrations. Only one of these test reports could be retrieved. This study, from Anonymous (1990b), is a very thorough study where care is taken that the actual exposure concentrations were determined. It is a 96 h flow-through study with 30-34 days old juvenile *Pimephales promelas* with a wet weight of 49 to 177 mg and length of 15.4 to 21.8 mm. 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 are based on the average test concentrations of alpha-terpinene ranging from 1.05 to 4.82 mg/L. The reported LC50 and EC50 are 3.15 mg/L and 1.48 mg/L respectively. For the EC50 should be noted that it is based on the geometric mean of the NOEC and LOEC since at the LOEC 100% effect was observed. Nevertheless, these endpoints can be considered as reliable (Klimisch score: Ri=2) and will be used for classification purposes.

The analysis of test media showed the presence of additional substances (two hydrolysis peaks at 28% and 44% and an unknown at 6%), not being the parent compound. The authors of the study concluded these substances to be hydrolysis products. We do not agree with this conclusion, as alpha-terpinene does not hydrolyse (see section 5.1, Table 14). For the hydrolysis of an alkene to occur, strong acidic conditions are required which is not the case here (Chemgapedia 2016). In our view, the additional substances may be either oxidation or hydration products. The molar mass of formed compounds was indicating an incorporation of a water molecule in the compound (the weight of the product is 18 mass units higher). This reaction only occurs directly after addition to water since the ratios between the parent and products is the same between stock and test solutions. In either case, formed metabolites are expected to be more polar than the parent compound, having lower toxicity (See section 7.1). The test concentrations of alpha-terpinene and hydration products are expressed as alpha-terpinene. This is the only aquatic study that reports the presence additional components other than the parent substance after exposure to water.

QSAR based (neutral organics) LC50 values for fish could be generated with ECOSAR v1.11 available in EPIsuite. Based on the log Kow value of 4.25, LC50 values of 1.07 and 1.36 were estimated for fresh and saltwater fish respectively. These estimations are in the same order of magnitude as the experimental values. Taking into account that the experimental values are based on the actual measured exposure concentrations, these will be considered for classification purposes.

This section on the analogue substance is provided for informational purposes

Data on acute toxicity to fish are available for alpha-terpinene, the data presented under this heading will therefore not be used for classification purposes but are only presented support of the read-across and for informational purposes. Experimental EC50 values for *Pimephales promelas* are available in the OECD toolbox (LMC ASIS 2014) and PAN database (Kegley et al. 2014). These values range from 0.2 to 35 mg/L. Considering the high volatility of the substance the original test reports should be assessed in order to ensure that the toxicity endpoints are based on the actual exposure concentrations. Most of these test reports cited in these databases could be retrieved (Anonymous 1990a, 1990b, 1997).

The study, from Anonymous (1990b) is a very thorough study where care is taken that the actual exposure concentrations were determined. It is a 96 h flow-through study with 30-34 days old juvenile *Pimephales promelas* with a wet weight of 49 to 177 mg and length of 15.4 to 21.8 mm. 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 was performed in two tests with d-limonene from two different sources. The test concentrations were analysed every 24 hours and the toxicity endpoints are based on the average test concentrations of d-limonene ranging from 0.18 to 1.11 mg/L for test 1 and 0.25 to 1.89 for test two. The reported LC50 and EC50 for 96 hours of exposure are both 0.702 mg/L for test 1 and respectively 0.720 and 0.688 mg/L for test 2. These endpoints can be considered as reliable and will be used for classification purposes.

The analysis of test media showed the presence of additional substances (8–11%), not being the parent compound. The authors of the study concluded these substances to be hydrolysis products. We do not agree with hydrolysis conclusion, as d-limonene does not hydrolyse (see Table 14 in section 5.1). For the hydrolysis of an alkene to occur, strong acidic conditions are required which is not the case here (Chemgapedia 2016). The additional substances may be either oxidation or hydration products. The molar mass of formed compounds was indicating an incorporation of a water molecule in the compound (the weight of the product is 18 mass units higher). This reaction only occurs directly after addition to water since the ratios between the parent and products is the same between stock and test solutions. In either case, formed metabolites are expected to be more polar than the parent compound, having lower toxicity (See Annex 7.2). The test concentrations of d-limonene and hydration products are expressed as d-limonene. This is the only aquatic study that reports the presence of additional components other than the parent substance after exposure to water.

The study of Anonymous (1990a) is the same as the first test from Anonymous (1990b) and in the publication of Anonymous (1997) no data on d-limonene could be found. Therefore these references are not further discussed in this report. One more acute study with fish is mentioned in the OECD toolbox, the original reference of this could not be retrieved but in the toolbox was also mentioned that the exposure concentrations were not measured and therefore could already be concluded that the endpoint would not be reliable for the purpose of classification. In the public literature, other references (e.g.) are available where d-limonene is tested as component in a commercial product. Since in the tests with these products the effects of other components cannot be excluded, these studies are also not taken into account.

QSAR generated information

In addition to the studies above, the registrant has submitted a QSAR generated endpoint (KREATiS 2015a) for the dossier of d-limonene. This endpoint was calculated with the iSafeRat® Holistic HA-QSAR and was supplemented with a QMRF document. This QSAR resulted in a 96 h LC50 of 0.459 mg/L with confidence limit of 0.40 - 0.52 mg/L. In addition, the dossier submitter calculated QSAR based (neutral organics) LC50 values for fish with ECOSAR v1.11 available in EPIsuite. Based on the log Kow value of 4.38, LC50 values of 0.845 and 1.041 mg/L were estimated for fresh and saltwater fish respectively. These estimations are in the same order of magnitude as the experimental values.

5.5.1.2 Long-term toxicity to fish

No long-term experimental data for fish are available. QSAR based (neutral organics) NOEC values for fish could be generated with ECOSAR v1.11 available in EPIsuite. Based on the log Kow value of 4.25, NOECs of 0.094 and 0.41 mg/L were estimated for fresh and saltwater fish respectively (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log Kow value used was within the domain of the freshwater QSAR. The QSAR for the saltwater fish is based on only two endpoints and its endpoint is therefore considered unreliable.

Read across with the analogue substance

For the dossier of d-limonene, the registrant has submitted an early life stage study on *Pimephales promelas*, this study is summarised below.

Reference	:	Anonymous (2015)	water solubility	:	4.0-5.7
type of study	:	Early life stages	species	:	Pimephales promelas, embryos
year of execution	:	2013	exposure duration	:	8 days (4 days post hatch)
GLP statement	:	Yes	nominal concn.	:	0, 2.5*%, 5.3%,11%, 32% and 48.6% of saturation
			Time weighted mean measured concn.		0, *, 0.059, 0.19, 0.37 and 0.67 (mg/L)
Guideline	:	OECD 212	dosing method	:	Renewal
test substance	:	d-limonene	acceptability	:	Reliable with restriction (Klimisch score of 2)
Purity	:	minimum p>99%	NOEC	:	0.059 mg/L (growth, measured) (EC10 between 0.37 and 0.67 mg/L)
			NOEC		0.19 mg/L (appearance)
			EC10		0.32 (survival)
			NOEC		0.37 (survival)

*The 2.5% solutions were not analysed as they were considered not relevant for the determination of the EC or NOEC values.

Embryos were used in an early life stages test to evaluate the sub-lethal effects of d-limonene. The substance was tested at the following nominal concentrations: 0 , 2.5%, 5.3%, 11.0%, 23.2% and 48.6% of a saturated solution of the test item in test medium. Time weighted average test concentrations were 0.059, 0.19, 0.37 and 0.67 mg/L for the nominal concentrations of 5.3%, 11.0%, 23.2% and 48.6%. The 2.5% solutions were not analysed, as they were not relevant for the determination of the EC or NOEC values. Thirty eggs (3 replicates of 10 eggs each) were exposed to each concentration of the test item and the control. The test vessels were 100 ml flasks sealed with PTFE coated screw caps. No aeration was used. The test medium was prepared by dilution of a saturated solution, renewal was performed at day 3 and 6 and test concentrations were analysed at start and termination of the experiment and before and after each renewal. Duration of the test was 8 days (4 days post hatch). The test was carried out at 23.5-25.3°C, a light: dark regime of 16:8 was maintained and each test concentration was tested in triplicate. Dissolved oxygen, temperature and pH were measured at the beginning, renewal and end of the test. The validity criteria specified in the test guidelines were met.

At termination of the test, the growth of the hatched larvae was determined and during the test, hatching, survival, abnormal appearance, and behaviour was observed and recorded daily. Actual measured test concentrations were used in the data analysis of NOEC, LOEC, LC10 and LC50 values. LC10 and LC50 values for the endpoint survival was calculated on the basis of the analytical results, by use of the standard procedure for Probit analysis and NOEC and LOEC values were estimated by use of Students T-test. Special considerations were taken considering the volatility of the test substance and endpoints are based on time weighted mean measured concentrations. The analyses of the test concentrations seem to be prone to uncertainty as test concentrations appear to increase between renewals. It is noted that due to the volatility and lipophilicity of the compound, d-limonene is a difficult substance to determine in the water phase and to assure a constant concentration during the exposure period. Therefore, the reported fluctuations in the test concentrations are considered acceptable but it reduces the reliability of the results and a Klimisch score of 2 (=reliable with restriction) is assigned.

The results show slight to moderate effect on the appearance and behaviour of fish at 0.37 mg/L and increased hatching rate at 0.67 mg/L (highest concentration). Statistically significant chronic effects were observed for survival rate at 0.67 mg/L (100% at mortality) and on the growth rate at the end of the test at, 0.19 and 0.37 mg/L. The NOEC and LOEC for growth rate are determined to be 0.059 and 0.19 mg/L, respectively. The effects on growth rate observed at 0.19 and 0.37 mg/L was less than 10% and more than 10% for 0.67 mg/L (the data did not allow the calculation of EC10 and EC50). For survival, an EC10 value of 0.32 mg/L was determined. A NOEC for survival was not

given in the report but up to 0.37 mg/L the mortalities were not significantly different from the control, therefore the NOEC for survival is considered to be 0.37 mg/L. An overview of the observed mortalities at test termination is given in the table below.

Saturated solution of *d*-Limonene (%)	Total number of eggs			Day 8 - 2013.10.23											
				Malformed larvae			Total number of dead eggs and larvae			Total number of hatched larvae			Total alive		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Control	10	10	10	-	-	-	3	3	2	7	7	8	7	7	8
2.5	10	10	10	-	-	-	1	0	7	9	10	3	9	10	3
5.3	10	10	10	-	-	-	2	2	0	8	9	10	8	8	10
11	10	10	10	-	-	-	1	2	0	10	9	10	9	8	10
23.2	10	10	10	-	1	-	2	4	5	10	9	9	8	6	5
48.6	10	10	10	-	-	-	-	-	-	-	-	-	0	0	0

Tests performed according to OECD test guideline 210 are preferred because they cover more sensitive life stages and as such are considered to be more sensitive. Nevertheless, a study according to OECD test guideline 212 is also considered a chronic study because the CLP guidance (section I.2.1.2) indicates that chronic studies can vary from 7 days to over 200 days. Furthermore, in the REACH guidance (R.7.8.4.1), OECD test guideline 212 is listed as a chronic study.

Where EC10 values are available, they are preferred over NOEC values for the same endpoint (ECHA 2015; OECD 2006). The $NOEC_{growth}$ of 0.059 mg/L was concluded with a statistically significant effect on growth of 4%, observed at two consecutive concentrations of 0.19 and 0.37 mg/L. The use of the $NOEC_{growth}$ of 0.059 mg/L is considered inappropriate for classification purposes as EC10 values are preferred over NOECs and for this endpoint, it is certain that the effects for growth at the 0.1 mg/L threshold will be limited and the EC10 will be higher than 0.37 mg/L. The next lowest value is the NOEC of 0.19 mg/L for appearance and behaviour but this data is only recorded for support of mortality data and it is not used for classification purposes. Therefore, the endpoint to use for classification purposes will be the EC10 of 0.32 mg/L for survival. **This study is considered as key study for read-across to alpha-terpinene.**

QSAR generated information

In addition to the laboratory study, the registrant has also submitted a QSAR generated endpoint (KREATiS 2015b). This endpoint was calculated with the iSafeRat® HA-QSAR for chronic aquatic toxicity and was supplemented with a QMRF document. This QSAR resulted in a 28 day NOEC of 0.080 mg/L with confidence limit of 0.056 – 0.11 mg/L. In addition, the dossier submitter calculated QSAR based (neutral organics) NOEC values for fish with ECOSAR v1.11 available in EPIsuite (US-EPA 2012). Based on the log Kow value of 4.38, NOECs of 0.073 and 0.34 mg/L were estimated for fresh and saltwater fish respectively (ECOSAR generates ChV values, these are converted to a NOEC by: $NOEC = ChV/\sqrt{2}$). The log Kow value used was within the domain of the freshwater QSAR. The QSAR for the saltwater fish is based on only two endpoints and its endpoint is therefore considered unreliable.

5.5.2 Aquatic invertebrates

5.5.2.1 Short-term toxicity to aquatic invertebrates

One peer reviewed publication is available that presents toxicity data for alpha-terpinene to *Daphnia magna* (Park et al. 2011). In the test 24 h old daphnids were exposed to alpha-terpinene glass tanks. The actual test concentrations were not monitored and the reported endpoint of 8.45 mg/L is based on nominal concentrations. A separate residue test was performed with a solution of 100 mg/L which was measured after 2 and 7 days. This test could be used to determine time weighted average concentration but since the concentration in the residue test exceed the water solubility with a factor of 18 it is considered not representative for the reported endpoint. Because of the low water solubility and high vapour pressure of alpha-terpinene, the reported EC50 is likely to be an underestimation of the actual toxicity of the compound and the endpoint is considered unreliable (Ri=3). The OECD toolbox and the PAN database (Kegley et al. 2014) both contain one EC50 for *Daphnia magna* (both LC50 = 1.85 mg/L). Considering the high volatility of the substance, the original test reports should be assess in order to ensure that the toxicity endpoints are based on the actual exposure concentration. Only the test report given in the OECD toolbox could be retrieved. This study, from , is a very thorough study where care is taken that the actual exposure concentrations were determined. It is a 48 h flow-through study with <24 hours old *Daphnia magna*. The test volume of 20 °C 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 are based on the average test concentrations of alpha-terpinene ranging from 1.36 to 5.89 mg/L. The reported LC50 and EC50 are both 1.85 mg/L. For these endpoints should be noted that they are calculated as the geometric mean of the NOEC and LOEC since at the LOEC 100% effect was observed. Nevertheless, these endpoints should be considered as reliable (Ri=2) and will be used for classification purposes.

The analysis of test media showed the presence of additional substances, not being the parent compound. The authors of the study concluded these substances to be hydrolysis products. We do not agree with this conclusion, as alpha-terpinene does not hydrolyse (see section 5.1, Table 14). For the hydrolysis of an alkene to occur, strong acidic conditions are required which is not the case here (Chemgapedia 2016). In our view, the additional substances may be either oxidation or hydration products. The molar mass of formed compounds was indicating an incorporation of a water molecule in the compound (the weight of the product is 18 mass units higher). This reaction only occurs directly after addition to water since the ratios between the parent and products is the same between stock and test solutions. In either case, formed metabolites will be more polar than the parent compound, having lower toxicity (See Annex I). The test concentrations of alpha-terpinene and hydration products are expressed as alpha-terpinene.

QSAR based (neutral organics) LC50 values for daphnids and mysids (saltwater) could be generated with ECOSAR v1.11 available in EPIsuite. Based on the log Kow value of 4.25, LC50 values of 0.75 and 0.22 mg/L were estimated for daphnids and mysids respectively. The log Kow value is within the domain of the QSARs (max log Kow of 6.4).

The QSAR and experimental endpoints are in the same order of magnitude. Taking into account that the experimental values are based on the actual exposure concentrations, these are considered preferable over the QSAR endpoints.

<u>This section on the analogue substance is provided for informational purposes</u>

Data on acute toxicity to aquatic invertebrates are available for alpha-terpinene, the data presented under this heading will therefore not be used for classification purposes but are only presented support of the read-across and for informational purposes. For the dossier of d-limonene, the registrant has submitted three study reports with acute toxicity tests on Daphnia magna. These studies are summarised below:

Reference	:	Betat (2013b)	water solubility	:	4.0 mg/L
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	2012	exposure duration	:	48 hours
GLP statement	:	yes	nominal conc.	:	0.2 - 1.2 mg/L
Guideline	:	OECD 202, EU C.2	dosing method	:	Renewal
test substance	:	d-limonene	acceptability	:	Reliable (Klimisch score of 1)
Purity	:	96.3%	48-h EC50	:	0.307 mg a.s./L (0.257-0.354, 95% c.i.) (mean measured)

Juveniles of D. magna were exposed to six test concentrations of d-limonene (0.2, 0.3, 0.4, 0.6, 0.8 and 1.2 mg/L). The test concentrations were prepared from a saturated solution of the test substance in water and the medium was renewed after 24 hours. The test was performed in 20 ml flasks sealed with screw caps. Test temperature was 20°C, light:dark regime was 16:8h and no aeration was performed during the test but dissolved oxygen was >60 % of the air saturation value. pH ranged from 6.86 to 7.68. Four replicates were performed per test concentration and control containing five daphnids. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken at t=0, t= 24 h (before after renewal) and t=48 h. Observations were made at 24 and 48 hours.

A 48-h EC50 for mobility of 0.307 mg/L (0.257-0.354, 95% conf.int.) based on mean measured concentrations was reported. Special considerations were taken considering the volatility of the test substance. The results are assigned a Ri of 1 (=reliable). The 48-h EC50 value from this study is used for classification purposes

Reference	:	Delpit (2014)	water solubility	:	5.46 mg/L
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	2013	exposure duration	:	48 hours
GLP statement	:	yes	nominal conc.	:	0.2 - 1.2 mg/L
Guideline	:	OECD 202, EU C.2	dosing method	:	Renewal
test substance	:	d-limonene	acceptability	:	Reliable (Klimisch score of 1)
Purity	:	95.5%	48-h EC50	:	0.456 mg a.s./L (0.353-0.551, 95% c.i.) (mean measured)

Juveniles of *D. magna* were exposed to six test concentrations of d-limonene (nominal: 0.5, 0.7, 1.0, 1.5, 2.1 and 3.0 mg/L). The test concentrations were prepared from a saturated solution of the test substance in water and the medium was renewed after 24 hours. The test was performed in 20 ml flasks sealed with screw caps. Test temperature was 20°C, light:dark regime was 16:8h and no aeration was performed during the test but dissolved oxygen was >3 mg/L. pH ranged from 7.52 to 8.31. Four replicates were performed per test concentration and control containing five daphnids. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken at t=0, t= 24 h (before after renewal) and t=48 h. Observations were made at 24 and 48 hours.

A 48-h EC50 for mobility of 0.456 mg/L (0.353-0.551, 95% conf.int.) based on mean measured concentrations was reported. Special considerations were taken considering the volatility of the test substance. The results are assigned a Ri of 1 (=reliable). The 48-h EC50 value from this study is used for classification purposes

Reference	:	Bjørnestad (2013)	water solubility	:	"very low"
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	2013	exposure duration	:	48 hours
GLP statement	:	yes	nominal conc.	:	19.8 - 100% of saturation
Guideline	:	OECD 202, ISO 6341	dosing method	:	Renewal
test substance	:	d-limonene	acceptability	:	Reliable (Klimisch score of 1)
Purity	:	>99%	48-h EC50	:	0.51 mg a.s./L (0.46-0.59, 95% c.i.) (mean measured)

Juveniles of D. magna were exposed to six test concentrations of d-limonene derived by dilution of a saturated stock solution. The stock solution was prepared by siphoning off the mid fraction of a solution with excess of the test compound (1 g/L). The nominal test concentrations were 19.8, 29.6, 44.4, 66.7 and 100% of the stock solution. The test medium was renewed after 24 hours. The test was performed in 42 ml flasks sealed with PTFE coated screw caps and a minor headspace. Test temperature was 21°C ± 0.8, light:dark regime was 16:8h and no aeration was performed during the test but dissolved oxygen saturation was 100 % in all tested concentrations. pH of the test solution was 7.8 ± 0.5. Four replicates were performed per test concentration and control containing five daphnids. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken at t=0, t= 24 h (before after renewal) and t=48 h. Observations were made at 24 and 48 hours.

A 48-h EC50 for mobility of 0.51 mg/L (0.46-0.59, 95% conf.int.) based on mean measured concentrations was reported. Special considerations were taken considering the volatility of the test substance. The results are assigned a Ri of 1 (=reliable). The 48-h EC50 value from this study is used for classification purposes

In addition to the reported studies from the registrant, one peer reviewed publication is available that presents toxicity data for d-limonene to *Daphnia magna* (Park et al. 2011). In the test 24 h old daphnids were exposed to d-limonene in glass tanks. The actual test concentrations were not monitored and the reported endpoint of 7.85 mg/L is based on nominal concentrations. A separate residue test was performed with a solution of 100 mg/L which was measured after 2 and 7 days. This test could be used to determine time weighted average concentration but since the concentration in the residue test exceed the water solubility with a factor of 8 it is considered not representative for the reported endpoint. Because of the low water solubility and high vapour pressure of d-limonene, the reported EC50 is likely to be an underestimation of the actual toxicity of the compound and the endpoint is considered unreliable (Ri=3). The OECD toolbox and the PAN database (Kegley et al. 2014) both contain EC50 values for *Daphnia magna* (ranging from 0.275 to 69.6 mg/L). Considering the high volatility of the substance, the original test reports should be assessed in order to ensure that the toxicity endpoints are based on the actual exposure concentration. Where available, the original references were retrieved (Anonymous 1990b; May Passino and Smith 1987; Park et al. 2011).

The study, from Anonymous (1990b) is a very thorough study where care is taken that the actual exposure concentrations were determined. It is a 48 h flow-through study with <24 hours old *Daphnia magna*. The test volume of 200 ml and a temperature of 20 °C 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 was performed in to tests with d-limonene from two different sources. The test concentrations were analysed every 24 hours and the toxicity endpoints are based on the average test concentrations of d-limonene ranging from 0.24 to 1.35 mg/L for test 1 and 0.29 to 1.63 for test two. For test 1, the reported LC50 for 48 hours of exposure is 0.924 mg/L. For test 2 an LC50 of 0.577 mg/L and an EC50 for mobility of 0.421 mg/L is reported. For the LC50 of the first test and EC50 of the second test should be noted that they are calculated as the geometric mean of the NOEC and LOEC since at the LOEC 100% effect was observed. Nevertheless, these endpoints should be considered as reliable (Ri=2) and will be used for classification purposes.

The analysis of test media showed the presence of additional substances (8–11%), not being the parent compound. The authors of the study concluded these substances to be hydrolysis products. We do not agree with this conclusion, as d-limonene does not hydrolyse (see section 5.1, Table 14). For the hydrolysis of an alkene to occur, strong acidic conditions are required which is not the case here (Chemgapedia 2016). In our view, the additional substances may be either oxidation or hydration products. The molar mass of formed compounds was indicating an incorporation of a water molecule in the compound (the weight of the product is 18 mass units higher). This reaction only occurs directly after addition to water since the ratios between the parent and products is the same between stock and test solutions. In either case, formed metabolites are expected to be more polar than the parent compound, having lower toxicity (See section 7.2). The test concentrations of d-limonene and hydration products are expressed as d-limonene.

The study of May Passino and Smith (1987) tested toxicity of d-limonene to *Daphnia magna* in a static test system and reported an EC50 of 69.6 mg/L based on nominal concentrations. Because of the low water solubility and high vapour pressure of d-limonene, the reported EC50 is likely to be an underestimation of the actual toxicity of the compound. Therefore the endpoint is considered to be unreliable and will not be used for classification purposes.

On the ECHA dissemination site one study (Author not disseminated 2007) is presented that is not available in the dossier nor has its endpoints been discussed in this report. The study is performed according to OECD test guideline 202 in a static test set-up. The test concentrations are only confirmed by analysis at the start of the test and the endpoints are based on nominal concentrations. The EC50 reported for 48 hours is 0.36 mg/L. Because of the low water solubility and high vapour pressure of d-limonene, the reported EC50 is likely to be an underestimation of the actual toxicity of the compound since it is no based on time-weighted-average concentrations. Therefore the endpoint is considered to be unreliable and will not be used for classification purposes.

QSAR generated information

In addition to the laboratory study, the registrant has also submitted a QSAR generated endpoint for d-limonene (KREATiS 2015c). This endpoint was calculated with the iSafeRat® Holistic HA-QSAR and was supplemented with a QMRF document. This QSAR resulted in a 48 h EC50 of 0.62 mg/L with confidence limit of 0.55 – 0.69 mg/L. In addition, the dossier submitter calculated QSAR based (neutral organics) LC50 values for daphnids and mysids (saltwater) with ECOSAR v1.11 available in EPIsuite. Based on the log Kow value of 4.38, LC50 values of 0.577 and 0.154 mg/L were estimated for daphnids and mysids respectively. The log Kow value is within the domain of the QSARs (max log Kow of 6.4). The QSAR and experimental endpoints are in the same order of magnitude.

5.5.2.2 Long-term toxicity to aquatic invertebrates

No long-term experimental data for aquatic invertebrates are available. QSAR based (neutral organics) NOEC values for daphnids and mysids could be generated with ECOSAR v1.11 available in EPIsuite (US-EPA 2012). Based on the log Kow value of 4.25, NOECs of 0.092 and 0.071 mg/L were estimated for daphnids and mysids respectively (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log Kow value is within the domain of the QSARs (max log Kow of 8) but it should be noted that the QSAR for the mysids is based on only two endpoints and its endpoint is therefore considered unreliable.

Read across with the analogue substance

For the dossier of d-limonene, the registrant has submitted a chronic toxicity test on Daphnia magna. This study is summarised below:

Reference	:	Kamper (2016b)	water solubility	:	4.0-5.7 mg/L
type of study	:	Reproduction toxicity study	Species	:	*Daphnia magna*
year of execution	:	2016	exposure duration	:	21 days
GLP statement	:	yes	nominal conc.	:	2.5 - 16% of saturation
Guideline	:	OECD 211	dosing method	:	Renewal
test substance	:	d-limonene	acceptability	:	Reliable (Klimisch score of 1)
Purity	:	>99%	NOEC	:	0.08 mg a.s./L
			EC10		0.153 mg a.s./L (0.083-0.0.222, 95% c.i.) (mean measured)

Juveniles of D. magna were exposed to five test concentrations of d-limonene derived by dilution of a saturated stock solution. The stock solution was prepared by siphoning off the mid fraction of a solution with excess of the test compound. The nominal test concentrations were 2.5, 4.0, 6.5, 10 and 16% of the stock solution. Ten daphnids (female <24 hours) were exposed to each test concentration and each animal was placed in an individual test flask of 50 ml that was thereafter sealed with a PTFE-coated screw cap. Renewal was performed every Monday, Wednesday and Friday by transferring the test animal to a new flask containing fresh prepared test solution. The animals were fed (algae) at each renewal. The test temperature was 19.9°C ± 0.1, light:dark regime was 16:8h and no aeration was performed during the test but dissolved oxygen saturation was 100 % in all tested concentrations. pH of the test solution was 7.8 ± 0.5. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken at start and termination of the test and at each renewal (before after renewal) from an additional test flask containing no daphnids and algae (feed). Observations were made at each renewal and at termination. The parameters monitored were: number of offspring, mortality of parents, time for first offspring, dead offspring. After submission of the study report, additional analysis was performed on the highest tested concentration (Kamper 2016a). The results of this analysis are also included here. Time weighted average test concentrations were 23, 50, 80, 173 and 288 µg/L. Mortality of the parents in the control was at most 10%. An EC10 of 157 µg/L and a NOEC of 80 µg/L were determined based on the number of life offspring. Special considerations were taken considering the volatility of the test substance and endpoints. The results are assigned an Ri of 1 (=reliable). The EC10 value from this study is used for classification purposes. It is scientifically preferred since it is based on interpolation of the concentration effect data while the NOEC is dependent of the test design. **This study is considered as key study for read-across to alpha-terpinene.**

QSAR generated information

In addition to the laboratory study, the registrant has also submitted a QSAR generated endpoint for *D. magna* (KREATiS 2015e). This endpoint was calculated with the iSafeRat® HA-QSAR for chronic aquatic toxicity and was supplemented with a QMRF document. This QSAR resulted in a 21 day NOEC of 0.050 mg/L with confidence limit of 0.035 – 0.070 mg/L. In addition, the dossier submitter calculated QSAR based (neutral organics) NOEC values for daphnids and mysids with ECOSAR v1.11 available in EPIsuite (US-EPA 2012). Based on the log Kow value of 4.38, NOECs of 0.074 and 0.005 mg/L were estimated for daphnids and mysids respectively (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log Kow value is within the domain of the QSARs (max log Kow of 8) but it should be noted that the QSAR for the mysids is

based on only two endpoints and its endpoint is therefore considered unreliable. The QSAR and experimental endpoints for *D. magna* are in the same order of magnitude.

5.5.3 Algae and aquatic plants

In the OECD toolbox experimental NOECS for *Pseudokirchneriella subcapitata* are given of <0.2 and <2.66 mg/L. The original test report of these values has been assessed. These endpoints are based on a static test where after 24 hours all of the test compound had dissipated form the test solution but a dissipation curve is not available. Although no significant effects were observed at any test concentration, time weighted average test concentrations cannot be determined. It is also unclear how the endpoints in the OECD toolbox were derived from the test results. Therefore these endpoints are considered unreliable (Ri=3) and they will not be used for classification purposes.

QSAR based (neutral organics) LC50 values for daphnids and mysids (saltwater) could be generated with ECOSAR v1.11 available in EPIsuite . Based on the log Kow value of 4.25, an LC50 values of 1.31 mg/L and a NOEC of 0.39 mg/L were estimated for algae (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log Kow value is within the domain of the QSARs (max log Kow of 8.0).

Read across with the analogue substance

For the dossier of d-limonene, the registrant has submitted two algal toxicity tests. These studies are summarised below:

Reference	:	Betat (2013a)	water solubility	:	3.4-5.7 mg/L
type of study	:	Growth inhibition study	Species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2012	exposure duration	:	72 hours
GLP statement	:	yes	nominal conc.	:	0.2 - 2.0 mg/L
Guideline	:	OECD 201, EU C.3	dosing method	:	Static
test substance	:	d-limonene	acceptability	:	Reliable with restrictions (Klimisch score of 2)
Purity	:	96.3%	72 h EC50 72 h EC10	:	0.32 mg a.s./L (0.291-0.355, 95% c.i.) 0.174 mg a.s./L (0.137-0.202, 95% c.i.) (growth rate, mean measured)

Algal cells of *Pseudokirchneriella subcapitata* were exposed to an aqueous solution of d-limonene at nominal concentrations of 0.2, 0.3, 0.5, 0.8, 1.3 and 2.0 mg/L. The stock solution was prepared by sampling the bottom and mid fraction of a solution with excess of the test compound which was thereafter directly diluted to obtain the test solutions. Inoculation occurred with such an amount of algae that the initial concentration in the test vessels was 5 x 10^3 cells/ml. The test flasks were sealed with a fritted glass stopper. Incubation occurred under continuous shaking. The test temperature was 23.0 - 23.2°C, mean light intensity was 5474 lux and did not vary more than 15%. pH of the test solution ranged from 7.68 - 10.21, variation was observed most at the end of the test. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken at start and termination of the test from all concentrations and biotic and abiotic control. Cell density was counted daily and increased 114 times within 72 hours. Geometric mean measured test concentrations were 0.134, 0.189, 0.306, 0.536 and 0.938 mg/L for the nominal concentrations of 0.3, 0.5, 0.8, 1.3 and 2.0 mg/L. For the nominal concentration of 0.2 mg/L, at start, the concentrations was already below the detection limit (LOD) and an actual concentration could not be determined. For the nominal concentration of 0.3 and 0.5 mg/L the concentration was also below the LOD in the biotic systems. Because of this, it is unclear if the mean concentrations are a good representative for the actual

exposure concentration since it is unclear how the actual decline in the exposure concentrations develops. This lowers the reliability of the derived endpoints especially because the endpoints are at the level of these test concentration. Endpoints are based on the mean measured concentrations and results for the lowest test concentrations were not included. For growth rate an EC50 of 0.320 mg/L and an EC10 of 0.174 mg/L was derived. For yield the EC50 and EC10 were 0.214 and 0.149 mg/L respectively. The results are assigned an Ri of 2 (=reliable with restrictions) because of the high variation in the pH at the end of the test and the uncertainty in the lower test concentrations. The EC50 and EC10 value for growth rate from this study are used for classification purposes.

Reference	:	Seierø (2015)	water solubility	:	4.0-5.7 mg/L
type of study	:	Growth inhibition study	Species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2014	exposure duration	:	72 hours
GLP statement	:	yes	nominal conc.	:	7, 10, 16, 24, 35, 53 and 80% of saturation
			Time weighted mean measured conc. (48 h)		0.09, 0.14, 0.23 and 0.30 mg/L for 7 - 24%*
			Time weighted mean measured conc. (72 h)		0.05, 0.08, 0.12 and 0.17 mg/L for 7 - 24%*
Guideline	:	OECD 201, ISO 8692	dosing method	:	Static
test substance	:	d-limonene	acceptability	:	48 h: Reliable with restrictions (Klimisch score of 2) 72 h: Unreliable (Klimisch score of 3)
Purity	:	>99%	48 h EC50	:	0.25 mg a.s./L (0.24-0.27, 95% c.i.)
			48 h EC10		0.14 mg a.s./L (0.13-0.16, 95% c.i.)
			72 h EC50		0.15 mg a.s./L (0.15-0.16, 95% c.i.)
			72 h EC10		0.09 mg a.s./L (0.08-0.09, 95% c.i.) (for all: growth rate, mean measured)

*The 35, 53 and 80% solutions were not analysed because at 24% already 100% effect was observed.

Algal cells of *Pseudokirchneriella subcapitata* were exposed to an aqueous solution of d-limonene at nominal concentrations of 7, 10, 16, 24, 35, 53 and 80% of a saturated solution of the test item in test medium. The stock solution was prepared by sampling the mid fraction of a solution with excess of the test compound which was thereafter directly diluted to obtain the test solutions. The initial concentration of algae in the test vessels was 2.5 x 10^3 cells/ml, this amount was chosen to enable exponential growth throughout the incubation period. The test was carried out with minor headspace in 42 mL glass vials sealed with PTFE coated caps. Incubation occurred under continuous shaking. The test temperature was 22.3 ± 0.1°C, mean light intensity was 60-120 µmol/m^2/sec. pH of the test solution ranged from 7.9 - 9.4, variation was observed most at the end of the test. The control consisted of the same dilution water, test conditions and test organisms, but no test substance. Samples for chemical analysis of the test item were taken every 24 hours from all concentrations and control, frozen (-20°C) and sent frozen to an external laboratory for analysis where they were kept refrigerated until analysis. Analysis was performed via headspace analysis and detection with GC-MS. The storage conditions were checked for difference between frozen storage or refrigerated storage, no significant differences in analytical results were found between the two methods of storage. Cell density was counted daily and control growth rate was 1.7 per day over 72 hours. Geometric mean measured test concentrations were determined only for the nominal concentrations of 7, 10, 16 and 24%, because at 24% already 100% effect was observed, higher test concentrations were not analysed. These geometric mean concentrations were 0.9, 0.14, 0.23 and 0.30 mg/L over 0-48 hours and 0.05, 0.08, 0.12 and 0.17 mg/L over 0-72 hours respectively. For all concentration apart from 24%, the concentration at 72 hours was below the LOD, these were included in the calculations of the geometric mean as 0.005 mg/L. An overview of all measured concentrations is given in Table 32 below.

Table 32: Results of the chemical analysis (mg/L) of subsamples from the test solutions without algae and the calculated geometric mean concentrations (mg/L).

nominal test concentration (% of saturated stock solution)	t = 0 h	t = 24 h	t = 48 h	t = 72 h	Geometric mean 0-48 hours	Geometric mean 0-72 hours
Control	< 0.010	-	-	<0.010	-	<0.010
7%	0.08	0.12	0.06	<0.010	0.09	0.05
10%	0.13	0.15	0.12	<0.010	0.14	0.08
16%	0.20	0.29	0.17	<0.010	0.23	0.12
24%	0.36	0.31	0.24	0.010	0.30	0.17

Reported endpoints are based on the mean measured concentrations. Growth inhibition and growth were calculated for each test concentration relative for the control without addition of test item. ECx values for growth and yield were determined using the computer program TOXEDO and NOEC and LOEC values were estimated by the computer program Dunnett's procedure as the highest tested concentration at which no significant inhibition was observed. For growth rate an 72 hour EC50 of 0.15 mg/L and an EC10 of 0.09 mg/L was derived. For yield the EC50 and EC10 were 0.09 and 0.05 mg/L respectively. As all tested concentrations caused significant inhibition on the yield, no NOEC could be determined.

Because the concentrations in the 72 hour samples were below the limit of detection, it was recommended in the report to use the 48 hour endpoints rather than the 72 hour endpoints. It is however not explained what would have caused this decrease in detectability. The test concentrations seems not to decrease over the first 48 hours, with even increases (up to 50%) for most test concentrations between t=0 and t=24. A rapid decline was then observed over the last 24 hours. These fluctuations in concentration are inconsistent and it is strange that no evaporation seems to occur over the first period followed by a massive evaporation over the last period. This is also not explained in the report and deviations from the test protocol that could explain this observation are also not given in the report. The decline over the last 24 hours indicate significant changes in the test conditions that does indeed indicate that endpoints derived over 72 hours of exposure are not reliable. Where it concerns the proposed use of endpoints derived over 48 hours, the analytical result of the test concentrations show a high fluctuation in the test concentrations over this period, for example and increase for the 16% test solution from 0.20 mg/L to 0.29 mg/L. This suggests a high uncertainty in the analysis of the samples taken at different time points. This shows that the analysis of the test concentrations is prone to uncertainty but due to the volatility and lipophilicity of the compound, it is a difficult substance to determine in the water phase and the results are considered best achievable. Therefore, the reported fluctuations in the test concentrations are considered acceptable but it reduces the reliability to Ri 2 (= Reliable with restrictions). In this view together with the fact that the endpoints from the study of Seierø (2015) are in the same order of magnitude, the use of results of 48 hours exposure for classification purposes is supported. **This study is considered as key study for read-across to alpha-terpinene.**

For additional endpoints, the OECD toolbox was checked for additional toxicological data, experimental NOECS for *Pseudokirchneriella subcapitata* are given of <0.05 and <1.5 mg/L. The original test report of these values has been assessed. These endpoints are based on a static test where after 24 hours all of the test compound had dissipated form the test solution but a dissipation curve is not available. Although no significant effects were observed at any test concentration, time

weighted average test concentrations cannot be determined. It is also unclear how the endpoints in the OECD toolbox were derived from the test results. Therefore these endpoints are considered unreliable (Ri=3) and they will not be used for classification purposes.

QSAR generated information

In addition to the laboratory study, the registrant has also submitted a QSAR generated endpoint for algae for d-limonene (KREATiS 2015d). This endpoint was calculated with the iSafeRat® Holistic HA-QSAR and was supplemented with a QMRF document. This QSAR resulted in a 72 hour EC50 for growth rate of 0.50 mg/L with confidence limit of 0.42 – 0.60 mg/L. In addition, the dossier submitter calculated QSAR based (neutral organics) LC50 values for algae with ECOSAR v1.11 available in EPIsuite (US-EPA 2012). Based on the log Kow value of 4.38, an LC50 values of 1.07 mg/L and a NOEC of 0.32 mg/L were estimated for algae (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log Kow value is within the domain of the QSARs (max log Kow of 8.0). The QSAR and experimental endpoints are in the same order of magnitude.

5.5.4 Other aquatic organisms (including sediment)

A few studies are available where alpha-terpinene has been tested on *Aedes aegypti* and/or *Aedes albopictus* (Cheng et al. 2009a; Cheng et al. 2009b; Park et al. 2011). In these studies, the exposure concentrations exceed the water solubility of alpha-terpinene and the endpoints are based on nominal concentrations. Furthermore the tests were performed in paper or polypropylene cups which could have caused sorption of the test substance making it even less available in the water phase. The reported endpoints are in the range of 12.5 to 28.1 mg/L but considering the shortcomings of the studies, they are likely an underestimation of the actual toxicity of alpha-terpinene to these mosquitos. The endpoints will therefore not be used for classification purposes and are not included in the summary table.

This section on the analogue substance is provided for informational purposes

In the above mentioned studies d-limonene was also tested, For d-limonene, the reported endpoints are in the range of 19.8 to 50 mg/L but for the same reasons as given above, these endpoints are considered unreliable and were not used for classification purposes of d-limonene and are therefore not taken over for read-across purposes. For d-limonene, a few additional studies were available where d-limonene was tested on *Aedes aegypti* and/or *Aedes albopictus* (Giatropoulos et al. 2012; Liu et al. 2013; Santos et al. 2011; Silva et al. 2008). In these studies that were water-only tests, the exposure concentrations exceed the water solubility of d-limonene and the endpoints are based on nominal concentrations. Also for these studies several shortcomings were noted and they are likely to present an underestimation of the actual toxicity of d-limonene to these mosquitos.

5.6 Comparison with criteria for environmental hazards (sections 5.2 – 5.5)

CLP - Acute aquatic hazards

Table 33: Overview of reliable acute endpoints that can be used for classification purposes

Method	Results (mg/L)	Remarks	Reference
Short-term fish toxicity according to ASTM E729 method; GLP not reported.	96 h LC50 = 3.15 96 h EC50 = 1.48	*Pimephales promelas* **alpha-Terpinene**	Anonymous (1990b)
Short-term invertebrate toxicity according to ASTM E729 method; GLP not reported.	48 h LC50 = 1.85 48 h EC50 = 1.85	*Daphnia magna* **alpha-Terpinene**	
Aquatic toxicity to algae according to OECD guideline 201	72 h ErC50 = 0.32 72 h ErC10 = 0.174	*P. subcapitata* endpoint based on mean measured concentration Ri=2 **Read-across: d-limonene**	Betat (2013a)
Aquatic toxicity to algae according to OECD guideline 201	48 h EC50 = 0.25 48 h EC10 = 0.14 72 h EC50 = 0.15 72 h EC10 = 0.09	*P. subcapitata* endpoint based on mean measured concentration The endpoints for 72 hours are considered unreliable **Read across: d-Limonene**	Seierø (2015)

For alpha-terpinene there are reliable acute data for only two trophic levels (fish and daphnia). The lowest endpoint for fish is 1.48 mg/L and for daphnia it is 1.85 mg/L. Data for algae are not available. As discussed under section 5.1, it is considered that read-across from d-limonene for classification of alpha-terpinene is a justifiable realistic worst-case scenario. Therefore, the experimental data for d-limonene are used to fill gaps in the ecotoxicological data for alpha-terpinene. In the case of acute toxicity this considers only the algae toxicity. The experimental endpoints for alpha-terpinene complemented with the endpoints for d-limonene are considered preferable over the QSAR generated endpoints.

Since the experimental values for algae read across from d-limonene is 0.25 mg/L (the lowest available endpoint for 48 hours of exposure), it is concluded that alpha-terpinene does fulfil the criteria for classification as Aquatic Acute Cat. 1. An M factor of 1 is warranted based on the EC50 of 0.25 mg/L between 0.10 and 1 mg/L.

CLP - Chronic aquatic hazards

Table 34: Overview of reliable chronic endpoints that can be used for classification purposes

Method	Results (mg/L)	Remarks	Reference
Chronic toxicity to fish according to OECD test guideline 212	NOEC growth = 0.059 (EC10 between 0.37 and 0.67 mg/L) NOEC hatching = 0.37 NOEC behaviour = 0.19 EC10 survival = 0.32 NOEC survival = 0.37	*Pimephales promelas* endpoints based on mean measured concentration; EC10 for growth could not be statistically determined. **Read across: d-Limonene**	Anonymous (2015)
Chronic invertebrate toxicity according to OECD test guideline 211	21 day EC10 = 0.153	*Daphnia magna* renewal test, endpoint based on mean measured concentration **Read across: d-Limonene**	Kamper (2016a, 2016b)
Aquatic toxicity to algae according to OECD guideline 201	72 h ErC50 = 0.32 72 h ErC10 = 0.174	*P. subcapitata* endpoint based on mean measured concentration Ri=2 **Read-across: d-limonene**	Betat (2013a)
Aquatic toxicity to algae according to OECD guideline 201	48 h EC50 = 0.25 48 h EC10 = 0.14 72 h EC50 = 0.15 72 h EC10 = 0.09	*P. subcapitata* endpoint based on mean measured concentration The endpoints for 72 hours are considered unreliable **Read across: d-Limonene**	Seierø (2015)

Alpha-terpinene has a high potential for bioaccumulation and is considered rapidly degradable.

No experimental chronic toxicity endpoints are available for alpha-terpinene. As discussed under section 5.1, it is considered that read-across from d-limonene for classification of alpha-terpinene is a justifiable realistic worst-case scenario. Therefore, the experimental data for d-limonene is used to fill gaps in the ecotoxicological data for alpha-terpinene, in this case all endpoints for chronic toxicity. As considered for the acute classification the QSAR endpoints are not preferable. Therefore the chronic classification is based on the chronic endpoints and data on biodegradation

read-across form d-limonene. The relevant chronic endpoints for fish is 0.32 mg/L, for daphnia this is 0.153 mg/L and for algae this is 0.14 mg/L (the lowest available endpoint for 48 hours of exposure).. The lowest values of 0.14 mg/L is between 0.1 and 1 mg/L and the substance is considered rapidly biodegradable. Based on the criteria set out in CLP, Annex I, section 4.1, Table 4.1.0(b) (ii), alpha-terpinene, in read-across from d-limonene, fulfils the criteria for classification as Aquatic Chronic 3.

5.7 Conclusions on classification and labelling for environmental hazards (sections 5.2 – 5.5)

Conclusions on classification and labelling for environmental hazards of alpha-terpinene.

	CLP regulation	
	Classification	M-factor
Resulting harmonised classification.	Aquatic Acute category 1. H400: Very toxic to aquatic life Aquatic Chronic category 3. H412: Harmful to aquatic life with long lasting effects.	M = 1

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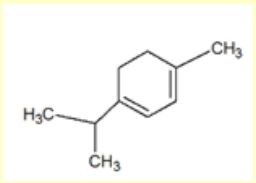
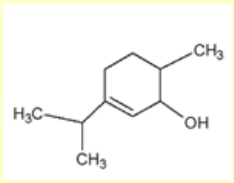
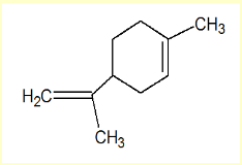
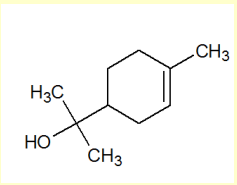
7 ANNEX

7.1 Comparison of aquatic acute toxicity data for alpha-terpinene and identified substance in the Anonymous (1990b) study

	Alpha-Terpinene (purity 22% in test water)	**Possible hydrate product: p-menth-3-en-2-ol**
Structure		
Physical chemical properties	LogKow = 4.25 Water solubility = 5.63 Molecular Weight= 136.23	LogKow = 3.296 Water Solubility = 360.2 mg/l Molecular Weight = 154.25
Acute Toxicity : Experimental lowest experimental value (mg/L)		
Fish	1.48	No data
Daphnia	1.85	No data
Algae	No data	No data
Acute Toxicity : Estimated toxicity data, ECOSAR (mg/L)		
Fish	1.07	8.689
Daphnia	0.75	5.561
Algae	1.31	6.794

ECOSAR predications are provided when experimental data is not available.

7.2 Comparison of aquatic acute toxicity data for d-limonene and identified substance in the Anonymous (1990b) study.

	d-limonene (purity 67% in test water)	**Possible hydrate product: p-menth-3-en-8-ol (alpha Terpineol) CAS number 98-55-5**
Structure		
Physical chemical properties	LogKow = 4.38 Water solubility = 12.3 mg/L Molecular weight = 136.23	LogKow = 3.28 Water solubility = 360.6 Molecular weight = 154.25
Acute Toxicity : Experimental lowest experimental value (mg/L)		
Fish	0.695	70* (geometric average)
Daphnia	0.307	73* (nm)
Algae	0.15	68* (TWA)
Acute Toxicity : Estimated toxicity data, ECOSAR (mg/L)		
Fish	0.845	8.068
Daphnia	0.577	5.180
Algae	1.07	6.416

*ECHA dissemination site: aquatic toxicity tests are carried out with Terpineol multi (a multi-constituent substance with alpha-Terpineol and gamma-Terpineol as constituents).

ECOSAR predications are provided when experimental data is not available.