Committee for Risk Assessment

RAC

Annex 1

Background document

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

1-isopropyl-4-methylbenzene; p-cymene

EC Number: 202-796-7
CAS Number: 99-87-6

CLH-O-0000001412-86-273/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 1-isopropyl-4-methylbenzene; *ρ-cymene*

EC Number: **202-796-7**

CAS Number: **99-87-6**

Index Number:

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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:	***1-isopropyl-4-methylbenzene;*** ***ρ-cymene***
EC number:	***202-796-7***
CAS number:	***99-87-6***
Annex VI Index number:	**-**
Degree of purity:	***> 80%***
Impurities:	***see Confidential Annex***

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	Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) Flam. Liq. 3 (H226: Flammable liquid and vapour) Acute Tox. 3 (H331: Toxic if inhaled) Aquatic Acute 1 (H400; M=1, Very toxic to aquatic life) Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways) Flam. Liq. 3 (H226: Flammable liquid and vapour) Acute Tox. 3 (H331: Toxic if inhaled) Aquatic Acute 1 (H400; M=1, Very toxic to

	aquatic life) 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	Conclusive but not sufficient for classification
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	Conclusive but not sufficient for classification
	Acute toxicity - dermal			None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox. 3 (H331)		None	
3.2.	Skin corrosion / irritation			None	Inconclusive

3.3.	Serious eye damage / eye irritation			None	Data lacking
3.4.	Respiratory sensitisation			None	Data lacking
3.4.	Skin sensitisation			None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity			None	Inconclusive
3.6.	Carcinogenicity			None	Data lacking
3.7.	Reproductive toxicity			None	Data lacking
3.8.	Specific target organ toxicity –single exposure			None	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure			None	Inconclusive
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:

GHS pictograms: GHS02, GHS06, GHS08 and GHS09

Signal word: Danger

Hazard statements:
H226: Flammable liquid and vapour.
H304: May be fatal if swallowed and enters airways
H331: Toxic if inhaled
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

ρ-Cymene 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

ρ-Cymene is one of the ingredients of the active substance terpenoid blend QRD460. The terpenoid blend, consisting of *p*-cymene, d-limonene and α-terpinene, is accepted as an active substance for plant protection products. However, as it is a mixture and not a substance harmonised classification of terpenoid blend is not possible. Therefore, a CLH proposal for the three ingredients (*ρ-cymene*, d-limonene, α-terpinene) will be submitted separately.

Data on *ρ-cymene* were collected from the DAR of terpenoid blend, the registration dossier of *ρ-cymene* and other publically available data through a search using several databases including e-chemportal, PubMed, ToxNet and publications such as the US Environmental Protection Agency report on screening hazard characterization of *ρ-cymene* (EPA 2012) and the flavour and fragrance high production volume consortia robust summary for *ρ-cymene* (EPA 2002, 2005).

ρ-Cymene has been assessed in the OECD High Production Volume (HPV) program (OECD 2009) and it is in the US EPA's HPV list (production and/or import volume greater than one million pounds per year) (EPA 2002, 2005).

In addition, the presence of *ρ-cymene* 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 but found. No indications that the substance can be classified in additional hazard classes were found.

Flammability

ρ-Cymene has a flash point of 47.2°C which is higher than 23 °C but lower than 60 °C (Annex I, Table 2.6.1, CLP), therefore classification as Flam. Liq. 3 (H226) according to regulation (EC) 1272/2008 (CLP regulation) is warranted.

Acute Toxicity

ρ-Cymene has an inhalation mouse LC_{50} less than 9.7 mg/L. According to the criteria in CLP Annex I, 3.1.2.6 (Decision logic for classification of substances), Category 3 inhalation applies to an Inhalation (vapour) $LC_{50} > 2$ but ≤ 10 mg/L. Therefore, classification as Acute Tox. 3 (H331) according to regulation (EC) 1272/2008 (CLP regulation) is warranted.

Aspiration toxicity

ρ-Cymene has a Kinematic viscosity @ 40°C of 7.1 mm^2/s and is a hydrocarbon which results in classification of Asp. Tox 1 (H304).

Aquatic toxicity

Aquatic Acute 1 (H400) is warranted based on QSAR estimated mysid 96 h LC50 of 0.327 mg/L; which is ≤ 1 mg/L (Annex I, Table 4.1.0, CLP). An M-factor of 1 is warranted as >0.1 to ≤ 1 mg/L (Annex I, Table 4.1.3, CLP).

ρ-Cymene was shown to be rapidly biodegradable with degradation after 14 days amounting to 88 ± 6.2% based on oxygen uptake (BOD).

Aquatic Chronic 3 (H412) is warranted based on an algal 72 h NOE$_b$C of 0.51 mg/L, a fish experimental 40-d NOEC of 0.690 mg/L, and a QSAR estimated daphnid NOEC of 0.117 mg/L which are below the ≤ 1 mg/L threshold and the substance is rapidly degradable.

For this reason, the dossier submitter considers a harmonized classification of *ρ-cymene* of Flam. Liq. 3 (H226: Flammable liquid and vapour), Acute Tox. 3 (H331: Toxic if inhaled), Asp. Tox 1 (H304: May be fatal if swallowed and enters airways), Aquatic Acute 1 (H400: Very toxic to aquatic life) with M factor of 1, and Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects).

2.3 Current harmonised classification and labelling

There is no current harmonised classification and labelling for *ρ-cymene* according to Annex VI of CLP regulation.

2.4 Current self-classification and labelling

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

Classification		Labelling		Specific Concentration limits, M-factors	# of Notifiers	Total number of notifiers	Percent (%)	Notes
Hazard Class and Category Code	Hazard Statement Code	Hazard Statement Code	Pictograms, Signal Word Code(s)					
Acute Tox. 4 oral	H302	H302	GHS02 GHS07 GHS08 GHS09 Dgr		5	1276	0.4	None
Acute Tox. 4 dermal	H312	H312			1	1276	0.1	
Acute Tox. 4 inhalation	H332	H332			1	1276	0.1	
Aquatic Chronic 1	H410	H410			1	1276	0.1	
Aquatic Chronic 2	H411	H411			1145	1276	89.7	
Aquatic Chronic 3	H412	H412			4	1276	0.3	
Asp. Tox. 1	H304	H304			1120	1276	87.8	
Eye Irrit. 2	H319	H319			44	1276	3.4	
Flam. Liq. 3	H226	H226			1273	1276	99.8	
Skin Irrit. 2	H315	H315			111	1276	8.7	
Skin Sens. 1A	H317	H317			1	1276	0.1	
STOT SE 3	H335 (Inhalation)	H335 (Inhalation)			74	1276	5.8	
Not Classified	-	-			1	1276	0.1	

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.

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

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

ρ-Cymene is currently not classified according to Annex VI of CLP-regulation.

ρ-Cymene belongs to the family of terpenes, which are active ingredients in insecticides. The terpenes can cause disruption of respiration causing insect death with insects that are more active or have larger spiracles likely to be more affected by the substances. Terpenes, including *ρ-cymene* are strong insecticides repelling insects such as thrips and whitefly. *ρ-Cymene* 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 it is a mixture and not a substance harmonised classification of terpenoid blend is not possible. Therefore, a CLH proposal for the three ingredients (*ρ-cymene*, d-limonene, α-terpinene) will be submitted.

Given that *p-cymene* 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

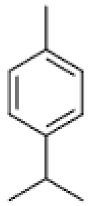
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	202-796-7
EC name:	*p-cymene*
CAS number (EC inventory):	99-87-6
CAS number:	99-87-6
CAS name:	benzene, 1-methyl-4-(1-methylethyl)
IUPAC name:	1-isopropyl-4-methylbenzene
CLP Annex VI Index number:	-
Molecular formula:	$C_{10}H_{14}$
Molecular weight range:	134.22 g/mol
Other names:	1-methyl-4-propan-2-ylbenzene; 4-isopropyltoluene; 1-methyl-4-isopropyl-benzene; para-Cymene

Structural formula:



p-Cymene

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
p-cymene	see confidential annex	see confidential annex	

Current Annex VI entry: None

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential	see confidential annex	see confidential annex	

Current Annex VI entry: None

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: n/a

1.2.1 Composition of test material

The composition of the test material concerns *ρ-cymene* with unknown purity unless otherwise specified in the study summaries.

1.3 <u>Physico-chemical properties</u>

Table 9: 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 transparent liquid Colourless to pale yellow liquid	(EPA 2009) (Good Scents Company website) [a]	Measured: method not known Purity not provided Comment DAR: Acceptable as background information (not relied on); Not GLP.
Melting/freezing point	-67.9 °C	(EPA 2002; INCHEM 1997) (CRC Handbook 1986) [a]	Measured: method not known Purity not provided Comment DAR: Acceptable as background information (not relied on); GLP not reported.
Boiling point	176 - 177.1 °C 175 °C	(EPA 2002) (LyondellBasell 2010b) [a]	Measured: method not known Purity not provided Method: Thermal analyser Purity: 99.20% Comment DAR: Acceptable; Data from SDS. Description of used method is not sufficient. However, since this a commonly used terpene, the information provided is considered acceptable; Not GLP.
Relative density	0.8573 g/cm^3 at 20°C 0.854 g/cm^3 at 25°C	(TOXNET 2014) (LyondellBasell 2010b) [a]	Measured: method not known Purity not provided Measured: method not known Purity: 99.20% Comment DAR: Data from SDS; Since a study performed with formulation is available, this information is considered supplementary (not relied on); Not GLP.
Vapour pressure	1.46 mm Hg at 25 °C (195 Pa); 1.50 mm Hg at 20 °C (200 Pa 266.64 Pa at 20 °C (2.0 torr)	(EPA 2002) (LyondellBasell 2010b) [a]	Measured: method not known Purity not provided Measured: method not known Purity: 99.20% Comment DAR: Acceptable; Data from SDS. Data is acceptable for the use in the environmental risk assessment; Not GLP.
Surface tension	28.09 dyne/cm = 0.02809 N/m at 20°C	(TOXNET 2014)	Measured: method not known Purity not provided

	28.5 ± 3 dyne/cm = 0.0285 ± 0.003 N/m	(Good Scents Company website) [a]	Measured: method not known Purity not provided Comment DAR: Acceptable as background information (not relied on).
Water solubility	23.35 mg/L at 25°C	(Banerjee et al. 1980) [a]	Measured: Distilled water was mixed with an excess of *ρ-cymene* by constant or intermittent shaking in a sealed stainless steel centrifuge tube and allowed to equilibrate (usually within 1 week). Afterwards, the tube was centrifuged (10,000 ppm, 60 minutes) and water samples were taken and analyzed by GC. The test was conducted at least twice and the analysis of samples was conducted in duplicate. Purity not provided Comment DAR: Acceptable; Not GLP.
Partition coefficient n-octanol/water	Log K_{ow} = 4.1	(Banerjee et al. 1980)	Measured: At a temperature of 23±1.5 °C, a mixture of purified octanol and water was shaken for 30 minutes and separated by centrifugation (10,000 rpm, 30 minutes). *ρ-cymene* was dissolved in the water-saturated octanol and then added to a steel tube which was then sealed and the contents were equilibrated by shaking for 4-5 minute intervals, 10 minutes apart. Afterwards, the tube was centrifuged (10,000 rpm, 30 minutes) and the octanol and water layers were sampled and analyzed by GC. The octanol sample was diluted with methanol prior to analysis. The test was conducted in duplicate. Purity not provided.
	Log P_{ow} = 5.08	(Bradbury 2004) [a]	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 Rapporteur reassessed the original study report: Method used is not OPPTS 830.7570 (= estimation by HPLC). The study was conducted in triplicate by dispersing pure *ρ-cymene* (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	47.2°C	(EPA 2014)	Measured: equilibrium method closed up Purity not provided
	52°C	(LyondellBasell 2010b) [a]	Measured: Tag closed cup Purity: 99.20% Comment DAR: Acceptable; Data from SDS. Description of used method is not sufficient. However, since this a commonly used terpene, the information provided is considered acceptable; Not GLP.
	47°C	(Polarome MSDS 2009b) [a]	Measured: method not known Purity not provided Comment DAR: Acceptable as background information (not relied on); Not GLP.

Flammability	*ρ-cymene* is highly flammable (flash point 47.2°C)	(EPA 2014)	Measured: equilibrium method closed up Purity not provided
Explosive properties	Examination of the structure indicates that there are no chemical groups associated with explosive properties. Not explosive	(TOXNET 2014) (LyondellBasell 2010b) [a]	Method: statement Purity: 90.4% Comment DAR: Acceptable as background information (not relied on). Based on the structure of the substance, explosive behaviour is not expected.
Self-ignition temperature	817°C	(NOAA 1999)	Measured: method not known Purity not provided
Oxidising properties	*ρ-cymene* does not contain any functional group associated with oxidising properties listed in the Guidance for the implementation of REACH R.7a table R.7.1-29.		
Granulometry			
Stability in organic solvents and identity of relevant degradation products	In accordance with column 1 of REACH Annex IX, the stability in organic solvents study does not need to be conducted as the stability of the substance is not considered to be critical.		
Dissociation constant	In accordance with section 1 of REACH Annex XI, the dissociation constant study does not need to be conducted as the substance does not contain any functional groups that dissociate and therefore testing does not appear scientifically necessary.		
Kinematic viscosity	7.1 mm^2/s at 40°C	(SDS 2013)	Measured: method not known Purity not provided
Henry's law constant	1.11×10^3 Pa m^3/mol	(EPA 2012)	Measured: method not known

	(1.1 x atm m^3/ mol)		Purity not provided
	1.38 x 10^3 Pa m^3/mol (1.36 x 10^{-2} atm m^3/mol)	(DAR 2013) [a]	Calculated (Vp*mw/S_w)
	5.5 x 10^2 - 1.1 x 10^3 Pa m^3/mol	(Sander 2015)	Measured (headspace method), calculated (Vp*mw/S_w) and QSAR values. Purity not provided

[a] As summarised in the DAR (Volume 3, annex B.1-5), May 2014.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

ρ-Cymene is an ingredient of the plant protection product terpenoid blend. It is accepted as an active substance for plant protection products. *ρ-Cymene* is a very versatile chemical which can be used in a wide variety of applications including polishes and sanitation goods such as soaps and detergents.

As stated on ECHA's dissemination site, *ρ-Cymene* is also manufactured and/or imported in the European Economic Area for industrial use resulting in the manufacture of another substance. It has been registered as intermediate.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flammability	*ρ-cymene* is highly flammable (flash point 47.2°C)	(EPA 2014)	Measured: equilibrium method closed up Purity not provided
Flash point	47.2°C	(EPA 2014)	Measured: equilibrium method closed up Purity not provided

3.1 Physical and Chemical Properties

3.1.1 Summary and discussion of physical chemical properties

ρ-Cymene is a flammable substance (flash point 47.2°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)

ρ-Cymene 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 *ρ-cymene* for flammability as Flam. Liq.3 (H226: Flammable liquid and vapour) is required.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

p-Cymene is a colourless transparent liquid at 20 °C and 101.3 kPa.

A summary of relevant physico-chemical studies/statements submitted by Dossier Submitter is provided in the table below:

Method	Results	Remarks	Reference
Explosive properties	Examination of the structure indicates that there are no chemical groups associated with explosive properties. Not explosive	Purity: 90.4 % Statement: Based on the structure of the substance, explosive behaviour is not expected	LyondellBasell, 2010b TOXNET 2014
Self-ignition temperature	436 °C (817 °F)	Measured: method not known Purity not provided	NOAA, 1999
Oxidising properties	p-cymene does not contain any functional group associated with oxidizing properties listed in the Guidance for the implementation of REACH R.7a table R.7.1-29.	-	-
Flash point	47.2 °C	Measured: equilibrium method closed up Purity not provided	EPA, 2014
	52 °C	Measured: Tag closed cup Purity: 99.20 % Comment DAR: Acceptable; Data from SDS. Description of used method is not sufficient. However, since this a commonly used terpene, the information provided is considered acceptable; Not GLP.	LyondellBasell, 2010b
	47 °C	Measured: method not known Purity not provided	Polarome MSDS 2009b according to DAR for substance terpenoid blend QRD460 (Volume 3, annex B.1-5), May 2014

p-Cymene has a flash point of 47.2 °C which is higher than 23 °C but lower than 60 °C (Annex I, Table 2.6.1, CLP), therefore classification as Flam. Liq. 3; H226 according to regulation (EC) 1272/2008 (CLP regulation) is proposed by the Dossier Submitter (DS).

p-Cymene does not possess oxidising or explosive properties.

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Based on the data provided, RAC agrees with the DS that p-cymene fulfils the criteria for classification in **Cat. 3 for flammability (Flam. Liq. 3; H226 – Flammable liquid and vapour)**.

No chemical groups associated with explosive properties present in the molecule, therefore p-cymene should not be classified as explosive substance.

p-Cymene does not contain either oxygen, fluorine or chlorine and therefore should not be classified as an oxidising liquid.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The metabolism of *ρ-cymene* was studied in rats and guinea. Following intragastric (100 mg/kg) or inhalation (550 mg/m^3) dosage urinary metabolite excretion was nearly complete within 48 h, amounting to 60-80% dose. Evaluation of urinary excretion in both species revealed in total 18 metabolites, of which 16 were identified in rats and 6 in guinea pigs. No ring-hydroxylation of ρ-*cymene* was detected in rats, but guinea-pigs formed small amounts of carvacrol and hydroxycarvacrol. Oxidation of both the methyl and isopropyl groups of ρ-*cymene* occurred extensively in both species. The following types of metabolites were formed: monohydric alcohols, diols, mono- and di-carboxylic acids and hydroxyacids. Conjugation with glycine of the cumic acid formed was extensive in guinea-pigs.pigs following intragastric exposure of 100 mg/kg bw. No ring-hydroxylation of *ρ-cymene* was detected in rats, but guinea pigs formed small amounts of carvacrol and hydroxycarvacrol. Oxidation of both the methyl and isopropyl groups of *ρ-cymene* occurred extensively in both species. The following types of metabolites were formed: monohydric alcohols, diols, mono- and di-carboxylic acids and hydroxyacids. Following an oral dose of 100 mg/kg bw of *ρ-cymene* to male rats, the principal urinary metabolites were *p*-isopropylbenzoic acid (19 % of the administered dose) and 2-*p*-carboxyphenylpropionic acid (16 %). Other less important urinary metabolites included 2-*p*-tolylpropan-1-ol (8 %), 2-*p*-tolylpropan- 2-ol (9 %), 2-*p*-carboxyphenylpropan-2-ol (9 %), 2-*p*-(hydroxymethyl)phenylpropionic acid (4 %), 2-*p*-carboxyphenylpropan-1-ol (11 %), *p*-isopropylbenzoylglycine (2 %), *p*isopropylbenzyl alcohol (1 %), and 2-*p*-tolylpropionic acid (1 %) Anonymous (1983). When the same dose was given to male guinea pigs, similar urinary metabolites were identified, however in different quantities. The primary urinary metabolite in guinea pigs was *p*-isopropylbenzoylglycine (31 %), indicating that conjugation with glycine was more prevalent in guinea pigs than in rats. Another major metabolite in guinea pigs was 2-*p*-tolylpropan-2-ol (14 %) (Anonymous 1983). In addition, whereas ring hydroxylation of *ρ-cymene* was not reported in rats (Anonymous 1983) and rabbits (Anonymous 1981a), trace amounts of the ring hydroxylation metabolites hydroxyl-*ρ-cymene* and hydroxycarvacrol (dehydroxyl-*ρ-cymene*) were detected in guinea pig urine. Ring hydroxylation in guinea pigs only occurred in *ortho* position to the methyl group (Anonymous 1983).Conjugation with glycine of the cumic acid formed was extensive in guinea pigs. Therefore, upon an oral dose of 100 mg/kg bw of *ρ-cymene* given to male Wistar rats or Dunkin Hartley guinea pigs, 80 % or 71 %, respectively, was excreted in the urine within the following 48 h in the form of extractable metabolites. The authors speculated that the rest of the dose was either excreted *via* the faeces or as unextractable metabolites in the urine (Anonymous 1983).

ρ-Cymene was metabolized in rabbits and the following four optically active metabolites, 2-(p-tolyl)-1-propanol (3': R/S = 65:35), 2-(p-tolyl)propanoic acid (5': R/S = 0:100), *p*-(2-hydroxy-1-methylethyl)benzoic acid (6': R/S = 91:9) and p-(1-carboxyethyl)benzoic acid (8': R/S = 30:70), were isolated in addition to three optically inactive metabolites, 2-(p-tolyl)-2-propanol (2), p-isopropylbenzoic acid (4'), and *p*-(1-hydroxy-1-methylethyl)benzoic acid (7'). The presumed metabolic pathways of *ρ-cymene* in rabbits were confirmed by the administration of the intermediate metabolites (2, 3', 4', and 5'). The enantiomeric ratios of the metabolites, 3' and 6', suggested that omega-hydroxylations of the isopropyl group in 1 and 4' occurred preferentially at the pro-S methyl group. In the metabolism of *ρ-cymene*, the S-isomers are predominant in the propanoic acid derivatives, but the R-isomers are rich in the propanol derivatives. It is of interest that the metabolism of 4', however, produced predominantly the corresponding propanol derivative (6'; R/S = 91:9) and propanoic acid derivative (8'; R/S = 80:20) possessing the same R-configuration. Some optically active *ρ-cymene* derivatives were also synthesized as standard compounds (Anonymous 1992).

The main metabolites in the urine of rabbits given a single oral dose of 670 mg *ρ-cymene*/kg bw were *p*-cymen-9-ol and *p*-cymen-8-ol (50 % and 28 %, respectively, of the neutral metabolites). Acidic metabolites identified were alpha-*p*-tolylpropionic acid, alpha-tolyl-alpha-hydroxylpropionic acid, *p*-isopropylbenzoic acid and *p*-1-hydroxyisopropylbenzoic acid. Ring hydroxylation did not occur (Anonymous 1981a).

Anonymous (1999) studied the metabolite pattern of *ρ-cymene* in rats following oral doses equivalent to 50 and 200 mg/kg bw. The major metabolites in 0-48 h urine after administration of the 50 mg/kg bw dose were 2-*p*-tolypropan-2-ol (39 % of recovered dose) and 2-*p*-carboxphenylpropan-2-ol (19 %). The former metabolite is the product of allylic hydroxylation of the isopropyl substituent, while the latter metabolite is the product of allylic hydroxylation of both the isopropyl substituent and the methyl substituent. Minor metabolites in rat urine were 2-*p*-carboxyphenylpropan-1-ol (10 %), 2-*p*-carboxyphenylpropionic acid (14 %), and *p*-isopropylbenzoic acid (17 %). A large percentage of the urinary metabolites at this dose was conjugated (66 % conjugated vs 34 % free) both to glucuronic acid and glycine. The same metabolites were observed after the high dose, but conjugation was considerably reduced (18 % conjugated vs 82 % free), suggesting saturation of the conjugation pathway (Anonymous 1999).

ρ-Cymene was oxidised at the isopropyl side-chain yielding 2-(*p*-tolyl)-2- propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate (EFSA 2006).

Skin absorption: ρ-Cymene is well absorbed through the skin. In studies with ^{14}C-labelled *ρ-cymene*, the penetration observed was 254 µg/cm^2 in 60 min (Anonymous 1968). Absorption by the skin was more rapid than with toluene, *p*-xylene or ethylbenzene.

Human information

No human studies were found.

4.1.2 Summary and discussion on toxicokinetics

ρ-Cymene is readily absorbed and excreted in different species. Lower absorption was observed via inhalation. *ρ-cymene* is extensively metabolised. See Section 4.1.1 for more details.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 11a: Summary table of relevant acute oral toxicity studies

Method	Results	Remarks	Reference
Rat Oral Dose: unspecified doses Purity: not provided N= 10/sex/dose	LD_{50}: 4750 mg/kg bw (95% confidence limits: 3720-6060)	2 (reliable with restrictions) Key study	(EPA 2005, 2012)
Rat Oral Dose: 620, 940, 1400, 2100, 3200, 4700, 7100 or 10,700 mg/kg bw. Purity: not provided N= 1-3/dose	LD_{50}: 3200 mg/kg bw	2 (reliable with restrictions) Key study	(EPA 2005, 2012)
Mouse oral: unspecified exposure regimen Dose: no data Purity: not provided N= not provided	LD_{50}:1695 mg/kg bw	4 (not assignable) supporting study experimental result	Various MSDS sheets*

*https://ca.vwr.com/store/asset?assetURI=https://ca.vwr.com/stibo/hi_res/eng_ca/94/56/8179456.pdf
http://www.chemcd.com/product/msds212d1b0f5d194761e3095e2dfad42ffd(25155-15-1).pdf
http://www.uww.edu/riskmanagement/msds/data/ρ-*cymene*-_98p_acros_organics_n.v._3.18.03.pdf

Key studies
Male and female rats (strain not specified; 1 – 3/sex/dose) were dosed by oral gavage with 620, 940, 1400, 2100, 3200, 4700, 7100 or 10,700 mg/kg bw. Following a 14-day observation period, all rats in the 620, 940, 1400 and 2100 mg/kg bw groups survived and 1/2, 2/2, 3/3 and 1/1 had died in the 3200, 4700, 7100 and 10,700 mg/kg bw groups, respectively. An LD_{50} of 3200 mg/kg bw was determined in this study. Prior to death, rats showed typical signs of intoxication: depression, tremor, lethargy, and muscular weakness. Necropsy was reported to show hyperemic lungs with scattered areas of hemorrhage, atelectasis and emphysema, partially digested blood and food in the stomach, petechial hemorrhages in the glandular stomach with hyperemic mucosa, bloody mucus in the upper small intestine and clear mucus in the lower small intestine, pale and mottled liver, congested liver, and distended urinary bladder (EPA 2005, 2012).

Osborne-Mendel rats (10/sex/dose) were administered various unspecified doses of the test substance. Rats were monitored for up to 2 weeks. Rats showed depression shortly following dosing and also coma, bloody lacrimation, diarrhea with irritable, scrawny appearance during the observation period. An LD_{50} of 4750 mg/kg bw (95% confidence limits: 3720-6060 mg/kg bw) was determined in this study. (EPA 2005, 2012).

Supporting study

Various MSDS sheets have reported an LD_{50} in mice of 1695 mg/kg bw but the study where this LD_{50} was based on could not be found in the literature (Table 11a*). Without the specific details of the study, an evaluation of the quality of the study cannot be made.

4.2.1.2 Acute toxicity: inhalation

Table 11b: Summary table of relevant acute inhalation toxicity studies

Method	Results	Remarks	Reference
Guinea pig, rat and mouse Inhalation Dose: 9.7 mg/L (vapour) for 5 h Purity: not provided N= 2-3 per species	LC_{50} (guinea pig) > 9.7 mg/L LC_{50} (rat) > 9.7 mg/L LC_{50} (mouse) < 9.7 mg/L	2 (reliable with restrictions) Key study	(EPA 2005, 2012)

Key study

Guinea pigs, rats and mice (2 – 3 animals per species, sex not indicated) were exposed by inhalation to a concentration of 9.7 mg/L (vapour) for 5 hours. Animals were observed for 1 week following exposure. No deaths were reported in guinea pigs or rats. All exposed mice died during or within 24 hours of exposure. The reported LC_{50}s were as follows: LC_{50} (guinea pig) > 9.7 mg/L; LC_{50} (rat) > 9.7 mg/L; LC_{50} (mouse) < 9.7 mg/L. For guinea pigs, rats and mice, signs reported during the first 30 minutes were those typical of irritation: excitement, pawing at the eyes and nose, increased blinking, squinting, and eye closure. Guinea pigs and rats showed tremors and clonic convulsions from which the animals fully recovered the day after. In addition, mice exhibited equilibrium loss and chronic convulsions with intervals of coma. One mouse died after 3.9 hours and another died after 4.8 hours. The 3rd mouse was comatose at termination of exposure and died during the night. Necropsies showed hyperemic lungs, mottled liver, and pale kidneys. In addition, it appeared that the heart had stopped in systole. None of these effects were reported in rats and guinea pigs at the same atmospheric concentration (EPA 2005, 2012).

4.2.1.3 Acute toxicity: dermal

Table 11c: Summary table of relevant acute dermal toxicity studies

Method	Results	Remarks	Reference
Rabbit Dermal Dose: 5000 mg/kg bw Purity: not provided N= 10 (number per sex not reported)	LD_{50} > 5000 mg/kg bw	2 (reliable with restrictions) Key study	(EPA 2005, 2012)
Rabbit Dermal Dose: 5140 mg/kg bw Purity: not provided N= 1.	no mortality in single test animal	3 (not reliable) Supporting study	(EPA 2005, 2012)

Key study
Ten rabbits (sex not specified; 10/sex/dose) were exposed to *ρ-cymene* at a dermal dose of 5000 mg/kg bw. Animals were observed for 14 days. No animals died during the observation period. Skin irritation was observed and was graded as follows: slight redness (3/10), moderate redness (7/10), slight edema (3/10), and moderate edema (7/10). The reported LD_{50} was greater than 5000 mg/kg bw (EPA 2012). +add reference EPA 2005

Supporting study
One rabbit (sex not indicated) was exposed to ρ-cymene at a dermal dose of 5144 mg/kg bw. Undiluted ρ-cymene was applied to the shaven abdominal skin (10 x 15 cm area) in 1 mL doses every hour for a total of 6 mL over a 6-hour exposure period. The animal was observed for 1 month after exposure. Slight hyperemia of the skin was observed after 1 hour and persisted approximately 4 hours after which a slight subcutaneous edema developed. After the exposure period, the skin still was slightly edematous and over the next 5 days, it was slightly thickened, hyperemic and showed fine cracks. After the first week, the skin began to return to normal and within the month is was normal with hair growth. There was no mortality in this single test animal (EPA 2005, 2012).

4.2.1.4 Acute toxicity: other routes

Data on other routes were not available.

4.2.2 Human information

No relevant information available

4.2.3 Summary and discussion of acute toxicity

Table 11d: Summary of all the relevant oral, dermal and inhalation LD_{50} values

	***p*-Cymene (CASRN 98-87-6)**
Acute Oral Toxicity LD_{50} (mg/kg)	(rats) **3200-4750**
Acute Dermal Toxicity LD_{50} (mg/kg)	(rabbits) **>5000**
Acute Inhalation Toxicity LC_{50} (mg/L)	(mouse) **< 9.7** (rat, guinea pigs) **>9.7**

For the oral LD_{50}, two key studies were reported. Male and female rats (1 – 3/dose) were dosed by oral gavage with 620, 940, 1400, 2100, 3200, 4700, 7100 or 10,700 mg/kg bw. Following a 14-day observation period, all rats in the 620, 940, 1400 and 2100 mg/kg bw groups survived and 1/2, 2/2, 3/3 and 1/1 had died in the 3200, 4700, 7100 and 10,700 mg/kg bw groups, respectively. An LD_{50} of 3200 mg/kg bw was determined in this study (EPA 2005, 2012). Osborne-Mendel rats (10/sex/dose) were administered various unspecified doses of the test substance. Rats were monitored for up to 2 weeks. An LD_{50} of 4750 mg/kg bw was determined in this study (EPA 2005, 2012). Various MSDS sheets have also reported an LD_{50} in mice of 1695 mg/kg bw but the study where this LD_{50} was based on could not be found in the literature (Table 11a*). Without the specific details of the study, an evaluation of the quality of the study cannot be made. Therefore the oral LD_{50} for rats is between 3200 and 4750 mg/kg bw.

For the inhalation LC_{50}, one key study was reported. Guinea pigs, rats and mice (2 – 3 animals per species, sex not indicated) were exposed by inhalation to a concentration of 9.7 mg/L (vapour) for 5 hours. Animals were observed for 1 week following exposure. No deaths were reported in guinea pigs or rats. All exposed mice died during or within 24 hours of exposure. The reported LC_{50}s were as follows: LC_{50} (guinea pig) > 9.7 mg/L; LC_{50} (rat) > 9.7 mg/L; and LC_{50} (mouse) < 9.7 mg/L (EPA 2005, 2012). As the quality of the tests in these three species is comparable and it is unknown which species is the most relevant for humans, the most sensitive species is used for determination of the classification in line with the Guidance on the CLP criteria version 4.1 chapter 3.1.2.3.2. Evaluation of non-human data.

For the dermal LD_{50}, one key study was reported. Ten rabbits (number per sex not reported) were exposed to *ρ-cymene* at a dermal dose of 5000 mg/kg bw. Animals were observed for 14 days. No animals died during the observation period. The reported LD_{50} was greater than 5000 mg/kg bw (EPA 2012).

4.2.4 Comparison with criteria

For the oral LD_{50}, classification is not warranted because the LD_{50}s from the key studies were between 3200-4750 mg/kg bw; both outside the border for Acute oral Category 4 of 300 to 2000 mg/kg bw.

For the inhalation LC_{50}, classification for Acute inhalation Category 3 is warranted because the mouse LC_{50} of less than 9.7 mg/L (vapour) is >2 but ≤ 10 mg/L. Although lower concentrations were not tested, the absence of overt toxicity in rats exposed to 1.23 mg/L for 6 hours/day, 5 days a week for 4 weeks indicates that classification in category 1 or 2 is not justified (see 4.12).

For the dermal LD_{50}, classification is not warranted because the LD_{50} from the key study of greater than 5000 mg/kg bw is outside the border for Acute dermal Category 4 of 1000 to 2000 mg/kg bw.

4.2.5 Conclusions on classification and labelling

Classification of *ρ-cymene* for acute inhalation toxicity as Acute Tox. 3 (H331: Toxic if inhaled) is required.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

Summary of relevant acute oral toxicity studies provided in CLH report for p-cymene (modified from Table 11a of the CLH report):

Method	Results	Remarks	Reference
Rat (Osborne-Mendel) Oral Dose: unspecified doses Purity: not provided N= 10/sex/dose	LD_{50}: 4 750 mg/kg bw (95 % confidence limits: 3 720-6 060)	2 (reliable with restrictions) Key study	EPA, 2005, 2012
Rat Oral gavage Dose: 620, 940, 1 400, 2 100, 3 200, 4 700, 7 100 or 10 700 mg/kg bw. Purity: not provided N= 1-3/dose	LD_{50}: 3 200 mg/kg bw	2 (reliable with restrictions) Key study	EPA, 2005, 2012
Mouse oral: unspecified exposure regimen Dose: no data Purity: not provided N= not provided	LD_{50}: 1 695 mg/kg bw	4 (not assignable) supporting study experimental result	Various SDSs

For the oral LD_{50}, two key studies were reported. In the second study, male and female rats (1-3/dose) were dosed by oral gavage with 620, 940, 1 400, 2 100, 3 200, 4 700, 7 100 or 10 700 mg/kg bw. Following a 14-day observation period, all rats in the 620, 940, 1 400 and 2 100 mg/kg bw groups survived and 1/2, 2/2, 3/3 and 1/1 had died in the 3 200, 4 700, 7 100 and 10 700 mg/kg bw groups, respectively. An LD_{50} of 3 200 mg/kg bw was determined in this study (EPA, 2005, 2012). In the first study, Osborne-Mendel rats (10/sex/dose) were administered various unspecified doses of the test substance. Rats were monitored for up to 2 weeks. An LD_{50} of 4 750 mg/kg bw was determined in this study (EPA, 2005, 2012). Various Safety Data Sheets (SDSs) have also reported an LD_{50} in mice of 1 695 mg/kg bw but the study where this LD_{50} was based on could not be found in the literature (Table above). Without the specific details of the study, an evaluation of the quality of the study cannot be made. Therefore the oral LD_{50} for rats is between 3 200 and 4 750 mg/kg bw.

No classification of p-cymene was proposed by the DS for acute oral toxicity.

Inhalation route

For the inhalation LC_{50}, one key study was reported for p-cymene (purity: not provided). Guinea pigs, rats and mice (2-3 animals per species, sex not indicated) were exposed by inhalation to a concentration of 9.7 mg/L (vapour) for 5 hours. Animals were observed for 1 week following exposure. No deaths were reported in guinea pigs or rats. All exposed mice died during or within 24 hours of exposure. The reported LC_{50}s were as follows: LC_{50} (guinea pig) > 9.7 mg/L; LC_{50} (rat) > 9.7 mg/L; and LC_{50} (mouse) < 9.7 mg/L (EPA, 2005, 2012). As the quality of the tests in these three species is comparable and it is unknown which species is the most relevant for humans, the most sensitive species was used for determination of the classification in line with the Guidance on the CLP criteria version 5 chapter 3.1.2.3.2.

Therefore, classification of p-cymene for acute inhalation toxicity as Acute Tox. 3; H331 – Toxic if inhaled) was proposed by the DS.

Dermal route

Summary of relevant acute dermal toxicity studies provided in CLH report for p-cymene (modified from Table 11c of the CLH report):

Method	Results	Remarks	Reference
Rabbit Dermal Dose: 5 000 mg/kg bw Purity: not provided N=10 (number per sex not reported)	LD_{50} > 5 000 mg/kg bw	2 (reliable with restrictions) Key study	EPA, 2005, 2012
Rabbit Dermal Dose: 5 140 mg/kg bw Purity: not provided N=1	no mortality in single test animal	3 (not reliable) Supporting study	EPA, 2005, 2012

For the dermal LD_{50}, the key study indicated in the table above was used. Ten rabbits were exposed to p-cymene at a dermal dose of 5 000 mg/kg bw. Animals were observed for 14 days. No animals died during the observation period. The reported LD_{50} was greater than 5 000 mg/kg bw (EPA, 2012). Therefore, no classification of p-cymene was proposed by the DS for acute dermal toxicity.

Comments received during public consultation

Concerning acute inhalation toxicity of p-cymene, one MSCA noted that "*considering that the mouse is the most sensitive species, results from the reported rat studies cannot be regarded as adequate justification for excluding categories 1 and 2*".

One company (the lead registrant) agreed with the conclusion that no classification and labelling is required for acute oral and dermal toxicity, but not with the proposal to classify as

Acute Tox. 3 (toxic if inhaled). Their argument was based on the effects having been seen exclusively in the mouse, and not in the rat (or guinea pig).

The DS responded as follows: "*as the quality of the acute inhalation toxicity studies with the three species is comparable and it is unknown which species is the most relevant for humans, the most sensitive species is used for determination of the classification (in line with the Guidance on the CLP criteria version 5.0 (July 2017) chapter 3.1.2.3.2)*".

Assessment and comparison with the classification criteria

For the oral LD_{50}, classification is not warranted because the LD_{50}s from the key studies were between 3 200-4 750 mg/kg bw; both outside the border for Acute oral Category 4 of 300 to 2 000 mg/kg bw.

Classification for acute inhalation toxicity in Category 3 is considered warranted because the mouse LC50 is less than 9.7 mg/L (vapour) which is > 2 but ≤ 10 mg/L, but lower concentrations were not tested. Considering that the only results from the reported mouse study were that the mouse LC_{50} is less than 9.7 mg/L, classification in categories 1 and 2 for acute inhalation toxicity cannot be excluded but with the available data it is very speculative. In addition, exposure time for all tested animals was 5 hours, which is longer than the 4-hour experimental exposure period required in the classification criteria for acute inhalation toxicity (CLP Regulation, 3.1.1.1), overestimating the toxicity. RAC also notes that and no deaths in guinea pigs or rats were seen after exposure to p-cymene at 9.7 mg/L, so the LC_{50} value in rats and guinea pigs can be estimated to be higher than 9.7 mg/L. Based on the data available, classification in category 3 could be supported. Since the exact values of LC_{50} in mice has not been determined, all exposed mice died within 24 hours after 5 hour inhalation exposure at 9.7 mg/L (vapour), RAC agrees to use the standard acute toxicity point estimate (ATE) value for category 3, i.e. 3 mg/L, for cymene (see table 3.1.2 of Regulation 1272/2008).

For the dermal LD_{50}, classification is not warranted because the LD_{50} value from the key study is greater than 5 000 mg/kg bw, which is outside the range for acute dermal toxicity, Category 4 (1 000 to 2 000 mg/kg bw).

RAC supports of DS proposal for classification of p-cymene for acute inhalation toxicity as **Acute Tox. 3; H331 – Toxic if inhaled,** with an **ATE value of 3 mg/L,** and no classification for acute oral or dermal toxicity.

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

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the acute toxicity studies as summarised in chapter 4.2.1 no specific effects on target organs were observed.

4.3.2 Comparison with criteria

Substances should be classified for STOT-SE when specific target organ toxicity (Cat 1 or 2) or narcotic effects or respiratory tract irritation (Cat 3) are observed.
As no specific organ effects fulfilling the classification criteria for specific organ toxicity – single exposure (STOT SE) were observed after single acute exposure via the oral, inhalation or dermal route, classification of *ρ-cymene* for STOT-SE is not required.

4.3.3 Conclusions on classification and labelling

Classification for Specific target organ toxicity – single exposure (STOT SE) is not required for *ρ-cymene*.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

In the acute toxicity studies (described above) no specific effects on target organs were observed, therefore no classification of p-cymene was proposed by the DS for STOT SE.

Comments received during public consultation

One company (the lead registrant) agreed with the proposal for no classification of p-cymene for STOT SE.

Assessment and comparison with the classification criteria

Substances should be classified for STOT-SE when specific target organ toxicity (Category 1 or 2) or narcotic effects or respiratory tract irritation (Category 3) are observed.

As no specific organ effects fulfilling the classification criteria for STOT SE were observed after single acute exposure via the oral, inhalation or dermal route, **classification of p-cymene for STOT SE is not required**.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit Dermal Dose: 5000 mg/kg. Purity: not provided N= 10 Observation period: 14 d	Slight redness: 3/10 Moderate redness: 7/10 Slight edema: 3/10 Moderate edema: 7/10	2 (reliable with restrictions) Key study	(EPA 2005, 2012)
Rabbit (Albino) Dermal Dose: 6 ml/kg bw or 5144 mg/kg bw Purity: 100% N=1 Observation period: 1 h- 1 month	After 1 h: Slight hyperemia of the skin which persisted for ~4h. After 4 h: Slight subcutaneous edema After 6 h: Slightly edematous skin 5 d: Skin was slightly thickened, hyperemic and showed fine cracks 1 month: skin began to return to normal	3 (not reliable) Supporting study	(EPA 2005, 2012)

4.4.1.1 Non-human information

Key Study

Acute exposure of 10 rabbits dermally treated with 5000 mg/kg bw of ρ-*cymene* and observed for 14 days. No rabbits died. Skin irritation was graded as follows: slight redness (3/10), moderate redness (7/10), slight edema (3/10), and moderate edema. No information is available on the quantitative skin irritation scores and whether the effects were observed immediately after exposure or continued until the end of the 14-d observation period (EPA 2005, 2012).

Supporting Study

Acute Exposure of undiluted ρ-*cymene* was applied to the shaven abdominal skin (10 x 15 cm area) of one single albino rabbit in 1 mL doses every hour for a total of 6 mL over a 6-hour exposure period. The rabbit was observed for 1 month following treatment. Slight hyperemia of the skin was observed after 1 hour and persisted approximately 4 hours after which a slight subcutaneous edema developed. After the exposure period, the skin still was slightly edematous and over the next 5 days, it was slightly thickened, hyperemic and showed fine cracks. After the first week, the skin began to return to normal and within the month it was normal with hair growth (EPA 2005, 2012). No quantitative information on the scores of skin irritation are available.

4.4.1.2 Human information

ρ-cymene is reported to be a primary skin irritant; contact with the undiluted liquid can produce erythema, dryness and defatting, the intensity depending on the dose and duration of contact (EPA 2005, 2012; TOXNET 2014). However, no study data are available to support this statement.

4.4.1.3 Summary and discussion of skin irritation

See sections 4.1.1.1 and 4.1.1.2.

4.4.1.4 Comparison with criteria

Classification is required when (1) a mean score at or above 2.3 is observed for erythema/eschar or for oedema from gradings at 24, 48 and 72 hours in 2 or more out of 3 animals; or (2) inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or (3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Based on the available data for *ρ-cymene*, all of these criteria are not met and classification is not warranted. Although the acute dermal toxicity data (rabbit) provide some evidence for skin irritation, no quantitative information is available to make a comparison with the criteria. Further, the statement on skin irritation in humans is not supported with data.

4.4.1.5 Conclusions on classification and labelling

Although there is some evidence for skin irritation, the available data is limited and classification for p-*cymene* cannot be sufficiently justified.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Animal data

In the first of the two acute dermal toxicity studies relevant for skin irritation (cf. CLH report, section 4.4, Table 12) 10 rabbits were dermally treated with 5 000 mg/kg bw of p-cymene and observed for 14 days. No rabbits died. The skin irritation results were described as follows: slight redness (3/10), moderate redness (7/10), slight oedema (3/10), and moderate oedema (7/10). No information was available on the quantitative skin irritation scores and whether the effects were observed immediately after exposure or continued until the end of the 14-day observation period (EPA, 2005, 2012).

In the second acute dermal toxicity study, undiluted p-cymene was applied to the shaven abdominal skin (10×15 cm area) of a single albino rabbit in 1 mL doses every hour for a total of 6 mL over a 6-hour exposure period. The rabbit was observed for one month following treatment. Slight hyperaemia of the skin was observed after 1 hour, persisting for approximately 4 hours, after which a slight subcutaneous oedema developed. After the exposure period, the skin was still slightly oedematous and over the next 5 days, it was slightly thickened, hyperaemic and showed fine cracks. After the first week, the skin began to return to normal and within the month it was normal with hair growth (EPA, 2005, 2012). No quantitative information on the scores of skin irritation are available.

Human information

p-Cymene was reported to be a primary skin irritant; contact with the undiluted liquid can produce erythema, dryness and defatting, the intensity depending on the dose and duration of contact (EPA, 2005, 2012). However, no study data were available to support this statement.

No classification of p-cymene was proposed by DS for skin corrosion/irritation.

Comments received during public consultation

One company (the lead registrant) agreed with no classification for skin irritation.

Assessment and comparison with the classification criteria

Based on the available data for p-cymene, none of the criteria are met and classification is therefore not warranted. Although the acute dermal toxicity data (rabbit) provide some evidence for skin irritation, no quantitative information is available to make a comparison with the criteria. Furthermore, the statement in the CLH report (referred to above) on skin irritation in humans is not supported by data.

RAC agrees with the DS's proposal for **no classification of p-cymene for skin corrosion/irritation due to inconclusive data.**

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 sensititsation

Table 13: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Human Dermal Concentration: 4% Purity: not provided N= 25 Observation period: not provided	No sensitisation reactions were reported	2 (reliable with restrictions) Key study	(TOXNET 2014)

4.6.1.1 Non-human information

No relevant information available.

4.6.1.2 Human information

A maximization test carried out on 25 volunteers showed that a 4% concentration of ρ-*cymene* in petrolatum produced no sensitisation reactions (TOXNET 2014).

4.6.1.3 Summary and discussion of skin sensitisation

See section 4.6.1.2.

4.6.1.4 Comparison with criteria

Although the available data are limited, no classification is warranted given that ρ-*cymene* did not induce any sensitisation reactions in 25 volunteers at a concentration of 4%.

4.6.1.5 Conclusions on classification and labelling

Data is conclusive but does not warrant classification for skin sensitisation of *ρ-cymene*

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Animal data

No relevant information available.

Human data

A maximization test carried out on 25 volunteers showed that a 4 % concentration of p-cymene (purity not provided) in petrolatum produced no sensitisation reactions (EPA, 2005). There were no data on skin sensitisation properties of the substance at concentrations higher than 4 %.

Comments received during public consultation

One company (the lead registrant) agreed with no classification for skin sensitisation.

Additional key elements

During the preparation of this opinion it was noted that in the parallel dossier, submitted by the same DS, on alpha-terpinene in one of the publication (Rudbäck *et al.*, 2012) the results of a Local Lymph Node Assay (LLNA) with p-cymene were provided, because p-cymene is one of the oxidation product of alpha-terpinene after exposure to air.

Table 2*. Summary table of Rudbäck et al., 2012, skin sensitisation study*

Method	Results			Remarks	Reference
In vivo LLNA Female CBA/Ca mice (around 8 weeks old)	Concentrations of p-cymene (% w/v) 0 (control) 0.1 1 5 10 25	3[H]thymidine incorporation (dmp/lymph node) 655 565 787 905 1 823 1 497	Stimulation Index (SI) - 0.9 1.2 1.4 2.8 2.3 EC3 > 30 % w/v	Klimisch score 2 (reliable with restrictions)	Rudbäck *et al.*, 2012

None of p-cymene concentrations tested in this LLNA (0.1-30 % w/v) induced a stimulation of cell proliferation above 3, i.e. a positive response in the LLNA. Nevertheless, it cannot be excluded that a positive response in this assay could be produced by higher concentrations of p-cymene.

Assessment and comparison with the classification criteria

Taking into account the information described above, RAC concludes that the data/information provided are not sufficient for assessing skin sensitisation of p-cymene, therefore **no classification is warranted due to inconclusive data**.

4.6.2 Respiratory sensititsation

Not considered in this report

4.7 Repeated dose toxicity

Not considered in this report

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See section 4.12 for a description of a subacute inhalation neurotoxicity study in rats.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The only available repeated dose toxicity study with *ρ-cymene* (i.e. subacute inhalation neurotoxicity study in rats; see section 4.12) did not result in clear toxic effects. Further data is lacking, therefore the criteria for classification of STOT RE are not met.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

There is no evidence of specific organ toxicity – repeated exposure, therefore classification for STOT RE is not required for *ρ-cymene*.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal According to the DS no relevant information on oral, inhalation or dermal repeated dose toxicity are available. However, this hazard can be assessed based on the subacute inhalation neurotoxicity study in male Long-Evans rats (described in detail in the CLH report, section 4.12.1.1; Neurotoxicity). In this study (non-GLP, not performed according to recognised international guidelines) rats were housed 2 per cage and subjected to a 12-hour light cycle, and exposed to p-cymene (purity 99 %) vapour at doses of 0, 50, or 250 ppm (approximately 0.25 and 1.23 mg/L) for 6 h/day, 5 days/week for 4 weeks during the dark cycle. There was no overt toxicity in the treated rats and no effect on body weight or terminal weight of the brain, cerebellum or whole brain. There was also no effect on regional enzyme activities, regional protein synthesis or regional neurotransmitter concentrations. At up to 250 ppm, p-cymene exposure did not produce signs of overt toxicity in male rats exposed for 4 weeks with an 8-week recovery period (EPA, 2005). According to the DS, the results of the only available repeated dose toxicity study with p-cymene do not warrant classification for STOT RE.

Comments received during public consultation

The lead registrant agreed with the conclusion that based on the data available no classification with regard to STOT RE is triggered. However, following completion of the on-going OECD TG 422 study, the classification might require a reassessment depending on the results obtained.

Assessment and comparison with the classification criteria

In the neurotoxicity study, rats were exposed to p-cymene at concentrations (0.25 and 1.23 mg/L) below the guidance value of ≤ 0.6 mg/L for STOT RE 1 and below the guidance value of ≤ 3 mg/L for STOT RE 2. The treatment did not affect the body weight or terminal weight of the cerebellum or whole brain. However, no data on effect of p-cymene on behaviour of animals, performance in functional, behavioural tests or on effects in other internal organs of exposed rates were provided in the scanty description of this study. Taking into account the deficiency of the reported study in the assessment of repeated dose toxicity, RAC concludes that **no classification for STOT RE is warranted for p-cymene due to inconclusive data.**

4.9 Germ cell mutagenicity (Mutagenicity)

Table 14: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Paper disk method, bacterial reversion assay E.coli Sd 4-73 Test concentrations: 0.01 – 0.025 ml/plate Purity: not provided Positive control substance: not mentioned	No increase in the frequency of revertants	2 (reliable with restrictions) Key study Experimental result	(EPA 2005; TOXNET 2014)
Sprague-Dawley rats Gavage 0.5 ml of ρ-*cymene* (approximately 1706 mg/kg bw) urine was collected for 24 h N = 2 Positive control substances: sodium azide (TA100), picrolonic acid (TA98) and aflatoxin B1 (activation of S9 fraction)	No increase in mutant frequency was observed.	2 (reliable with restrictions) Supporting study Experimental result	(Anonymous 1979)

4.9.1 Non-human information

4.9.1.1 In vitro data

Key study

Escherichia coli strain Sd-4-73 was cultured overnight at 36°C in an aerated nutrient broth containing 20 µg/mL streptomycin. Plates were prepared and ρ-*cymene* was added by applying to a paper disk (0.01-0.025 mL or small crystal), which was then placed on the agar. Relative mutagenicity, defined as "an approximate ratio of the number of colonies on the plate containing the mutagen to the number of colonies on the control plate", was calculated. Potent mutagens had relative mutagenicities of greater than 3 and weak and doubtful mutagens had relative mutagenicities between 1.5 and 3. ρ-*Cymene* produced no increase in the frequency of reversion from streptomycin dependence to independence in Sd-4-73 E. coli (EPA 2005; TOXNET 2014).

4.9.1.2 In vivo data

In a study designed to investigate the mutagenicity in vivo-in vitro of urinary metabolites of a number of food additives, Sprague-Dawley rats were given 0.5 ml of ρ-*cymene* (approximately 1706 mg/kg bw) by gavage and urine was collected for 24 h. Three types of urine samples were tested in the Ames assay with *S. typhimurium* strains TA98 and TA100 with metabolic activation: a direct urine sample, a urine-ether extract, and the aqueous fraction of the urine–ether extract. The urine samples of rats treated with ρ-*cymene* did not show any evidence of mutagenicity, either in the presence or absence of beta-glucuronidase (Anonymous 1979; EFSA 2015).

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

Two studies (one in vitro and one in vivo study) testing for the mutagenicity of p-*cymene* had negative results. These tests were non-standard but further data on this endpoint is lacking. .

4.9.5 Comparison with criteria

Considering that no positive response was observed in the available *in vitro* and *in vivo* mutagenicity tests, no classification is required for *ρ*-cymene.

4.9.6 Conclusions on classification and labelling

Classification for mutagenicity is not warranted for *ρ*-cymene due to the lack of observed mutagenicity in vitro or in vivo.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS presented in the CLH report two studies on mutagenicity of p-cymene reported in greater detail elsewhere (one in EPA, 2005 and the other in EFSA 2015; Anonymous, 1979) but none of them were reported to be performed in compliance with GLP or according to internationally recognised guidelines.

Based on results of these studies, the DS concluded that no classification for mutagenicity is warranted due to the lack of observed mutagenicity *in vitro* or *in vivo*.

Comments received during public consultation

The lead registrant agreed with the conclusion that based on the data available no classification with regard to mutagenicity or carcinogenicity is triggered.

Assessment and comparison with the classification criteria

In the first, non-GLP and performed not according to recognized guideline study (EPA, 2005) p-cymene produced no increase in the frequency of reversion from streptomycin dependence to independence in *Escherichia coli* strain Sd-4-73 cultured *in vitro*.

In the second, non-GLP, and performed not according to recognized guideline study (EFSA 2015; Anonymous, 1979) the Sprague-Dawley rats were given 0.5 mL of p-cymene (approximately 1 706 mg/kg bw) by gavage and urine was collected for 24 h. Three types of urine samples were tested in the Ames assay with *S. typhimurium* strains TA98 and TA100 with metabolic activation: a direct urine sample, a urine-ether extract, and the aqueous fraction of the urine–ether extract. The urine samples of rats treated with p-cymene did not show any evidence of mutagenicity, in either the presence or absence of beta-glucuronidase.

None of the tests reported in the CLH report meets criteria of well conducted, sufficiently validated test, performed according to methods described in Regulation (EC) No 440/2008. They are also not listed among the tests recommended in section 3.5.2.3. of the CLP Regulation for assessment of mutagenicity to be used for classification for heritable effects in human germ cells. Therefore, RAC is of the opinion that **no classification for germ cell mutagenicity for p-cymene is warranted due to lack of data.**

4.10 Carcinogenicity

Not considered in this report

4.11 Toxicity for reproduction

Not considered in this report

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 15: Summary table of relevant neurotoxicity studies

Method	Results	Remarks	Reference
Long Evan rats (male), inhalation of p-*cymene* vapour Test concentrations: 0 – 250 ppm as vapour Rats exposed for 6 hours/day, 5 days/week for 4 weeks during dark. Allowed to recover for 8 weeks (with weekly body weight measurements) before decapitation. Endpoints include brain weight, neurotransmitter and enzyme activity.	No overt toxicity	2 (reliable with restrictions) Key study Experimental result	(EPA 2005; TOXNET 2014)

This study was designed to specifically examine the neurotoxic potential of inhaled ρ-*cymene*. Male Long-Evans rats were housed 2 per cage and subjected to a 12-hour light cycle. Exposure to ρ-*cymene* (purity 99%) vapour at doses of 0, 50, or 250 ppm (approximately 0.25 and 1.23 mg/L) for 6 hr/day, 5 days/wk for 4 weeks occurred during the dark cycle and rats were placed in stainless steel wire cages without food or water. Air exchange in the exposure chambers was 13 times/hour with a temperature of 23±2°C. ρ-*Cymene* concentration in the exposure chamber was measured every 10 minutes with an infrared gas cell spectrophotometer. An 8-week post-exposure observation period was included. During the study, body weight was recorded weekly. After the 8-week recovery period, rats were decapitated and the cerebellum was removed, weighed, and homogenized (4 mL ice cold 0.32 M sucrose). The remainder of the brain was also weighed and homogenized. Synaptosomes were prepared using gradient centrifugation. The 2 homogenates and the synaptosomes were processed for neurotransmitter analyses (i.e. determination of noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT)), and aliquots were taken for determination of enzyme activities (lactate dehydrogenase (LDH), acetylcholinesterase (AChE), and butylnylcholinesterase (BuChE)) and protein analysis. The researchers reported that there was no overt toxicity in the treated rats and no effect on body weight or terminal weight of the brain, cerebellum or whole brain. There was also no effect on regional enzyme activities, regional protein synthesis or regional neurotransmitter concentrations. The relative yield and total amount of synaptosomal protein were significantly reduced at 50 and 250 ppm in a concentration-related manner. Relative yield for control, 50 and 250 ppm = 16.4, 9.20, and 8.62 mg protein/g whole brain-cerebellum, respectively. Total amount for control 50, and 250 ppm = 29.1, 16.4, and 15.1 mg protein/g whole brain-cerebellum, respectively. The relative activity of LDH, AChE, and BuChE were significantly increased at 50 and 250 ppm. For control, 50 and 250 ppm, respectively: relative LDH activity = 2,7, 4.87, and 5.33 U/mg protein; relative AChE activity = 159, 291, and 288 mU/mg protein; relative BuChE activity = 209, 386, and 358 mU/mg protein. Total activity of LDH, AChE and BuChE were unaffected. In relation to the cytoplasmatic marker (LDH), the relative synaptosomal choline esterase activities (AChE and BuChE) were unaffected by ρ-*cymene* exposure. In relation to LDH, the relative synaptosomal concentrations of NA, DA, and 5-HT were unaffected by treatment. Relative to synaptosomal protein, relative NA and DA concentrations were significantly increased at 50 and 250 ppm; whereas 5-HT was unaffected. For control, 50, and 250 ppm, respectively: relative NA = 18.4, 34.4, and 31.3 pmol/mg synaptosomal protein; relative DA = 19.8, 38.0, and 36.8 pmol/mg synaptosomal protein; relative 5-HT = 8.98, 12.4, and 13.1 pmol/mg synaptosomal protein. Conversely, the total amount of NA and DA in the synaptosomal fraction was unaffected by treatment; whereas, the total amount of 5-HT was significantly decreased at 250 ppm. For control, 50, and 250 ppm, respectively: total amount of NA = 522, 544, and 461 pmol/whole brain-cerebellum; total amount of DA = 553, 600, and 541 pmol/whole brain-cerebellum; total amount of 5-HT = 255, 194, and 189 pmol/whole brain-cerebellum. At up to 250 ppm, ρ-*cymene* exposure did not produce signs of overt toxicity in male rats exposed for 4 weeks with an 8-week recovery period (EPA 2005; TOXNET 2014).

4.12.1.2 Immunotoxicity

No relevant information available.

4.12.1.3 Specific investigations: other studies

ρ-Cymene has a kinematic viscosity of 7.1 mm^2/s at 40°C (see section 1.3, table 9), which might indicate the potential for aspiration toxicity.

4.12.1.4 Human information

No relevant information available.

4.12.2 Summary and discussion

A study focusing on the neurotoxic potential of inhaled *ρ-cymene* in rats did not reveal relevant effects for classification.

ρ-Cymene has a kinematic viscosity of 7.1 mm^2/s at 40°C (see section 1.3, table 9), which might indicate the potential for aspiration toxicity.

4.12.3 Comparison with criteria

Aspiration toxicity:

Substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard. A substance is classified in category 1 for aspiration toxicity:

(a) based on reliable and good quality human evidence or

(b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C.

Given that *ρ-cymene* is a hydrocarbon and has a kinematic viscosity of 7.1 mm^2/s at 40°C, classification of *ρ-cymene* for category 1 Aspiration toxicity is warranted.

4.12.4 Conclusions on classification and labelling

Classification of *ρ-cymene* for Aspiration toxicity as Asp. Tox 1 (H304: May be fatal if swallowed and enters airways) is required.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

p-Cymene has a kinematic viscosity of 7.1 mm^2/s at 40 °C (method not known, purity not provided; SDS, 2013), which might indicate the potential for aspiration toxicity.

The DS proposed that p-cymene be classified as Asp. Tox. 1; H304 – May be fatal if swallowed and enters airways.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

According to the CLP Regulation, a substance is classified in category 1 for aspiration toxicity:

- based on reliable and good quality human evidence or

- if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40 °C.

Given that the only data available indicates that p-cymene is a hydrocarbon and has a kinematic viscosity of 7.1 mm^2/s at 40 °C, p-cymene, despite the limitations of this data, it should be **classified for Aspiration toxicity in Cat. 1 (Asp. Tox 1; H304 – May be fatal if swallowed and enters airways)**.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of Terpenoid blend which contains *ρ-cymene* were assessed in the Draft Assessment Report (10th October 2013), 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 *ρ-cymene* were taken from the DAR. However, considering that the DAR is for the Terpenoid Blend QRD 460 that contains *ρ-cymene*, but also α-terpinene and d-limonene, data on *ρ-cymene* as a single compound was limitedly available. Other publically available data were obtained by searching several databases including e-chemportal, PubMed, NITE, ToxNet and publications such as the US Environmental Protection Agency report on screening hazard characterization of *ρ-cymene* (EPA 2012) and the flavour and fragrance high production volume consortia robust summary for *ρ-cymene* (EPA 2002). 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.

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Method: statement in DAR	*ρ-cymene* does not contain any functional groups that are susceptible to hydrolysis.		(Habig 2010) [a]
Half-life in air relative rate method (295±2 K; atmospheric pressure); non-guideline; not GLP.	hydroxyl radicals: ~ 1 day nitrate radicals: ~34 days	at an atmospheric concentration of 5 x 10^{-11} OH/cm^3 at an atmospheric concentration of 2.4 x 10^8 NO_3/ cm^3	(Corchnoy and Atkinson 1990) [b] (Corchnoy and Atkinson 1990); (Atkinson et al. 1990) [b]
QSAR: AOPWIN v1.92a	hydroxyl radicals: 15 h.		(US-EPA 2012)
Ready biodegradability OECD 301C; not GLP; non-radiolabeled *ρ-cymene*; purity 95.3%	ready biodegradable	88.0±6.2% degradation after 14 days based on BOD.	(NITE 2015) (Klopman and Tu, 1997) [c] refers to a Japanese MITI test with *ρ-cymene*. Study details were not presented in the DAR. The Rapporteur reassessed the original Japanese study report (see 5.1.2.2 for details). The study

			is considered reliable. The data are assigned a Klimisch score of 1 and are used for classification.
QSAR: BIOWIN v4.10	Not ready biodegradable BIOWIN 3: weeks BIOWIN 5 and 6: not readily biodegradable		(US-EPA 2012)
Water degradation study non-standard study similar to OECD 309; not GLP; aerobic; dark; non-radiolabeled *ρ-cymene*; purity 99.1%	DT50 = 11.2 hours	1 natural water; continuous aeration; rapid evaporation to trapping solution. The reported DT50 is a dissipation half-life not degradation half-life.	Moser 2011 [c]
Soil degradation study OECD 307; not GLP; aerobic, dark; non-radiolabeled *ρ-cymene*; purity 99.1%	DT50 = <24 hours	1 soil; rapid evaporation to trapping solution & escape from trapping solution. The reported DT50 is a dissipation half-life not degradation half-life.	Moser 2010 [c]

[a] As summarised in the DAR (Volume 3, annex B.1-5), May 2014.
[b] As summarised in (TOXNET 2014), accessed November 2014.
[c] As summarised in the DAR (Volume 3, annex B.8), July 2013.

5.1.1 Stability

There are no experimental data available for hydrolysis of *ρ-cymene*. *ρ-cymene* contains no functional groups that can hydrolyze such as esters, amides or epoxides. The vapor pressure of *ρ-cymene* is high (1.95– 2.67 x 10^2 Pa) and its solubility in water is relatively low (23 mg/L) giving a high Henry's Law Constant (1.38 x 10^3 Pa m3/mole) which predicts a high rate of volatility from water (EPI Suite version 4.0.) (DAR 2013). Other sources confirm the magnitude of the Henry's Law Constant (see paragraph 5.2.2).

Partitioning from water and soil to air is also corroborated by the level III fugacity model that was presented in the DAR and that was run simulating application of *ρ-cymene* to a crop (see paragraph 5.2.3). Persistence in the total system or DT_{100} was predicted to be 46.4 hours, extremely rapid for a pesticide, because most of the *ρ-cymene* will partition to air and be degraded via interaction with hydroxyl radicals rapidly (DAR 2013). In the DAR, it was stated there was no literature discussing the nature of the degradation of *ρ-cymene* in air available. Thus, for *ρ-cymene*, the rate of degradation in air was estimated. The half-life in air was calculated to be 15 hours for *ρ-cymene* based on an OH concentration of 1.5 x 10^6 OH/cm^3 and a 12 hour day, using AOPWIN (v1.92a) in EPI Suite (v4.0).

The Hazardous Substances Data Bank (HSDB) reports the following literature data on atmospheric degradation of *ρ-cymene* (TOXNET 2014). The rate constant for the vapor-phase reaction of *ρ-cymene* with photochemically-produced hydroxyl radicals has been measured as 1.5 x 10^{-11} cm^3/molecule-sec at 25°C (Corchnoy and Atkinson 1990). This corresponds to an atmospheric half-life of about 1 day at an atmospheric concentration of 5x 10^{-11} OH/cm^3 (Corchnoy and Atkinson 1990). The rate constant for the vapor-phase reaction of *ρ-cymene* with nitrate radicals has been measured as 9.9 x 10^{-16} cm^3 /molecule-sec at 25°C (Corchnoy and Atkinson 1990). This corresponds to an atmospheric half-life of about 34 days at an atmospheric concentration of 2.4 x 10^8 nitrate radicals per cm^3 (Atkinson et al. 1990).

ρ-cymene has a UV absorption maxima at 274 nm (log epsilon = 2.74) and a rapidly decreasing log epilson of about 1.1 at 280 nm (V. Talrose et al.); although *ρ-cymene* may have minor absorption >290 nm, direct photolysis is not expected to be an important environmental fate process (TOXNET 2014).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

The BIOWIN v4.10 QSAR contained within EPI Suite™ version 4.11 (US-EPA 2012) consists of six models. *ρ-cymene* is predicted to biodegrade fast using linear (BIOWIN 1) and non-linear (BIOWIN 2) biodegradation models. Ultimate biodegradation, i.e., conversion of *ρ-cymene* 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, *ρ-cymene* was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. *ρ-cymene* 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 *ρ-cymene* will not be readily biodegradable, the overall conclusion is that *ρ-cymene* is estimated not to be readily biodegradable.

5.1.2.2 Screening tests

The DAR states that for *ρ-cymene* a peer reviewed study from open literature was available which showed biodegradability of the substance. In section B.8.1.1. of the DAR, the following summary was presented. The biodegradation potential of *ρ-cymene* was evaluated using the MITI test method OECD 301C. The result for *ρ-cymene* was reported in a publication by Klopman and Tu, 1997. Specifically, 100 mg/L of the test chemical is incubated with 30 mg/L of sludge for up to 28 days. Reported activity is described as final day biochemical oxygen demand (BOD), i.e., oxygen uptake. In the case of *ρ-cymene*, final day BOD was 88% indicating extensive biodegradation. The brief summary in the DAR did not allow concluding on the reliability of this study. The reference Klopman and Tu (1997) does not contain the study data, but refers to a study conducted at MITI (Ministry of International Trade and Industry, Japan). Therefore, the dossier submitter consulted the NITE (National Institute of Technology and Evaluation, Japan) database (NITE 2015) to retrieve the original study report (see Annex 8.2.1 for the translated report) from which the missing study details and results were retrieved.

<u>Ready biodegradation study</u> *NITE study*

Reference	:	(NITE 2015)	study type		OECD 301C
year of execution	:	1987	incubation time	:	14 days
GLP statement	:	No	nominal concentration	:	100 mg/L
Guideline	:	MITI test method equivalent to OECD 301C	Temperature	:	25±1°C
test substance	:	*ρ-cymene*	Degradability	:	88.0±6.2% based on BOD
Purity	:	95.3% (*ρ-cymene*;K-696C)	Metabolites		not reported
test system	:	respirometer	Acceptability	:	**acceptable**

The biodegradation potential of *ρ-cymene* was evaluated using the MITI test method OECD 301C. The purity of *ρ-cymene* (K-696C) was 95.3%. The test was conducted at 25±1°C, for 14 days. The activated sludge was prepared as specified in OECD 301C, i.e. it was collected from 10 municipal wastewater sewage treatment plants, mixed and adjusted to pH 7. The suspended solids concentration of the sludge inoculum was 6000 mg/L. Test vessels were 300-mL culture bottles that were improved for volatile substances. Six bottles were prepared: One inoculum blank (mineral medium only), one abiotic control (*ρ-cymene* in water at 100 mg/L), one positive control (alanine at 100 mg/L in mineral medium with 30 mg/L suspended solids), and three test vessels (*ρ-cymene* at 100 mg/L in mineral medium with 30 mg/L suspended solids). During the test, precipitation of activated sludge, pH, temperature and dissolved oxygen concentration were measured. The test was performed in a respirometer. Lime was used as CO_2 absorbent. Besides oxygen consumption, gas chromatography (GC) was used to measure the test substance, and dissolved organic carbon was measured using a total organic carbon (TOC) meter. Recovery for the GC was reported to be 99.2% (water + test substance), and 96.8% (sludge + test substance). There was no oxygen consumption in the abiotic control and very limited oxygen consumption in the inoculum blank amounting to 3.8 mg O_2/L after 14 days. The percent degradation (based on BOD) in the positive control amounted to 53 and 87% after 7 and 14 days, respectively. For the test substance average degradation after 14 days amounted to 88.0±6.2% based on BOD, 88.7±1.2 based on TOC, and 100±0.0% based on GC. The 10-day window was met for all three replicates based on BOD. Thus, all validity criteria were met, and *ρ-cymene* was shown to readily biodegradable. Considering the above, this study is considered reliable without restrictions. **The data are assigned a Klimisch score of 1, and are used for classification purposes**.

5.1.2.3 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. α-terpinene, *ρ-cymene* and d-limonene, individually in separate test vessels. The relevant sections of the DAR summaries that report on ***ρ-cymene*** as a single compound are provided below.

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

reference	:	Moser, F.	study type		non-standard study with natural lake water similar to OECD 309
year of execution	:	2011	incubation time	:	48 hours; 96 hours
GLP statement	:	yes	nominal concentration	:	1 mg/L
guideline	:	none	Temperature	:	18.1-21°C
test substance	:	d-limonene, *ρ-cymene*, α-terpinene	DT50	:	11.2 hours (for *ρ-cymene*)
purity	:	99.1% (*ρ-cymene*; lot # 812108)	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 α-terpinene, *ρ-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 were analysed immediately after application (hour 0) and after 1, 3, 6, 24 and 48 hours. Samples of ρ-*cymene* were also taken after 96 hours. Their respective trapping solutions were also analysed.

Application solutions were prepared with a concentration of 0.946 mg a.i./L (d-limonene, 0.993 mg a.i./L *ρ-cymene* and 1.01 mg a.i./L α-terpinene. 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 ρ-*cymene* of 88.2% (low concentration) and 81.2% (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 *ρ-cymene* are shown and discussed below.

The purity of the *ρ-cymene* was determined to be 96.8% , which is slightly lower from the value reported with the test item.

<u>Water Quality</u>*:* 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 *ρ-cymene* the extracted concentration at t=0 was 0.776 and 0.848 mg a.i./L resp., which correspond to a recovery of 78.1 and 85.3% of the initial concentration of 0.993 mg a.i./L, similar to recoveries in the method validation. The concentration ρ-*cymene* in the extracts decreased continuously to below the LOQ of 0.0246 mg a.i./L. ρ-*cymene* was found in the trapping solution in the range from 0.182 mg a.i./L after 6 hours to 0.423 mg a.i./L after 96 hours. Detailed results for *ρ-cymene* are given in table 8.4.3.2-03.

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

Table B.8.4.3.2-02 Concentration of *ρ-cymene* in extracts and trapping solutions

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	0.776	81.7	0.783	-
0	0.848		0.855	-
1	0.445	45.8	0.449	<LOQ
1	0.464		0.468	<LOQ
3	0.479	44.4	0.483	<LOQ
3	0.403		0.407	<LOQ
6	0.395	40.1	0.399	<LOQ
6	0.401		0.404	<LOQ
24	0.200	24	0.202	0.300
24	0.278		0.280	<LOQ
48	<LOQ		<LOQ	0.215
48	<LOQ		<LOQ	0.385
96	<LOQ		<LOQ	0.366
96	<LOQ		<LOQ	0.423

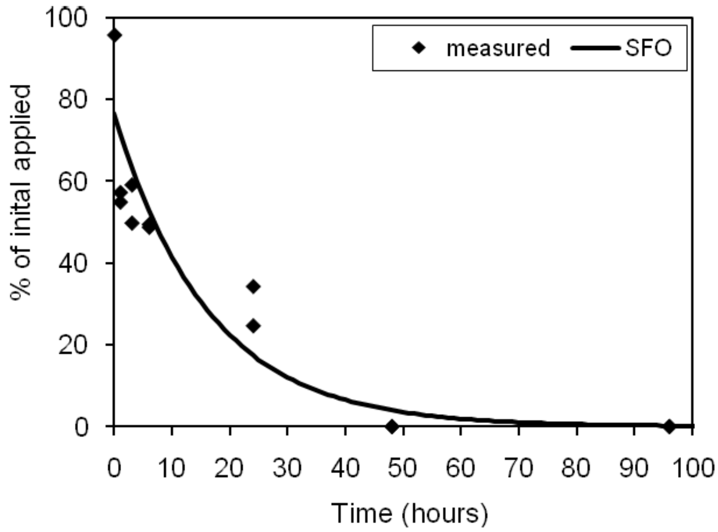
[a] The recovered concentration was calculated using the mg a.i./L divided by the purity of the test item, which was 99.1% for *ρ-cymene*

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

LOQ Limit of Quantification. Determined as 0.0246 mg a.i./L in extract and 0.199 mg a.i./L in trapping solution

Degradation rate: In figures B.8.4.3.2-02 the results of the kinetic fit using the FOCUS Kintetics spreadsheet are presented.

Figure 8.4.3.2-02 SFO degradation plot and error level Chi2 test of *ρ-cymene*.



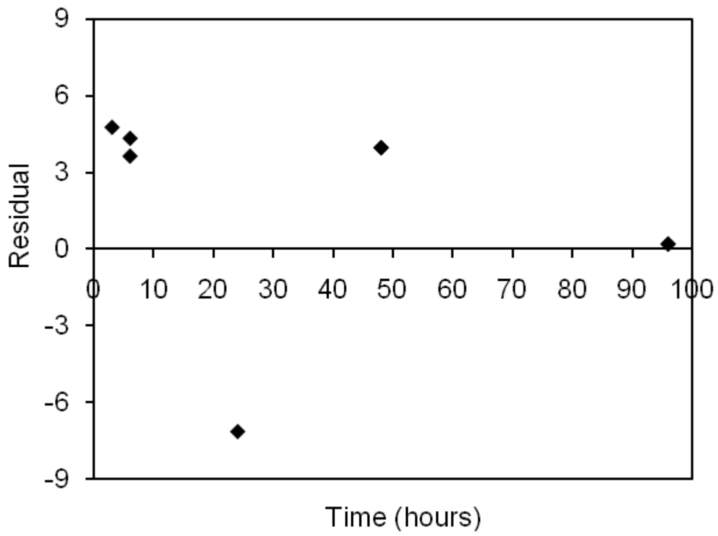


Table B.8.4.3.2-04 Summary of DT50 and DT90 values, SFO parameters and chi2 test.

	DT_{50} [hours]	**DT_{90} [hours]**	**M0 (fitted)**	**K (fitted)**	**Error level Chi^2 test**
ρ-*cymene*	11.2	37.4	76.72	0.062	23.8

<u>Conclusion:</u> ***ρ-cymene* volatilized from the natural water test systems rapidly with a DT50 of 11.2 and DT90s of 37.4 hours**. The trapping solution showed the presence of the test substance but no degradates. Degradates in the water were also not detected. Thus, **rapid escape (fugacity via volatility) appears to be the predominant pathway for *ρ-cymene* 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 *ρ-cymene* showed an increase in concentration of the a.i. in the trapping solution. The SFO fits shows for ρ-*cymene* a less optimal chi^2 value, however, the visual fit of the data is good. The distribution of residuals is less optimal again No t-test was performed.

RMS considers the DT_{50} value derived for ρ-*cymene* 11.2 hours.

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

Reference	:	Moser, F., 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	:	0.68 mg/kg *ρ-cymene*
guideline	:	OECD 307 (2002)	temperature	:	20°C
test substance	:	d-limonene, *ρ-cymene*, α-terpinene	DT50	:	<24 hours (for *ρ-cymene*)
purity	:	99.1% (*ρ-cymene*; lot # 812108).	metabolites	:	not applicable
soils	:	Sandy loam	acceptability	:	**acceptable**

The **aerobic soil degradation of** α-terpinene, ***ρ-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 α-terpinene, 0.68 mg/kg *ρ-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 analyzed after 0 and 7 hours, and 1, 2 and 3 days after application. The trap of the respective sample was analyzed too.

Duplicate samples for each test item were analyzed 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 analyzed by GC. The trapping solution was analyzed by GC without any further treatment. The analytical method was subject to validation as part of the study. The LOQ was 0.04 mg a.i./kg soil for *ρ-cymene*.

Only the results for *ρ-cymene* are shown and discussed below.

Table B.8.1.2.1-04 Concentration of ρ-*cymene*, in soil extracts and trapping solutions

Sample	Sample time	Concentration	Sample	Sample time	Concentration

	[hours]	**[mg a.i./kg]**
Soil extract	0	0.55 0.56
	7	<LOQ 0.10
	12	<LOQ <LOQ
	24	n.d. n.d.
	36	<LOQ n.d.

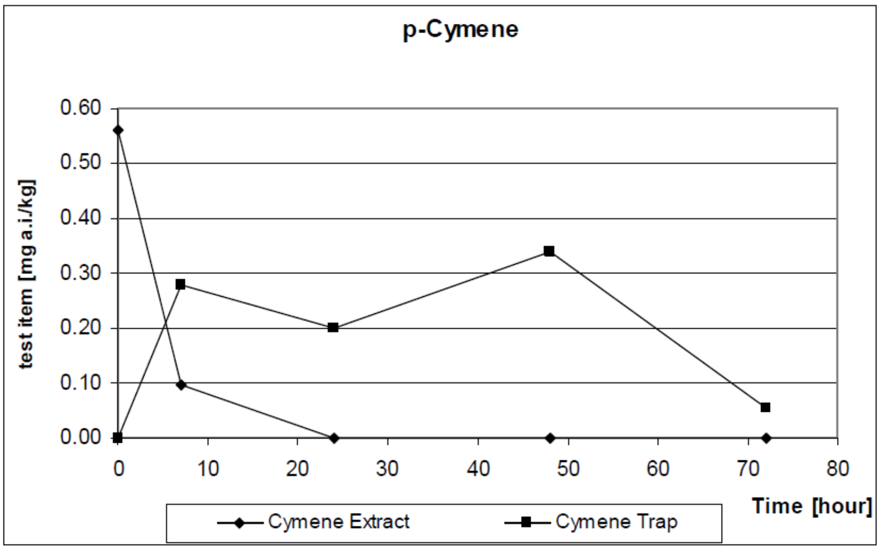
	[hours]	**[mg a.i./L]**
Trap	-	-
	7	0.27 0.29
	12	0.40 <LOQ[a]
	24	0.35 0.33
	36	<LOQ[a] 0.11

n.d. not detectable
na not applicable
[a] LOQ = 0.201 mg a.i./L. (concentration of lowest analytical standard)

In the soil extract of *ρ-cymene* of T0, 0.55 and 0.56 mg a.i./kg of the applied 0.59 mg a.i./kg were found. No degradation products were detected. 7 hours after application, the soil extract of one replicate contained 0.10 mg a.i./kg ρ-*cymene*, whereas the other replicated showed a concentration <LOQ (<0.04 mg a.i./kg). On day 1, both of the replicates with *ρ-cymene* showed concentrations <LOQ and from day 2 onwards there was no detectable residue.

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 8.1-02 Distribution of *ρ-cymene* in soil extract and trapping solution



It was concluded that *ρ-cymene* 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.

<u>RMS comment:</u> 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 (α-terpinene, ρ-*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**. The result show that α-terpinene, *ρ-cymene* and d-limonene disappear rapidly from the soil with a **DT_{50} of <24 hours**.

5.1.3 Summary and discussion of degradation

The biodegradation potential of *ρ-cymene* was evaluated using the MITI test method OECD 301C with degradation after 14 days amounting to 88 ± 6.2% based on oxygen uptake (BOD). Degradation was confirmed by dissolved organic carbon measurements (TOC), and chemical analysis (GC). Furthermore, the 10-day window was met. Therefore, *ρ-cymene* is considered readily biodegradable.

The available water and soil degradation studies with *ρ-cymene* 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 *ρ-cymene* evaporated to the trapping solution. No degradation products were detected. The DT50 was calculated to be 11.2 hours but the disappearance was considered to be caused by evaporation rather than biodegradation. The aerobic soil simulation study also used non-radiolabelled *ρ-cymene*, and evaporation to the trapping solution was shown as the predominant disappearance route. Therefore, these studies cannot be used to assess the biodegradability of *ρ-cymene*.

The BIOWIN QSAR predicts that *ρ-cymene* 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 *ρ-cymene* will not biodegrade rapidly. The BIOWIN QSAR estimate will not be used for classification as a reliable ready biodegradability study is available.

Considering the above, *ρ-cymene* is considered to be rapidly degradable for the purpose of classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

No experimental studies on the sorption behaviour of *ρ-cymene* in soil are available. In the DAR, the RMS agreed with the registrant that such a study cannot be performed based on the volatility of *ρ-cymene*. An estimated K_{oc} of 3614 L/kg was reported in the DAR that was calculated with the KOCWIN (v2.00) in EPI Suite (v4.0). The height of this value indicates that *ρ-cymene* should sorb relatively strong to soil and sediment.

5.2.2 Volatilisation

The vapor pressure of *ρ-cymene* is high (1.95 – 2.67 x 10^2 Pa) and its solubility in water is relatively low (23 mg/L) giving a high Henry's Law Constant (1.36 x 10^3 Pa m^3/mol) which predicts a high rate of volatility from water (EPI Suite version 4.0.). Similarly high Henry's Law Constant values have been reported for *ρ-cymene* by other sources, i.e. in the US-EPA report on screening hazard characterization of *ρ-cymene* a measured value of 1.11 x 10^3 Pa m^3/mol was reported (EPA 2012), and a recent peer-reviewed publication that compiled 17350 values of Henry's law constants for 4632 species, collected from 689 references reported for *ρ-cymene* eight Henry's law constants ranging 5.5 x 10^2 - 1.1 x 10^3 Pa m^3/mol in (Sander 2015). Of these eight values, two were experimentally determined using the headspace analysis method, i.e. 5.5 x 10^2 Pa m^3/mol by Hiatt (2013) and 1.1 x 10^3 Pa m^3/mol by (Kondoh and Nakajima 1997).

In a river, 1 meter deep and a current velocity of 1 meter/second and a wind velocity of 5 meters/second, the volatilization half-life of *ρ-cymene* is predicted to be 1.2 hours. In a lake 1 meter deep with a current velocity of 0.05 meters/second and a wind velocity of 0.5 meters/second, the volatilization half-life of *ρ-cymene* is predicted to be 111 hours (4.6 days [EPI Suite v4.0]).

The predictive model provides an idea as to the volatility of *ρ-cymene* from natural waters. Furthermore, a water study under static water conditions that was submitted in the DAR (see paragraph 5.1.2.3) showed that 90% of *ρ-cymene* is volatilized within 37.4 hours (DAR 2013).

5.2.3 Distribution modelling

The fugacity model in EPI Suite v4.0 is a Level III multimedia fate model using environmental parameters identical to those used in MacKay *et al*. 1992. The model is reduced to four main compartments, namely, air, water, soil and sediment. The distribution of the chemical and the environmental compartments depends on how the chemical is introduced in Level III. In the DAR, the application of *ρ-cymene* to a crop was simulated by assuming deposition from spraying plants was 90% to the air (representing a combination of what deposited on the crop foliage and what remained in the air following application), 1 % drift to an adjacent water body and the remainder (9%) reaching the soil and not the crop canopy. For *ρ-cymene*, the fugacity model outputs are provided in the Table B.8-03. Input parameters were based on estimations within EPI Suite except for vapour pressure and water solubility which were taken from the *ρ-cymene* database. The Henry's Law constant was calculated from these data. The complete EPI Suite modelling run can be found in Schocken 2011.

Table B.8-03. Fugacity model outputs for *ρ-cymene*.

Compartment	Mass Amount (%)	Half-life (hours)	Reaction (%)	Advection (%)
Air	41.2	17	77.9	19.1
Water	4.14	360	0.37	0.192
Soil	54.5	720	2.44	0
Sediment	0.161	3240	0.0016	0.00015

In the DAR it was noted that the main environmental compartment receiving *ρ-cymene* was air (see Level I) which also degraded *ρ-cymene* much faster than the soil, sediment and water compartments. It is notable that the environmental compartment distribution in Level III is based on reaching steady state conditions and not equilibrium in a closed system.

Persistence in the total system or DT_{100} was predicted to be 46.4 hours, extremely rapid for a pesticide, because most of the *ρ-cymene* will partition to air and be degraded via interaction with hydroxyl radicals (discussed further in paragraph 5.1.1) rapidly.

5.3 Aquatic Bioaccumulation

No experimental studies on the bioaccumulation potential of *ρ-cymene*. Estimated BCF values have been calculated with the BCFBAF (v3.01) in EPI Suite (v4.11).

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
QSAR: BCFBAF v3.01	236 L/kg wet-wt	regression based method; log K_{ow} = 4.1	(US-EPA 2012)
QSAR: BCFBAF v3.01	520.5 L/kg wet-wt	Arnot-Gobas (upper trophic) method incl. biotransformation rate estimates; log K_{ow} = 4.1	(US-EPA 2012)
QSAR: BCFBAF v3.01	1290 L/kg wet-wt	Arnot-Gobas (upper trophic) method assuming a biotransformation rate of zero; log K_{ow} = 4.1	(US-EPA 2012)

5.3.1 Aquatic bioaccumulation

In the DAR an experimentally determined log K_{ow} of 5.08 is reported. This study was considered unreliable by the dossier submitter (see Table 9 for details), and the outcome cannot be used for classification. The flavour and fragrance high production volume consortia (FFHPVC) (EPA 2002) reported an experimentally determined log K_{ow} of 4.1 (Banerjee et al. 1980). This study is considered reliable with restrictions, and is assigned a Klimisch score of 2. In support of the latter value are also QSAR estimated log K_{ow} values, i.e. KOWWIN (v1.68) in EPI suite predicts an estimated log K_{ow} of 4.00 (US-EPA 2012), LogP (v5.11.1) estimates a log P_{ow} of 3.81 (ChemAxon Ltd. 2010), ACD/LogP (v12.01) estimates a log P_{ow} of 4.02 ± 0.19 (ACD/Labs 2010) and ClogP (v1.5) estimates a log P_{ow} of 4.10 (BioByte Corp. 2006). It should be noted that for *p*-cymene a surface tension of 28.1 and 28.5 mN/m has been reported (see table 9). As these values are below 60 mN/m surface activity could be expected (ECHA 2014). However, the reliability of these surface tension values could not be assessed as only values are reported in the DAR, without reference to the applied methodology or study details. In the DAR it is stated that the surface tension values are acceptable as background information, but they were not relied on, suggesting there were doubts regarding their reliability. The dossier submitter considers it unlikely that *p*-cymene would display surface active properties, as the molecule has only polar groups. A surfactant would have both polar and non-polar parts. Therefore, the log K_{ow} is considered a valid descriptor for assessing the bioaccumulation potential of *p*-cymene, and is suitable to be used as input for QSAR models.

The DAR further stated that log P_{ow} values greater than 3 suggest the possibility of bioconcentration, and that the notifier claimed that these terpenes, because of their high volatility combined with their water insolubility, will not have sufficient residence time in water to provide significant exposure to fish or other aquatic organisms to trigger any meaningful risk. DT50 values are all < 1 day. It was further stated that it is also reasonable to conclude that naturally occurring substances such *ρ-cymene* will not have a propensity to bioaccumulate or bioconcentrate in aquatic organisms. However, there were some concerns about the information on degradation in the fate section, and extreme worst-case default values were used for dissipation. The DAR concluded that the argumentation above could not be confirmed by the fate section (Banerjee et al. 1980).

5.3.1.1 Bioaccumulation estimation

QSAR calculations can be performed with BCFBAF v3.01 in EPI Suite (US-EPA 2012). On the basis of the log K_{ow} of 4.1, BCF values of 236, 522 and 1290 L/kg wet-wt are estimated with the regression based method, Arnot-Gobas method incl. biotransformation rate estimates, and Arnot-Gobas method assuming a biotransformation rate of zero, respectively.

5.3.1.2 Measured bioaccumulation data

Bioconcentration in fish studies are not available for *ρ-cymene*. However, there is ADME data available for *ρ-cymene* in the DAR (see paragraph 4.1. for details) for different species, including the common brushtail possum, the koala and the rat (Anonymous 2002), rabbits (Anonymous 1992), guinea pigs and rats(Anonymous 1983), and rats (Anonymous 1999). It was reported that absorption is rapid for *ρ-cymene* (70-80%) in rats and guinea pigs with recovery within 48 hours. Thus, in mammals *ρ-cymene* appears to be readily metabolized to substances that are rapidly excreted within 48 hours.

5.3.2 Summary and discussion of aquatic bioaccumulation

According to the guidance (section 4.1.3.2.3.3), the log K_{ow} of 4.1 being higher than 4, indicates that the substance should be considered as having a 'high' potential for bioaccumulation.

5.4 Aquatic toxicity

Table 23: Summary of relevant information on aquatic toxicity

Method	**Results (mg/L)**	**Remarks**	**Reference**
Experimental endpoints			
Fish			
Short-term fish, 96 h OECD 203; GLP not reported.	**96 h LC50 = 2.0**	Medaka (*Oryzias latipes*) flow-through; purity not reported; mean measured concentrations. **Klimisch score = 2** **Key data**	(NITE 2015)
Short-term fish, 96 h US-EPA method for acute fish toxicity tests (EPA 1975); GLP not reported.	96 h LC50 = 48	Sheepshead minnow (*Cyprinodon variegatus*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(Anonymous 1981b)
Short-term fish, 96 h	96 h LC50 = 44	Bluegill fish (*Lepomis macrochirus*)	(EPA 1978)

method and GLP not reported.		static; purity not reported; nominal concentrations. **Klimisch score = 4**	
Long-term fish, 40 d equivalent to OECD 210; GLP not reported.	**40 d NOEC = 0.690**	Medaka (*Oryzias latipes*) flow-through; purity not reported; measured concentrations. **Klimisch score = 2** **Key data**	(NITE 2015)
Aquatic invertebrates			
Short-term invertebrate, 48 h acute invertebrate toxicity test (EPA 1975); GLP not reported.	48 h EC50 = 6.5	Water flea (*Daphnia magna*) static; purity ≥80%; nominal concentrations. **Klimisch score = 3**	(LeBlanc 1980)
Short-term invertebrate, 48 h US-EPA method for acute toxicity of freshwater and marine organisms (EPA, 2002), not GLP.	48 h EC50 = 3.52	Water flea (*Daphnia magna*) static; purity 95%; nominal concentrations. **Klimisch score = 3**	(H. M. Park et al. 2011)
Short-term invertebrate, 48 h method and GLP not reported.	96 h LC50 = 4.4	Opossum shrimp (*Americamysis bahia*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(EPA 1978)
Long-term invertebrate, 21 d OECD 211 (1998); GLP not reported.	21 day NOEC = 0.46	Water flea (*Daphnia magna*) semi-static; purity not reported; nominal concentrations. **Klimisch score = 3**	(NITE 2015)
Algae and aquatic plants			
Algal growth inhibition, 72 h OECD 201; GLP.	**72 h E_rC50 = 4.03** 72 h E_bC50 = 2.04 72 h NOE_bC = 1.40	Green alga (*Selenastrum capricornutum*) static; purity 99.6%; initial mean measured concentrations. **Klimisch score = 1** **acute = key data**	(Ward 2003)

		chronic = supporting data	
Algal growth inhibition, 72 h OECD 201; GLP not reported.	24-48 h E_rC50 = 5.1 24-48 h NOE_rC = 1.3 24-72 h E_rC50 = 6.7 24-72 h NOE_rC = 2.7 72 h E_bC50 = 3.7 **72 h NOE_bC = 0.51**	Green alga (*Selenastrum capricornutum*) static; purity not reported; mean measured concentrations. **Klimisch score = 2** **acute = supporting data** **chronic = key data**	(NITE 2015)
Algal growth inhibition, 96 h method and GLP not reported.	96 h LC50 = 22 96 h LC50 = 49	Diatom (*Skeletonema costatum*) Green alga (*Pseudokirchneriella subcapitata*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(EPA 1978)
Other (mosquito larvae tested in a water only test setup)			
Short-term insect larvae, 24 h non-guideline method, not GLP.	24 h LC50 = 43.3 24 h LC50 = 34.9	Yellow fever mosquito (*Aedes aegypti*) Tiger mosquito (*Aedes albopictus*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(Cheng et al. 2009c)
Short-term insect larvae, 24 h non-guideline method, not GLP.	24 h LC50 = 19.2 24 h LC50 = 46.7	Yellow fever mosquito (*Aedes aegypti*) Tiger mosquito (*Aedes albopictus*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(Cheng et al. 2009a)
Short-term insect larvae, 24 h non-guideline method, not GLP.	24 h LC50 = 37.1 24 h LC50 = 25.9	Yellow fever mosquito (*Aedes aegypti*) Tiger mosquito (*Aedes albopictus*) static; purity not reported; nominal concentrations. **Klimisch score = 3**	(Cheng et al. 2009b)
QSAR calculated endpoints			

Fish QSAR: ECOSAR v1.11 neutral organics	Short-term 96 h LC50 = 1.434 (freshwater) 96 h LC50 = 1.828 (saltwater) Long-term NOEC = 0.124 (freshwater) NOEC = 0.506 (saltwater)	based on log K_{ow} of 4.1 and water solubility of 23.35 mg/L. **Klimisch score = 2** **Supporting data**	(US-EPA 2012)
Invertebrates: daphnid / mysid QSAR: ECOSAR v1.11 neutral organics	Short-term **96 h LC50 = 0.327 (mysid)** **48 h LC50 = 0.988 (daphnid)** Long-term **16 d NOEC = 0.117 (daphnid)**	based on log K_{ow} of 4.1 and water solubility of 23.35 mg/L. **Klimisch score = 2** **Key data**	(US-EPA 2012)
Algae QSAR: ECOSAR v1.11 neutral organics	Short-term 96 h EC50 = 1.641 Long-term NOEC = 0.468	based on log K_{ow} of 4.1 and water solubility of 23.35 mg/L. **Klimisch score = 2** **Supporting data**	(US-EPA 2012)

5.4.1 Fish

There are three fish toxicity studies available. The FFHPVC reported a short-term fish toxicity test for *ρ-cymene* (EPA 2002). A summary of a short-term fish toxicity test for *ρ-cymene* was obtained from the NITE database (NITE 2015). In addition, the US EPA ECOTOX database contained a 96-h LC50 value for fish (EPA 2005).

5.4.1.1 Short-term toxicity to fish

<u>Short-term toxicity to fish</u> *FFHPVC reference (1 of 3)*

Reference	:	Anonymous (1981b)	water solubility	:	not reported
type of study	:	Acute toxicity study	species	:	Sheepshead minnow (*Cyprinodon variegatus*, 8-15 mm)
year of execution	:	1981	exposure duration	:	96 hours
GLP statement	:	Ambiguous	nominal concn.	:	10 -500 mg/L
Guideline	:	"Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (EPA, 1975)	dosing method	:	Static; use of solvent was not described
test substance	:	*ρ-cymene*	acceptability	:	Unreliable (Klimisch score of 3)
Purity	:	minimum purity of 80%	96-h LC50	:	48 mg a.s./L (36-64, 95% c.i.) (nominal)

Groups of 10 sheepshead minnows (*Cyprinodon variegatus*) were used in a 96-hour static test to evaluate the potential toxicity of *ρ-cymene*. The test vessels were either 4-L glass jars filled with 3 L of test water (filtered [5 µm] natural seawater) or 19-L glass jars filled with 15 L test solution. No aeration was used. The use of a solvent for *ρ-cymene* was not described. Dissolved oxygen was measured at the beginning of the test and daily thereafter. pH was measured in the low and high concentration groups at the beginning and end of the test. Specific nominal concentrations and/or measured concentrations were not reported in the FFHPVC robust summary, but the US-EPA Hazard Characterization Document of *ρ-cymene* reported that the nominal concentrations of *ρ-cymene* ranged from 10 to 500 mg/L (EPA 2012). LC50s at 24, 48, 72, and 96 hours were calculated with a computer program (Stephan, 1977) that determined the most appropriate statistical method (moving average angle analysis, probit analysis, or binomial probability) to apply. A 96-h LC50 of 48 mg/L based on nominal concentrations was reported (EPA 2002).The results were assigned a Klimisch score of 2 (=reliable with restrictions) in the FFHPVC report (EPA 2002). The report 96-h LC50 of 48 mg/L is much higher than the water solubility of 23.35 mg/L at 25°C (Banerjee et al. 1980). Furthermore, the **actual concentrations were not determined**, despite the fact that *ρ-cymene* has a high volatility. Therefore, it is more appropriate to consider this **endpoint unreliable and assign it a Klimisch score of 3**. The 96-h LC50 value from this study is **not used for classification purposes**.

Short-term toxicity to fish *NITE reference (2 of 3)*

Groups of 10 medaka's (*Oryzias latipes*) were used in a 96-hour flow-through test to evaluate the potential toxicity of *ρ-cymene*. Not reported if GLP compliant. The fish were kept in a volume of 9 L. Flow rate was 50 mL/min, and renewal rate was about 8 times/day. The type of water was not specified. The fish were not fed and there was no aeration. The nominal test concentrations were 1.0, 1.8, 3.2, 5.6 and 10 mg/L. Control and solvent control (not specified which solvent) were included. It is not reported if pH and dissolved oxygen were measured. Water samples were taken at the start and after 48 hours, and were analyzed using GC-MS. Since the measured concentration differed more than ±20% of the nominal test concentration, the **results were based on the measured test concentrations**. After 96 hours, mortality amounted to 0 and 100% in the 1.2 mg/L and 3.4 mg/L treatments, respectively. The 96 h LC50 was reported to be 2.0 mg/L. The above data has been retrieved from a MITI study summary (see Annex 8.2.4 for the translated study summary). Therefore, the **reliability of this study is assigned a Klimisch score of 2, reliable with restrictions**. The 96-h LC50 value form this study is used as **key data for classification purposes**.

Short-term toxicity to fish *US EPA ECOTOX reference (3 of 3)*

The US EPA ECOTOX database reported an experimental 96-h LC50 value of 44 mg/L for the bluegill fish *Lepomis macrochirus* (EPA 2015). The original study report was not available (EPA 1978). The brief summary reported that the study design was static, and that the effect concentrations were based on mortality. The purity of *ρ-cymene*, the type of medium, and the test temperature were not specified. Dissolved oxygen concentrations and pH were not measured, and it was not reported if a control was included. The followed test guideline was not reported. Furthermore, the effect concentration was based on nominal test concentrations. **The reliability of this study cannot be evaluated since there are too many missing elements**. Therefore, this study is assigned a Klimisch score of 4. The 96-h LC50 value from this study is **not used for classification purposes**.

Short-term toxicity to fish *ECOSAR estimates*

QSAR based (neutral organics) LC50 values for fish could be generated with ECOSAR v1.11 available in EPI suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, LC50 values of 1.434 and 1.828 were estimated for fresh and saltwater fish, respectively. The log K_{ow} is within the applicability domain (max log K_{ow} of 5.0 for fish 96-h LC50). The estimated LC50 values are similar to the experimental value for fish. The QSAR endpoints for short-term fish toxicity are considered reliable, and will be used as **supporting information for classification purposes**.

5.4.1.2 Long-term toxicity to fish

The summary of a long-term fish toxicity study with *ρ-cymene* was obtained from the MITI database.

<u>Long-term toxicity to fish</u> *NITE reference (1 of 1)*

An early life stage toxicity test was conducted with medaka (*Oryzias latipes*) to assess the toxicity of *ρ-cymene*. Not reported if GLP compliant. 60 fertilized eggs were used per test group. Five nominal test concentrations were tested, i.e. 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L. Control was included. Test temperature was 24 ± 1 °C. All treatments were tested in quadruplicate. The study design was flow-through. Exposure duration was 40 days (hatching after 31 days). Monitored endpoints were: hatching rate, time to hatch, developmental abnormalities, survival, toxic symptoms, body weight, and body length of surviving fry. Water quality parameters were monitored. Chemical analyses were performed, but it was not specified when. Since the measured test concentrations ranged 58.4 to 80.0% of nominal concentrations, the **effect concentrations were based on measured concentrations**. No significant effects were observed for the embryonic indicators; hatching rate, time to hatching and the developmental abnormalities rate. A significant effect was observed on the survival and growth after hatching of larval and juvenile fish at 1.44 mg/L. In addition, toxicity effects were observed. The LOEC was reported as 1.44 mg/L, and the NOEC as 0.690 mg/L. The above data has been retrieved from a NITE study summary (see Annex 8.2.5 for the translated study summary). Therefore, the **reliability of this study is assigned a Klimisch score of 2, reliable with restrictions**. The 40-d NOEC value form this study is used as **key data for classification purposes**.

<u>Long-term toxicity to fish</u> *ECOSAR estimates*

QSAR based (neutral organics) NOEC values for fish could be generated with ECOSAR v1.11 available in EPI Suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, NOECs of 0.124 and 0.506 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 K_{ow} value used was within the applicability domain (max log K_{ow} of 8.0 for ChV). The estimated NOEC values are similar to the experimental value for fish. The **QSAR endpoints for long-term fish toxicity are considered reliable, and will be used as supporting information for classification purposes**.

5.4.2 Aquatic invertebrates

The FFHPVC reported a short-term aquatic invertebrates toxicity test for *ρ-cymene* (EPA 2002). In addition, the US EPA ECOTOX database contained acute effect concentration for two aquatic invertebrate species (EPA 1978). The summary of a long-term daphnia toxicity study with *ρ-cymene* was obtained from the NITE database (NITE 2015).

5.4.2.1 Short-term toxicity to aquatic invertebrates

Short-term toxicity to aquatic invertebrates *FFHPVC reference (1 of 3)*

Reference	:	LeBlanc G.A. (1980)	water solubility	:	not reported
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	1980	exposure duration	:	48 hours
GLP statement	:	Ambiguous	nominal conc.	:	not reported
Guideline	:	"Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (EPA, 1975)	dosing method	:	Static; use of solvent was not described
test substance	:	*ρ-cymene*	acceptability	:	Unreliable (Klimisch score of 3)
Purity	:	minimum purity of 80%	48-h LC50	:	6.5 mg a.s./L (4.3-10, 95% c.i.) (nominal)

At the initiation of all tests, the dissolved oxygen concentration of diluent water was greater than 60%. Test was conducted at 22 ± 1°C. Addition of the test material to diluent water varied according to the water solubility of the chemical. The use of a vehicle (triethylene glycol, ethanol, acetone or dimethylformamide) was dependent on the solubility of the chemical. It was not stated whether a vehicle was used for *ρ-cymene*. Five to 8 nominal concentrations were tested. Within 30 minutes of solution preparation, soluble test materials were tested with 5 daphnids (<24 hours old) randomly placed in 3 150 mL jars containing test solution; otherwise 15 daphnia were placed in 2 L jars containing test solution. In either case, the jars were covered with plastic wrap held with an elastic band. The control consisted of the same dilution water, test conditions and test organisms, but no test substance or vehicle. When appropriate, a positive (solvent) control was included. Observations were made at 24 and 48 hours. The dissolved oxygen concentration, pH and temperature of test solutions were measured at the initiation and termination of the toxicity tests in the high, middle and low test concentrations and controls. Actual concentrations were not measured. LC50s and 95% confidence limits were determined using a moving average angle method, but if the data did not meet the requirements of this method a probit analysis was used and if this did not work, a binomial probability analysis was conducted. Results were limited to tabular reporting of LC50s. Measured dissolved oxygen concentrations ranged from 6.5-9.1 mg/L, measured pH values ranged from 6.7-8.1 and 7.4-9.4 for solutions with a hardness of 72 and 173 mg $CaCO_3$/L, respectively. A 48-h LC50 of 6.5 mg/L (4.3-10, 95% conf.int.) was reported. The results were assigned a Ri of 2 (=reliable with restrictions) in the FFHPVC report (EPA 2002). **Considering the high volatility of *ρ-cymene*, the LC50 should have been based on measured concentrations and not nominal concentrations**. The actual LC50 is likely to be much lower than the reported 48-h LC50 of 6.5 mg/L. Therefore, it is considered more appropriate to assign these data a **Klimisch score of 3, unreliable**. The 48-h EC50 value from this study is **not used for classification purposes**

Short-term toxicity to aquatic invertebrates *US EPA ECOTOX reference (2 of 3)*

Reference	:	H. M. Park et al. (2011)	water solubility	:	not reported
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	not GLP	nominal concn.	:	not reported

Guideline	:	"Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed." (EPA, 2002)	dosing method	:	Static; acetone was used as solvent
test substance	:	*ρ-cymene*	acceptability	:	Unreliable (Klimisch score of 3)
Purity	:	95%	48-h LC50	:	3.52 mg./L (3.22-4.22 95% c.i.) (nominal)

The US EPA ECOTOX database reported an experimental 48-h EC50 values of 3.52 mg/L for the water flea *Daphnia magna* (EPA 2015). The peer reviewed publication was available (H. M. Park et al. 2011). The test was conducted according to US-EPA guideline "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed." from 2002, with some modifications. Test substance was *ρ-cymene* (purity 95%). Stock solution was made in aceton (0.01% wt-vol). Control and solvent control were included. In the test, 24-h old *D. magna* neonates were exposed to *ρ-cymene* in 125-mL glass tanks filled with 100 mL water. The temperature was maintained at 20±1°C under a 16:8 h light:dark cycle. Five *D. magna* neonates were used per test. All the tests were repeated four times. Death and immobility were determined at 48 h after treatment. The actual test concentrations were not monitored and the **reported endpoint of 3.52 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. Since the concentration in the residue test exceed the water solubility of *ρ-cymene* (23.35 mg/L at 25°C) with a factor of 5 it is considered not representative for the reported endpoint. Because of the low water solubility and high vapour pressure of *ρ-cymene*, the reported EC50 is likely to be an underestimation of the actual toxicity of the compound. Therefore the **48-h EC50 value from this study is considered unreliable**, and the data are assigned a Klimisch score of 3. The 48-h EC50 value from this study is **not used for classification purposes**.

Short-term toxicity to aquatic invertebrates *US EPA ECOTOX reference (3 of 3)*

The US EPA ECOTOX database reported an experimental 96-h LC50 value of 4.4 mg/L for the opossum shrimp *Americamysis bahia* (EPA 2015). The original study report was not available (EPA 1978). The brief summary reported that the study design was static, and that the effect concentrations were based on mortality. The purity of *ρ-cymene*, the type of medium, and the test temperature were not specified. Dissolved oxygen concentrations and pH were not measured, and it was not reported if a control was included. The followed test guideline was not reported. Furthermore, the **effect concentration was based on nominal test concentrations**. Therefore, this study is **considered unreliable, and is assigned a Klimisch score of 3**. The 96-h LC50 value from this study is **not used for classification purposes**.

Short-term toxicity to aquatic invertebrates *ECOSAR estimates*

QSAR based (neutral organics) LC50 values for aquatic invertebrates could be generated with ECOSAR v1.11 available in EPI suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, LC50 values of 0.988 and 0.327 were estimated for daphnids (fresh water) and mysids (saltwater), respectively. The log K_{ow} is within the applicability domain (max log K_{ow} of 5.0 for Daphnid 48-h LC50, and Mysid 96-h LC50). **Thus, taking into account that the Daphnia experimental values are based on nominal concentrations, the QSAR endpoints are considered preferable, and classification will be based on the QSAR estimated endpoints**.

According to the CLP guidance 'QSARs can be relied upon to provide predictions of acute toxicity to fish, crustacea (Daphnia and Mysid) and algae for non-electrolytes, non-electrophilic, and otherwise non-reactive substances. Care should be taken when evaluating the toxicity of poorly water soluble substances, where the quoted toxicity may be greater than the water solubility' (version 4.0 November 2014). This is not the case with p-cymene.

For aquatic invertebrates, experimental EC50 values in the range of 3.5 to 6.5 mg/L are available. However, these EC50 values are based on nominal concentrations and are as such not considered reliable. The actual effects concentration would be expected to be lower given the substance properties (e.g. high volatility, log K_{ow} of 4.1). This is supported by the estimated LC50 values of 0.988 and 0.327 mg/L for daphnids and mysids, respectively.

We conclude the QSAR model for acute affects and predictions based on baseline toxicity (narcosis type organics) for daphnia is reliable (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R7b: Endpoint specific guidance (version 2.0, November 2014). Section R.7.8.5 Reliable QSAR results, page 42). Therefore, **QSAR endpoints for short-term daphnia will be used as key data for classification purposes**. A QSAR model reporting format (QMRF) and QSAR prediction reporting format (QPRF) is provided in Annex III.

5.4.2.2 Long-term toxicity to aquatic invertebrates

The summary of a long-term daphnia toxicity study with *ρ-cymene* was obtained from the MITI database.

Long-term toxicity to aquatic invertebrates *NITE reference (1 of 1)*

A 21-day *Daphnia magna* reproduction test with *ρ-cymene* was conducted according to OECD 211 (1998). Not reported if GLP compliant. Purity of the test substance was not reported. 10 daphnids were used per test group. Seven nominal test concentrations were tested, i.e. , 0.010, 0.022, 0.046, 0.10, 0.22, 0.46 and 1.0 mg/L. Control and solvent control were included. Type of solvent was not specified. Test temperature was 20 ± 1 ℃. Test volume was 80 mL. The study design was semi-static with full exchange of test ware every 48 hours. Feeding consisted of *Chlorella vulgaris* amounting to 0.15 mg C (carbon content organism)/day for each daphnid. Water samples were taken at the start, day 2, 6, 8, 14 and 16. Test substance was quantified using GC-MS. The test concentrations below 1.0 mg/L could not be measured as they were below the detection limit. Therefore, the effect concentrations are expressed based on nominal concentrations. The LC50 based on adult mortality, and the EC50 based on reproduction were reported to be ≥1.0 mg/L. The NOEC was reported to be 0.46 mg/L. The above data has been retrieved from a NITE study summary (see Annex 8.2.3 for the translated study summary). Considering that the **effect data are based on nominal test concentrations**, the study is **not considered reliable, and assigned a Klimisch score of 3**. The 21-d NOEC value form this study is **not used for classification purposes**.

Long-term toxicity to aquatic invertebrates *ECOSAR estimates*

QSAR based (neutral organics) NOEC values for aquatic invertebrates could be generated with ECOSAR v1.11 available in EPI Suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, a NOEC of 0.117 mg/L was estimated for daphnids (fresh water) (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log K_{ow} value used was within the applicability domain (max log K_{ow} of 8.0 for ChV).). **Thus, taking into account that the Daphnia experimental value are based on nominal concentrations, the QSAR endpoints are considered preferable, and classification will be based on the QSAR estimated endpoints**.

According to the CLP guidance 'QSARs can be relied upon to provide predictions of acute toxicity to fish, crustacea (Daphnia and Mysid) and algae for non-electrolytes, non-electrophilic, and otherwise non-reactive substances. Care should be taken when evaluating the toxicity of poorly water soluble substances, where the quoted toxicity may be greater than the water solubility' (version 4.0 November 2014). This is not the case with p-cymene.

For aquatic invertebrates, an experimental 21 day NOEC is reported of 0.46 mg/L. However, this NOEC is based on nominal concentrations and is as such not considered reliable. The actual effects concentration would be expected to be lower given the substance properties (e.g. high volatility, log K_{ow} of 4.1). This is supported by the estimated 16 day NOEC of 0.117 mg/L for daphnids. The estimated NOEC is considered to represent an upper estimate, as it estimates toxicity after 16 days of exposure, and will thus by default be above a 21 day value. Furthermore, the applied QSAR accounts only for baseline toxicity and other possible chronic effects are not considered.

We conclude the QSAR model for chronic affects and predictions based on baseline toxicity (narcosis type organics) for daphnia is reliable (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R7b: Endpoint specific guidance (version 2.0, November 2014). Section R.7.8.5 Reliable QSAR results, page 42). Therefore, **QSAR endpoints for long-term daphnia will be used as key data for classification purposes**. A QSAR model reporting format (QMRF) and QSAR prediction reporting format (QPRF) is provided in Annex III.

5.4.3 Algae and aquatic plants

There are three algal growth inhibition studies available The revised FFHPVC reported an algal growth inhibition test for *ρ-cymene* (EPA 2005). A summary of an algal growth inhibition test for *ρ-cymene* was obtained from the NITE database (NITE 2015). In addition, the US EPA ECOTOX database reported effect concentrations from a static algal toxicity test with *ρ-cymene* (EPA 1978).

Toxicity to algae *FFHPVC reference (1 of 3)*

Reference	:	Ward T. (2003)	water solubility	:	not reported
type of study	:	Acute toxicity study	Species	:	*Selenastrum capricornutum*
year of execution	:	2003	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	0, 0.65, 1.3, 2.5, 5.0 and 10.0 mg/L
Guideline	:	OECD 201	dosing method	:	Static
test substance	:	*ρ-cymene*	acceptability	:	Reliable without restrictions (Klimisch score of 1)
Purity	:	99.6%	48-h LC50	:	72 h EC50 = 2.04 (biomass) (initial measured) 72 h EC50 = 4.03 (growth rate) (initial measured) 72 h NOEC = 1.40 (biomass) (initial measured)

GLP-complaint algal growth inhibition test according to OECD TG 201. Test substance was *ρ-cymene* (purity 99.6%). Test species was the green algae *Selenastrum capricornutum* (UTEX 1648). Algae were maintained at test conditions for 14 days prior to the test. The culture was growing in at least 2 subcultures prior to the initiation of the test. In a range finding test, the number of cells/mL was 96% of controls at 0.10 mg/L, 94% at 1.0 mg/L, 11% at 10 mg/L,and <1% at 100 mg/L after three days. In the definitive test, algae was treated with nominal concentrations of 0, 0.65, 1.3, 2.5, 5.0 and 10.0 mg/L for 72 hours. pH was adjusted to 7.5 and solutions were exposed for 24 hours of light of intensity, 400-410 foot candles. The number of algal cells/mL as well as relative size, cell shapes, color, adherence and aggregation of cells was determined. At 24, 48, and 72 hours 3 treatment and 6 control vessels were sacrificed to determine the number of algal cells/mL. Concentrations were determined by HPLC. EC50 values determined by weighted least squares non-linear regression (Bruce and Versteeg, 1992); NOEC was determined using a one-way analysis of variance (ANOVA) and Bonferroni's test (Gulley et al. 1990). Initial mean measured concentrations 0.623, 1.40, 1.91, 3.52, and 5.32 mg/L; Final measured were 53-108% of nominal concentrations. **Results were expressed as initial mean measured concentrations**. Control algal populations grew at an acceptable rate (10,000 cells/ml) after 72 hours. Incubation temperatures were in the range from 23.2 to 24.0 C over the 72 hours and pH was unchanged by the test substance. At the conclusion of the test, samples of test media from each test vessel with maximal growth inhibition were combined with fresh media. After 48 hours incubation the number of cells increased from 1500 cells/mL to 1,1328,000 cells/mL at 3.52 mg/L suggesting that the toxic effects were algistatic. The acute toxicity of *ρ-cymene* measured as a 50% decrease in growth and reproduction of freshwater algae was estimated to be 72 hr E_rC50 = 4.03 mg/L based on average specific growth rate; 72-hr EC50 = 2.40 mg/L calculated using the number of cells/mL; 72-hr EC50 = 2.01 mg/L using the area under the growth curve. The 72-hr NOE_bC = 1.40 mg/ L is based on number of cells/mL. A 72-h NOErC value is not reported. Th**e study is considered reliable without restrictions**, and the data are assigned a Klimisch score of 1. **The 72-h E_rC50 value of 4.03 mg/L is used as key data, and the 72-h NOE_bC of 1.4 mg/L is used as supporting data for classification purposes**.

<u>Toxicity to algae</u> *NITE reference (2 of 3)*

An algal growth inhibition test according to OECD TG 201 (1984). Test species was the green algae *Selenastrum capricornutum* (ATCC22662). OECD medium was used. Test substance was *ρ-cymene* (purity not reported). The nominal test concentrations were: 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L. Control and solvent control were included. Type of solvent was not specified. All treatments were conducted in triplicate with about 1×10^4 cells/mL at the start. The test temperature was 23 ± 2 ° C. Not reported if water quality parameters were monitored. Water samples were taken at the start and end of the test, and analyzed using GC-MS. As the measured test concentration deviated more than 20% from the nominal test concentrations, **the effect concentrations are based on the mean measured test concentrations**. Section-by-section rates during the course of the test are reported. The following effect concentrations were reported: E_bC50 (0-72h) = 3.7 mg/L (95% C.I. of 3.1~4.1); E_rC50 (24-48h) = 5.4 mg/L (95% C.I. of 5.1~5.8); E_rC50 (24-72h) = 6.7 mg/L (95% C.I. of 5.6~6.8); NOE_bC (0-72h) of 0.51 mg/L; NOE_rC (24-48h) of 1.3 mg/L; and NOE_rC (24-72h) of 2.7 mg/L. The above data has been retrieved from a NITE study summary (see Annex 8.2.2 for the translated study summary). Therefore, the **reliability of this study is assigned a Klimisch score of 2, reliable with restrictions.** The EC50 and NOEC values based on algal growth rate for the 72 h period were not provided. The dossier submitter is unable to derive a 72-h ErC50 and 72-h NOErC because raw data are not available. As there are no NOE_rC values available, the **NOE_bC of 0.51 mg/L form this study is used as a key data for classification purposes**.

<u>Toxicity to algae</u> *US EPA ECOTOX reference (3 of 3)*

The US EPA ECOTOX database reported an experimental 96-h LC50 values of 49 and 22 mg/L for the green algae *Pseudokirchneriella subcapitata* and the diatom *Skeletonema costatum*, respectively (EPA 2015). The original study report was not available (EPA 1978). The brief summary reported that the study design was static, and that the effect concentrations were based on chlorophyll A concentrations. The purity of *ρ-cymene*, the type of medium, and the test temperature were not specified. Dissolved oxygen concentrations and pH were not measured, and it was not reported if a control was included. The followed test guideline was not reported. Furthermore, the **endpoints were based on nominal concentrations that exceed water solubility of *ρ-cymene***. Therefore, this **study is considered unreliable**, and the data are assigned a Klimisch score of 3. The 96-h LC50 value is **not used for classification purposes**.

Toxicity to algae *ECOSAR estimates*

QSAR based (neutral organics) EC50 and NOEC values for algae could be generated with ECOSAR v1.11 available in EPI Suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, an E_rC50 of 1.641 mg/L and a NOEC of 0.468 mg/L were estimated (ECOSAR generates ChV values, these are converted to a NOEC by: NOEC = ChV/√2). The log K_{ow} value used was within the applicability domain (max log K_{ow} of 6.4 and 8.0 for green algae EC50 and ChV, respectively). The estimated algal-E_rC50 value is in the same range as that of the experimental value, 4.03 mg/L. The **QSAR endpoints for algal toxicity are considered reliable, and will be used as supporting information for classification purposes**.

5.4.4 Other aquatic organisms (including sediment)

A few studies are available where *ρ-cymene* has been tested on the yellow fever mosquito (*Aedes aegypti*) and the tiger mosquito (*Aedes albopictus*) (Cheng et al. 2009a; Cheng et al. 2009c; Cheng et al. 2009b; H. M. Park et al. 2011; Y. K. Park et al. 2012). In short, 30-mL cups (paper/ polypropylene) were filled with water. Test substance was added resulting in a range of nominal test concentrations. Control and solvent control (DMSO) were included. In some studies, a positive control was included (e.g. chlorpyrifos). Each test was replicated 4 times. Test was started by adding 10 fourth-instar mosquito larvae to each cup. Larvae were not fed during the duration of the test. After 24 h of exposure, mortality was recorded. In these studies, **the exposure concentrations exceeded the water solubility of *ρ-cymene* 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. H. M. Park et al. (2011) did not report effect concentrations, just 100% mortality at 50 mg/L and 5% mortality at 25 mg/L, while Y. K. Park et al. (2012) reported a 24-h LC50 of 1.8 µL/100mL. The endpoints reported by (Cheng et al. 2009a; Cheng et al. 2009c; Cheng et al. 2009b) are in the range of 19.2 to 46.7 mg/L but considering the shortcomings of the studies, they are likely an underestimation of the actual toxicity of *ρ-cymene* to these mosquitos. The above discussed studies are **considered unreliable, and the data are assigned a Klimisch score of 3**. The endpoints from these **studies will not be used for classification purposes**.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

CLP - Acute aquatic hazards

Reliable studies are available for only two trophic levels: algae and fish. For algae, a reliable experimental 72 h E_rC50 of 4.03 mg/L (growth rate) was reported that is based on initial measured concentrations. This value is supported by the two EC_r50 values, i.e. 24-48 h EC_r50 of 1.3 mg/L, and 24-72 h EC_r50 of 2.7 mg/L, that were reported in the NITE algal growth inhibition study and that are based on mean measured values. For fish, an experimental 96 h LC50 of 2.0 mg/L was reported that is based on mean measured concentrations.

For aquatic invertebrates, experimental acute effect concentrations in the range 3.5 to 6.5 mg/L are available, but they are based on nominal concentrations. Considering the substance properties (e.g. high volatility, log K_{ow} of 4.1) this is not considered reliable. Therefore, the reliable QSAR generated values, i.e. a 96 h LC50 of 0.327 mg/L for mysids, and a 48 h LC50 of 0.988 mg/L for daphnids, are preferred for classification purposes.

The three values that are used for classification are an algal experimental 72 h EC_r50 of 4.03 mg/L, an experimental fish 96 h LC50 of 2.0 mg/L and QSAR estimated mysid 96 h LC50 of 0.327 mg/L Therefore, in accordance with table 4.1.0(a) (according to CLP guidance V4.1 June 2015, p. 503-505), *p*-cymene is classified as **Aquatic Acute 1**. According to Table 4.1.3, an **M-factor of 1** is warranted.

CLP - Chronic aquatic hazards

The biodegradation potential of *ρ-cymene* was evaluated using the MITI test method OECD 301C with degradation after 14 days amounting to 88 ± 6.2% based on oxygen uptake (BOD). Degradation was confirmed by dissolved organic carbon measurements (TOC), and chemical analysis (GC). Furthermore, the 10-day window was met. Therefore, *ρ-cymene* is considered rapidly biodegradable for classification purposes.

Reliable studies are available for only two trophic levels: algae and fish. For algae, 72 h NOEC values are only reported that are based on biomass (72 h NOE_bC) and not on growth rate (72 h NOE_rC). In regulation (EC) 1272/2008 (CLP regulation) the following is stated in note 2 "*Classification shall be based on the E_rC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no E_rC50 is recorded, classification shall be based on the lowest EC50 available*". There is, however, no reference made to NOEC values in the CLP regulation. In the guideline to the CLP regulation it also not indicated if note 2 is applicable to NOEC values. Considering that a 72 h NOE_rC is not reported, and that it cannot be calculated by the dossier submitter due to unavailability of raw data, the NOE_bC values will be used for classification. The 72 h NOE_bC of 0.51 mg/L is based on mean measured concentrations, and the 72 h NOE_bC of 1.4 mg/L is based on initial measured concentrations. These values are supported by the 24-48 h NOE_rC of 1.3 mg/L and the 24-72 h EC_r50 of 2.7 mg/L that are based on mean measured values. For fish, an experimental 40 d NOEC of 0.690 mg/L was reported that is based on measured concentrations.

For aquatic invertebrates, an experimental 21 d NOEC of 0.46 mg/L is available, but it is based on nominal concentrations. Considering the substance properties (e.g. high volatility, log K_{ow} of 4.1) this is not acceptable, and this endpoint is not considered reliable. Therefore, the reliable QSAR generated 16 d NOEC of 0.117 mg/L for daphnids is preferred for classification purposes.

The three values that are used for classification are an algal 72 h NOE_bC of 0.51 mg/L, a fish experimental 40-d NOEC of 0.690 mg/L, and a QSAR estimated daphnid NOEC of 0.117 mg/L. Therefore, in accordance with table 4.1.0(b)(ii) (according to CLP guidance V4.1 june 2015, p. 503-505), *p*-cymene is classified as **Aquatic Chronic 3**. According to Table 4.1.3, an M-factor is not warranted.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Acute aquatic toxicity

An algal experimental 72 h EC_r50 of 4.03 mg/L, an experimental fish 96 h LC50 of 2.0 mg/L and a QSAR estimated mysid 96 h LC50 of 0.327 mg/L, have been defined as the most appropriate values with respect to acute aquatic toxicity of *p*-cymene. These values are considered reliable, show the highest toxicity, and together represent three trophic levels. The classification proposal is based on the most critical endpoint, i.e. the mysid endpoint. Therefore, in accordance with table 4.1.0(a) (according to CLP guidance V4.1 June 2015, p. 503-505), *p*-cymene is classified as **Aquatic Acute 1**. According to Table 4.1.3, an **M-factor of 1** is warranted.

Chronic aquatic toxicity

An algal experimental 72 h NOE_bC of 0.51 mg/L, a fish experimental 40-d NOEC of 0.690 mg/L, and a QSAR estimated daphnid NOEC of 0.117 mg/L have been defined as the most appropriate values with respect to chronic aquatic toxicity of *p*-cymene. These values are considered reliable, show the highest toxicity, and together represent three trophic levels. The classification proposal is based on the most critical endpoint, i.e. the daphnid endpoint. *p*-Cymene is readily biodegradable and can be considered as rapidly degradable for classification purposes. For *p*-cymene there are no experimental bioaccumulation data, but as the log K_{ow} of 4.1 exceeds 4, *p*-cymene can be considered as having a 'high' potential for bioaccumulation. Therefore, in accordance with table 4.1.0(b)(ii) (according to CLP guidance V4.1 june 2015, p. 503-505), *p*-cymene is classified as **Aquatic Chronic 3**. According to Table 4.1.3, an M-factor is not warranted.

Conclusions on classification and labelling for environmental hazards of *p-cymene*.

	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 -

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1; H400 (M=1) and Aquatic Chronic 3; H412. The acute classification was based on a QSAR estimated mysid 96-hour LC_{50} of 0.327 mg/L. The value was in the range of > 0.1 to ≤ 1 mg/L leading to an M-factor of 1. The chronic classification was based on a QSAR estimated daphnid NOEC of 0.117 mg/L. The lowest fish and algae QSAR data were of the same order of magnitude. The substance was considered rapidly degradable and to have a high potential for bioaccumulation.

Degradation

There were no experimental data available for the hydrolysis of p-cymene. The substance contains no functional groups that could hydrolyse such as esters, amides or epoxides. The vapour pressure of p-cymene was high (1.95-2.67 × 10^2 Pa) and its solubility in water was relatively low (23 mg/L) giving a high Henry's Law Constant (1.38 × 10^3 Pa m^3/mol), which predicted a high rate of volatility from water. In a level III fugacity model simulating application on crops, persistence in the total system or DT_{100} was predicted to be 46.4 hours because most of the p-cymene will partition to air and be degraded via interaction with hydroxyl radicals rapidly. Direct photolysis was not expected to be an important environmental fate process. The overall conclusion from BIOWIN v4.10 QSAR contained within EPI Suite™ version 4.11, was that p-cymene was not readily biodegradable. The biodegradation potential of p-cymene was evaluated using the MITI test method OECD TG 301C. The purity of p-cymene was 95.3%. The test was conducted for 14 days. Test vessels were adapted for volatile substances. There was no oxygen consumption in the abiotic control and very limited oxygen consumption in the inoculum blank amounting to 3.8 mg O_2/L after 14 days. The percent degradation (based on BOD) in the positive control amounted to 53 and 87% after 7 and 14 days, respectively. For the test substance average degradation after 14 days amounted to 88.0 ± 6.2% based on BOD, 88.7 ± 1.2 based on TOC, and 100 ± 0.0% based on GC. The 10-day window was met for all three replicates based on BOD. Thus, all validity criteria were met and p-cymene was shown to be readily biodegradable. In an aquatic simulation study without sediment, p-cymene volatilised from the natural water test systems rapidly with a DT_{50} of 11.2 and DT_{90}s of 37.4 hours. The trapping solution showed the presence of the test substance but no degradation products. Degradants were also not detected in the water. Thus, rapid escape (fugacity via volatility) appears to be the predominant pathway for p-cymene in natural water. The DS concluded that p-cymene is rapidly degradable for classification purposes.

Bioaccumulation

There were no experimental BCF data available on the bioaccumulation potential of p-cymene.

There was a reliable experimentally determined log K_{ow} of 4.1 available. In support of this value, there were also QSAR estimated log K_{ow} values of 4.00, 3.81, 4.02 and 4.10 from KOWWIN, LogP, ACD/LogP and ClogP models, respectively. Although surface tension values of 28.1 and 28.5 mN/m had been reported for p-cymene, the DS considered it unlikely that p-cymene would display surface active properties, as the molecule has only non-polar groups. A surfactant would have both polar and non-polar parts. Therefore, the log K_{ow} was considered a valid descriptor for assessing the bioaccumulation potential of p-cymene. The DS concluded, based on the log K_{ow} of 4.1, that p-cymene has a high potential for bioaccumulation.

Aquatic toxicity

***Table**. Reliable aquatic toxicity data on p-cymene*.

Test	Test species	Result mg/L	QSARs for *p-cymene* (mg/L)
Fish			
p-cymene Short-term fish toxicity OECD TG 203 flow-through NITE 2015	*Oryzias latipes*	96 h LC_{50}: 2.0† based on mean measured concentrations	96 h LC_{50}: 1.434 (freshwater fish); 96-h LC_{50}: 1.828 (saltwater fish) (ECOSAR v.1.11)*
p-cymene Long-term fish toxicity Similar to OECD TG 210 flow-through NITE 2015	*Oryzias latipes*	40 day NOEC: 0.690 based on measured concentrations	Chronic NOEC: 0.124 (freshwater fish); 0.506 (saltwater fish) (ECOSAR v.1.11)*
Invertebrates			
			48 h LC_{50}: 0.988 (freshwater daphnids);96-h LC_{50}: 0.327 (saltwater mysids) (ECOSAR v.1.11)*
p-cymene	*Daphnia magna*		Chronic NOEC: 0.117, daphnids (ECOSAR v.1.11)*
Algae/Aquatic plants			
99.6% p-cymene OECD TG 201 (Alga, Growth Inhibition Test), GLP, static Ward 2003	*Selenastrum capricornutum*	72 h E_rC_{50} = **4.03** 72 h E_bC_{50} = 2.04 72 h NOE_bC = 1.40 based on initial mean measured concentrations	96 h EC_{50}*: 1.641; NOEC*: 0.468 (ECOSAR v.1.11)
p-cymene OECD TG 201 (Alga, Growth Inhibition Test), GLP, static NITE 2015	*Selenastrum capricornutum*	24-48 h E_rC_{50} = 5.1 24-48 h NOE_rC = 1.3 24-72 h E_rC_{50} = 6.7 24-72 h NOE_rC = 2.7 72 h E_bC_{50} = 3.7 72 h NOE_bC = **0.51**† mean measured concentrations	

* neutral organics, based on log K_{ow} 4.1
† solvent used, not specified

Acute Aquatic Toxicity

In an OECD TG 203 study, *Oryzias latipes* were used in a 96-hour flow-through test to evaluate the toxicity of p-cymene. The nominal test concentrations were 1.0, 1.8, 3.2, 5.6 and 10 mg/L. Control and solvent controls (not specified which solvent) were included. Since the measured concentration differed more than ±20% of the nominal test concentration, the results were based on the measured test concentrations. The 96 h LC_{50} was reported to be 2.0 mg/L. This value was used as a key data for classification.

QSAR based (neutral organics) LC_{50} values for fish were generated with ECOSAR v1.11 available in EPI suite. Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, 96-hour LC_{50} values of 1.434 and 1.828 mg/L were estimated for fresh and saltwater fish, respectively. The log K_{ow} was within the model applicability domain. The QSAR endpoints for short-term fish toxicity were considered reliable and were used as supporting information for classification purposes.

QSAR based (neutral organics) LC_{50} values for aquatic invertebrates were generated with ECOSAR v1.11 available in EPI suite. Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, 48-hour and 96-hour LC_{50} values of 0.988 and 0.327 were estimated for daphnids (fresh water) and for mysids (saltwater), respectively. The log K_{ow} is within the applicability domain. Thus, considering that there were no substance invertebrate data available, QSAR endpoints for short-term invertebrate toxicity were used as key data for classification purposes. A QSAR model reporting format (QMRF) and QSAR prediction reporting format (QPRF) were provided in Annex III of the CLH Report.

There were two reliable studies on algae toxicity. In a study performed according to the OECD TG 201 following GLP, p-cymene (purity 99.6 %) was tested with the green algae *Selenastrum capricornutum.* In the definitive test, algae were treated with nominal concentrations of 0, 0.65, 1.3, 2.5, 5.0 and 10.0 mg/L for 72 hours. Initial mean measured concentrations 0.623, 1.40, 1.91, 3.52, and 5.32 mg/L. Final measured concentrations were 53-108 % of nominal concentrations. Results were expressed as initial mean measured concentrations. Control algal populations grew at an acceptable rate (10 000 cells/ml) after 72 hours. The pH was unchanged by the test substance. At the end of the test, samples of test media from each test vessel with maximal growth inhibition were combined with fresh media. After 48 hours incubation, the number of cells increased from 1 500 cells/mL to 1 1328 000 cells/mL at 3.52 mg/L suggesting that the toxic effects were algistatic. The 72-h E_rC_{50} was 4.03 mg/L based on average specific growth rate. The 72-h E_rC_{50} value of 4.03 mg/L was used as key data for classification.

In the other algal growth inhibition test performed according to OECD TG 201 with *Selenastrum capricornutum*, the nominal test concentrations were: 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L. Control and solvent control were included. Type of solvent was not specified. All treatments were conducted in triplicate with about 1×10^4 cells/mL at the start. As the measured test concentration deviated more than 20 % from the nominal test concentrations, the effect concentrations were based on the mean measured test concentrations. Section-by-section rates during the test were reported. The EC_{50} and NOEC values based on algal growth rate for the 72 h period were not provided and only results based on biomass are available.

QSAR based (neutral organics) EC_{50} and NOEC values for algae were generated with ECOSAR v1.11 available in EPI Suite. Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, an EC_{50} of 1.641 mg/L was estimated. The log K_{ow} value used was within the applicability domain. The QSAR endpoint for acute algal toxicity were considered reliable and were used as supporting information for classification purposes.

Based on the Mysid QSAR derived 96h LC_{50} of 0.327 mg/L, the DS proposed classification as Aquatic Acute 1 (M=1).

Chronic Aquatic Toxicity

An early life stage toxicity test following OECD TG 210 was conducted with medaka (*Oryzias latipes*) to assess the toxicity of p-cymene. 60 fertilized eggs were used per test group. Five nominal test concentrations were tested, i.e. 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L. The study design was flow-through. Exposure duration was 40 days (hatching after 31 days). Monitored endpoints were: hatching rate, time to hatch, developmental abnormalities, survival, toxic symptoms, body weight, and body length of surviving fry. Since the measured test concentrations ranged 58.4 to 80.0 % of nominal concentrations, the effect concentrations were based on measured concentrations. No significant effects were observed for the embryonic indicators; hatching rate, time to hatching and the developmental abnormalities rate. A significant effect was observed on the survival and growth after hatching of larval and juvenile fish at 1.44 mg/L. The LOEC was reported as 1.44 mg/L, and the NOEC as 0.690 mg/L. The 40-d NOEC value from this study was used as key data for classification.

QSAR based (neutral organics) NOEC values for fish were generated with ECOSAR v1.11 available in EPI Suite (US-EPA 2012). Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, NOECs of 0.124 and 0.506 mg/L were estimated for fresh and saltwater fish respectively. The log K_{ow} value used was within the applicability domain. The QSAR endpoints for long-term fish toxicity were considered reliable and were used as supporting information for classification purposes.

There are no experimental data on chronic invertebrate toxicity available. QSAR based (neutral organics) NOEC values for aquatic invertebrates were generated with ECOSAR v1.11 available in EPI Suite. Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L, a NOEC of 0.117 mg/L was estimated for daphnids (fresh water). The log K_{ow} value used was within the applicability domain. Considering that there were no reliable experimental data on chronic invertebrate toxicity available, QSAR endpoints for chronic invertebrate toxicity were used as key data for classification. A QSAR model reporting format (QMRF) and QSAR prediction reporting format (QPRF) was provided in Annex III of the CLH Report.

The two reliable algae toxicity studies are described in detail under acute toxicity chronic algae toxicity on algae toxicity. Both studies were performed according to the OECD TG 201 with *Selenastrum caprocornutum*. In the first study, the 72-h NOE_bC = 1.40 mg/L is based on number of cells/ml. A 72-h NOE_rC value is not reported so the 72-h NOE_bC of 1.4 mg/L was used as supporting data for classification. In the other study, the EC_{50} and NOEC values based on algal growth rate for the 72 h period were not provided, with only biomass results available. As there were no 72-h NOE_rC values available, the NOE_bC of 0.51 mg/L from this study was used as a key data for classification.

QSAR values for chronic algae toxicity (neutral organics) were estimated with ECOSAR v1.11 available in EPI Suite. Based on the log K_{ow} value of 4.1 and a water solubility of 23.35 mg/L a NOEC of 0.468 mg/L were estimated. The log K_{ow} value used was within the applicability domain. The QSAR endpoint for chronic algal toxicity was considered reliable and was used as supporting information for classification purposes.

The DS proposed to classify as Aquatic Chronic 3 based on the daphniid QSAR NOEC of 0.117 mg/L and the substance being rapidly degradable.

Comments received during public consultation

Two Member States (MS) agreed with the Dossier Submitter (DS) proposal. One MS asked for QMRF and QPRF documents for QSAR predictions for Mysids in case the Mysid endpoint was to be used as key data for classification. They also wanted information of the training set substances. The DS confirmed that the mysid data is used as key data and added the QMRF and QPRF documents to the Response to Public Consultation Document. They informed that the training set of the Mysid acute QSAR contains 14 substances including benzene, toluene and ethylbenzene.

Regarding the chronic Daphnia magna study, the DS informed that they did not have sufficient details to calculate the mean measured concentrations which adds to the unreliability of the endpoint. The DS considered that the 16-day QSAR endpoint is the most realistic option for classification fully aware that the standard test duration is 21 days. They also preferred the 16-day value to the surrogate method with the QSAR generated endpoint. The training set of the chronic Daphnia QSAR contains 23 substances including benzene, toluene, xylene, ethylbenzene and an alkylbenzene.

One MS brought up that at present there was no valid chronic endpoint available for algae because the NOEC values available were not based on growth rate. They proposed to use the surrogate method for this endpoint. The DS did not agree because there were enough chronic data on algae even if the growth rate endpoint is not addressed.

An industry organisation was of the opinion that the QSAR modelling overestimates the aquatic toxicity potential of the compound and did not think the use of QSAR reliable for invertebrates. They also informed that they had submitted, in the context of a REACH registration, a new daphnia study including analytics, resulting in an EC_{50} value of 3.7 mg/L. As this data was not yet disseminated on the ECHA website, the DS was not able to review it. However, the information was available for review by RAC.

Additional key elements

During PC, an additional acute toxicity study on Daphnia following OECD TG 202 (GLP) was brought to the attention of RAC. The study was assessed by RAC and was considered to be both reliable and relevant for the classification and labelling of p-cymene. The study measured immobilisation of Daphnia after 48 hours at nominal concentrations of 0, 0.625, 1.25, 2.5, 5.0, 10 mg/L. At study end, concentrations of p-cymene deviated by more the 20 % from nominal (0, 0.30, 0.56, 1.30 2.30, 4.90 mg/L), so results are based on mean measured concentrations. All validity criteria were met. The 48 h EC_{50} for immobilisation was 3.7 mg/L.

As the study was not available to the DS and was not included in sufficient detail by the commenting party during PC, a targeted PC was launched for this additional study and two member states provided comments. One member state agreed that the study was reliable and relevant for consideration, whilst the second member state disagreed on the basis that a solvent (Dimethylformamide; DMF) was included in the study without sufficient justification and information as to the concentration used. In response, RAC considered that water solubilities in test systems can be significantly lower in toxicity test media and that on this basis, the use of a solvent could be justified. Furthermore, a solvent control had been conducted in the test, indicating no adverse effects from the solvent. On this bases, RAC concluded that the study is reliable and should be used for the classification of p-cymene. During the discussion at RAC, industry informed that the solvent concentration was available in the full study report and that it was within the specifications in the relevant OECD test guidelines.

Assessment and comparison with the classification criteria

p-Cymene was not susceptible to hydrolysis because it contains no functional groups that could hydrolyse such as esters, amides or epoxides. Direct photolysis was not expected to be an important environmental fate process. The biodegradation potential of p-cymene was evaluated using the MITI test method OECD TG 301C where average degradation after 14 days amounted to 88.0 ± 6.2% based on BOD, 88.7 ± 1.2 based on TOC, and 100 ± 0.0% based on GC. The 10-day window was met. All validity criteria were met, and p-cymene was shown to be readily biodegradable. RAC agrees with the DS that p-cymene should be considered as rapidly degradable.

There were no experimental data available on the bioaccumulation potential of p-cymene. There was a reliable experimentally determined log K_{ow} of 4.1 available supported by several estimations from different models. Based on this, RAC agrees with the DS's conclusion that p-cymene has a high potential for bioaccumulation.

As a consequence of the OECD TG 202 study on Daphnia using p-cymene, there are now reliable experimental data for acute toxicity in fish, *Daphnia* and algae, and these are preferentially used instead of the QSAR derived toxicity values. The lowest value is a 96-hour LC_{50} for fish of 2.0 mg/L. Consequently, RAC disagrees with the DS and **concludes that no classification for acute aquatic hazards is warranted**.

There are reliable experimental data on chronic toxicity for fish and algae. The lowest value is a 72-h NOE_bC = 0.51 mg/L resulting to Aquatic Chronic 3 classification for a rapidly degradable substance, based on CLP table 4.1.0(b)(ii). No values based on growth rate are available. As there is no chronic data available for invertebrates, the classification criteria for the surrogate method outlined in table 4.1.0(b)(iii) of CLP are used for this trophic level. This approach using substance data is preferred to use of QSAR derived values. The 48-h EC_{50} for Daphnia is 3.7 mg/L and as p-cymene is rapidly degradable but has a high potential for bioaccumulation, this leads to Aquatic Chronic 2 classification. As this is the most stringent outcome after comparison with the CLP criteria, classification as Aquatic Chronic 2 is warranted.

In conclusion, RAC considers that classification of p-cymene as **Aquatic Chronic 2; H411** is warranted.

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8 ANNEXES

8.1 CONFIDENTIAL ANNEX (1)

8.1.1 Composition of the substance (confidential information) & Full references on vertebrate animal studies

The contents of this annex have been included in a seperate (confidential) file.

8.2 ANNEX II

8.2.1 NITE – BIODEGRADATION STUDY

Page 1

Final report
The degradation test by microorganisms of the test substance K-696C
Chemicals Inspection and Testing Institute
Chemical Biotesting Center, Kurume Laboratory

Page 2 and 3 *contain GLP and Quality assurance statements: not translated*

Page 4 *contains the table of contents: not translated*

Page 5

Summary

1. Study title: The degradation test by microorganisms of the test substance K-696C

2. Degradation test

2.1 Test conditions

(1) The test substance concentration	100 mg/L
(2) Activated sludge concentration	30 mg/L (as suspended solids)
(3) Test solution volume	300 mL
(4) Test liquid culture temperature	25 ± 1 ° C
(5) Test liquid culture period	14 days

2.2 Measurement and analysis

(1) Measurement of biological oxygen demand (BOD) with closed system oxygen consumption measuring apparatus

(2) Analysis of dissolved organic carbon with total organic carbon meter (TOC)

(3) Analysis of the test substance by gas chromatography (GC)

3. Test results

(1) Degradability based on the BOD:	86%	83%	95%
(2) Degradability based on the TOC method:	88%	90%	88%
(3) Degradability based on the GC method:	100%	100%	100%

4. Stability of the test substance

Stability of the test substance under the storage conditions was confirmed.

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Final report

Study No. 20696C

1. Study title

The degradation test by microorganisms in the table entitled test substance K-696C

2. Sponsor

Name: Ministry of International Trade and Industry

Address: 1-3-1 Kasumigaseki, Chiyoda-ku, Tokyo, Japan

3. Testing facility

Name: Foundation Chemicals Inspection Association

Address: 19-14 Chuo-cho, Kurume-shi, Fukuoka, Japan

Tel: 0942-34-1500

Facility manager: ************

4. Purpose of the study

This study was conducted to evaluate biodegradability of the test substance (K-696C).

5. Test method

This study was conducted in accordance with the “Biodegradation Test of Chemical Substances” specified in the “Test Method Relating to New Chemical Substances (Kanpogyo No.5, Yakuhatsu No.615, 49 Kikyoku No.392, July 13, 1974)”.

6. Test period

(1) Start of the test: January 16, 1990

(2) Study period

Start of the use of activated sludge: November 13, 1989

Start of incubation: January 16, 1990

End of incubation: February 13, 1990

(3) End of the study: March 6, 1990

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7. Testing officials

Study director:	***************
Test personnel:	***************
Activated sludge management officer:	***************
Storage division manager:	***************
	March 6, 1987
Final report author:	***************

8. Approval of the final report March 8, 1987

Name: ***************

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9. Test substance

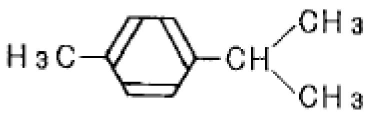
The test substance (K-1035) described herein is identified by following name, structure, etc.

9.1 Name

p-cymene

9.2 Structural formula, etc.

Structural formula



Molecular formula $C_{10}H_{14}$

Molecular weight 134.22

9.3 Purity

93% (see figure 9 by GC method)

9.4 Supplier and lot number

(1) Supplier: ***** (***** reagent)

(2) Lot number : APO2

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9.5 Identification

Structure of the test substance was identified by infrared spectroscopy, mass spectrometry and nuclear magnetic resonance spectroscopy.

9.6 physicochemical properties

Appearance	colorless transparent liquid
Boiling point*1	177 ℃
Specific gravity	0.856
Solubility	
water	5.0 mg/L
hexane	100 g/L or more
chloroform	100 g/L or more
ethyl acetate	100 g/L or more
methanol	100 g/L or more
tetrahydrofuran (THF)	100 g/L or more
dimethylformamide (DHF)	100g / L or more

Partition coefficient (n-octanol / water)

Log Pow = 4.44 (by the OECD method.)

Infrared absorption spectrum	(see figure 6)
Mass spectrum	(see figure 7)
Nuclear magnetic resonance spectrum	(see figure 8)

*1 from chemical encyclopedia (Kyoritsu Shuppan Co., Ltd.).

9.7 Storage conditions and stability under those conditions

(1) Storage conditions: Cool and dark place

(2) Stability: Stability of the test substance was confirmed by infrared spectra measured before and after incubation (see Figure 6).

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10 Preparation of the activated sludge

10.1 Sludge sampling sites and period

(1) Sites: Sludge samples were taken at following 10 sites around Japan:

- Fushikogawa Sewage Treatment Plant (Sapporo-shi, Hokkaido)
- Fukashiba Sewage Treatment Plant (Kashima-gun, Ibaraki)
- Nakahama Sewage Treatment Plant (Osaka-shi, Osaka)
- Ochiai Sewage Treatment Plant (Shinjuku-ku, Tokyo)
- Kitakami River (Ishinomaki-shi, Miyagi)
- Shinano River (Nishikanbara-gun, Nigata)
- Yoshino River (Tokushima-shi, Tokushima)
- Hiroshima Bay (Hiroshima-shi, Hiroshima)
- Dokai Bay (Kitakyushu-shi, Fukuoka)

(2) Period: September 1986

10.2 Collection of samples

(1) Municipal wastewater: returned sludge of the sewage treatment plants

(2) River, lake and sea: surface water and surface soil at water's edge having contact with the atmosphere

10.3 Mixing of old and new sludge

Portions of 500 mL each taken from filtrates of sludge samples collected at above sites were mixed with 5 L of filtrate of the old sludge which had been used for testing to make 10 L of a new sludge suspension. This suspension was adjusted to pH of 7.0 ± 1.0 and aerated in an incubation tank[*2].

[*2]: Filtered outdoor air was used for aeration.

10.4 Culturing

After stopping the aeration of the incubation tank to let the sludge settle for approximately 30 minutes, about one-third of the supernatant was replaced by equal amount of 0.1% synthetic sewage water[*3] before resuming the aeration. This procedure was repeated daily to prepare the activated sludge culture. The incubation temperature was 25 ± 2℃.

[*3]: Synthetic sewage water (0.1%) was prepared by dissolving glucose, peptone and potassium dihydrogen phosphate into deionized water at concentrations of 0.1% each and adjusting to pH of 7.0 ±1.0.

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10.5 Maintenance and use

Appearance of the supernatant and formation of the activated sludge flocks were observed. In addition, precipitability, pH, temperature and dissolved oxygen concentration of the activated sludge were recorded during incubation. Activated sludge used for the test was observed under an optical microscope as appropriate to confirm that no abnormalities were found in biota.

11 Conduct of the biodegradation study

11.1 Preparation of test

(1) Determination of suspended solid concentration in the activated sludge

Method: Carried out in accordance to JIS K 0102-1986 14.1.

Date: January 16, 1990

Result: Concentration of suspended solid was 6000 mg/L.

(2) Preparation of basal medium

Basal medium was prepared by mixing 3mL each of solutions A, B, C and D prescribed in Japanese Industrial Standard "Testing methods for industrial waste water: biochemical oxygen demand" (JIS K 0102-1985 21) and purified water to make a final volume of 1L and adjusting to pH of 7.0.

(2) Reference substance

Aniline (Showa Chemical Co., reagent grade) was used as a reference substance.

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11.3 Preparation of the test suspensions

Test suspensions were prepared in six separate bottles according to the procedures described below. Those bottles were incubated under the conditions described in section 11.3

(1) Spiking of test substance or aniline

(a) water and the test substance (1 bottle)

Transfer 300 mL of pure water into a culturing bottle and add the test substance at a concentration of 100 mg/L.

(b) activated sludge and the test substance (3 bottles)

Transfer 300 mL of basal medium into a culturing bottle and add the test substance at a concentration of 100 mg/L.

(c) activated sludge and aniline (1 bottle)

Transfer 300 mL of basal medium into a culturing bottle and add aniline at a concentration of 100 mg/L

(2) Inoculation of the activated sludge

(b), (c) and inoculum blank (one culturing bottle with only 300 mL basal medium) were inoculated with activated sludge that was prepared at a suspended solid concentration of 30mg/L.

11.2 Test liquid culture apparatus and conditions

(1) Test liquid culture apparatus

Closed system oxygen consumption measuring apparatus (coulometer; Ohkura Electric Co., Ltd)

Test vessel: 300ml culturing bottles (improved for volatile substances)

CO_2 gas absorber: Soda lime, No1 (manufactured by Wako Pure Chemical Industries, reagent first class)

Stirring method: rotary stirring with magnetic stirrer

(2)Environmental condition

Incubation temperature: 25±1℃

Incubation period: 14 days

Site: Equipment room No 6

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11.4 Analyses of the test suspensions

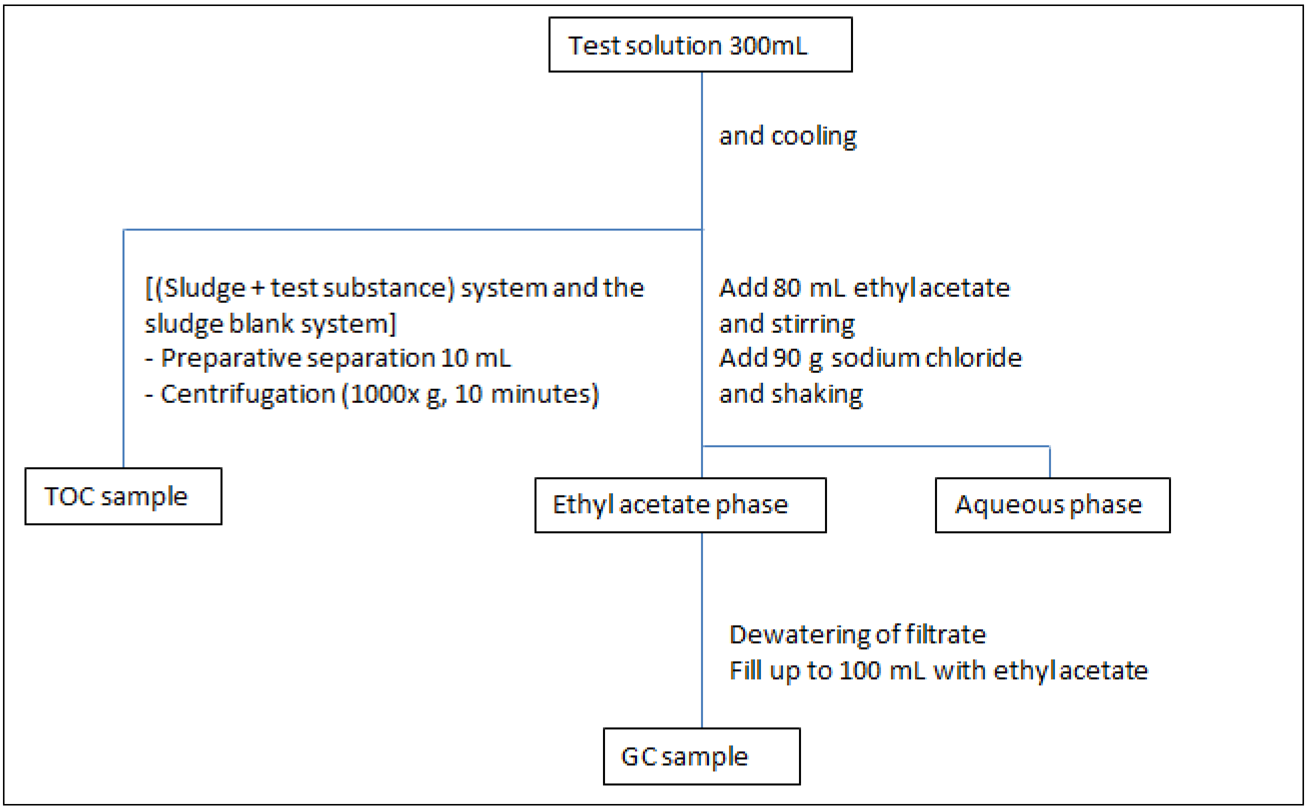
After the end of the incubation period, the test substance remaining in the test solution was analyzed. Also in the (sludge + test substance) system and the sludge blank system the test solution was analyzed for remaining dissolved organic carbon.

(1) Pre-treatment of the test solution

After the end of the incubation period, test suspensions of (sludge + test substance) system and the sludge blank system were pretreated according to the procedure described in the following flow chart to prepare samples for the GC analyses:

Note that (sludge + test substance) system and the sludge blank system were pretreated according to the procedure described in the following flow chart to prepare samples for the total organic carbon (TOC) meter:

Flow scheme:



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(2) Analysis of dissolved organic carbon by total organic carbon meter

[(Sludge + test substance) system and the sludge blank system]

Samples prepared in the pretreatment procedures were analyzed for dissolved organic carbon under the conditions shown below. Dissolved organic carbon in the test solution was calculated by comparing the peak height of the TOC sample relative to the peak height of the TOC sample of a standard solution (see Table 2 and Fig. 2). Incidentally, TOC standard solution was prepared by dissolving potassium hydrogen phthalate in purified water. Detection limit of the peak height was in consideration of the noise level two mm (dissolved organic carbon concentration 0.9 mg /L).

Quantitative conditions

Equipment manufactured by Shimadzu Corporation Ding TOC-10B

TC furnace temperature 900 ° C

Flow rate 200 ml / min.

(3) Samples prepared in the pretreatment procedures were analyzed for the test substance under the conditions shown below for GC samples. Concentrations in analytical samples were calculated from its peak height on the chromatogram relative to the peak height of 300.0 mg/L standard solution of the test substance (see Table 3 and Fig. 3). Measurement limit of the peak area was in consideration of the noise level 50W · sec (test substance concentration of 1.0 mg/ L) (see Figure 3).

(a) Analytical conditions

GC details were not translated

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(b) Calibration curve

A 150 mg portion of the test substance was dissolved in ethyl acetate to final volume of 100mL to give a 1500mg/L stock solution. This stock solution was diluted with ethyl acetate to make 75.0, 150 and 300 mg/L standard solutions. These standard solutions were then analyzed by GC under the conditions described above. A calibration curve was prepared based on the respective peak area and concentrations (see Figure 5).

(c) Recovery test

Recovery rates of the spiked test substance in (water + test substance) and (sludge + test substance) suspensions prepared as described in section 12.2 were determined. Suspensions were pretreated according to the procedure described in section 12.4.1 and analyzed by GC under the analytical conditions described in section 12.4.2. Recovery rates of duplicate samples are shown below (Table 4 shows recovery reference). The average recovery rates were used to correct concentrations of the test substance in analytical samples (see Table 4).

(Water + test substance) system recovery rate 99.2%

(Sludge + test substance) system recovery rate 96.8%

Page 16 *contains standard BOD, TOC and GC calculations: not translated*

Page 17

12 Test results

12.1 Appearance of the test suspensions

Appearance of the test suspensions were as follows:

	Test suspensions	Appearance
Start of test	(Water + test substance) system	Test substance was not dissolved. It was suspended in oil droplet form.
	(Sludge + test substance) system	Idem
End of test	(Water + test substance) system	Test substance was not dissolved. It was suspended in oil droplet form.
	(Sludge + test substance) system	The test substance was not confirmed. Growth of the sludge was observed.

12.2 Extent of degradation

Degradabilities of the test substance after 28 days of incubation were as follows:

	Decomposition rate (%)			Appendix table
	2	3	4	
Results from BOD	86	83	95	Table 1
Results from OC method	88	90	88	Table 2
Results from GC method	100	100	100	Table 3

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12.3 Confirmation of test conditions

Since the decomposition rate after 7, and14 days aniline obtained from BOD are respectively 53 and 87%, the test conditions of this test were confirmed to be effective.

13 Storage and retention of the test substance and records

13.1 Test substance

A 5g portion of the test substance was placed in a storage container, tightly sealed and stored in the sample storage room of the Kurume Laboratory for the period specified in paragraph 32 of the “Standard concerning Testing Facility Provided for in Article 3 of the Ordinance prescribing Test Items etc. Relating to New Chemical Substances.

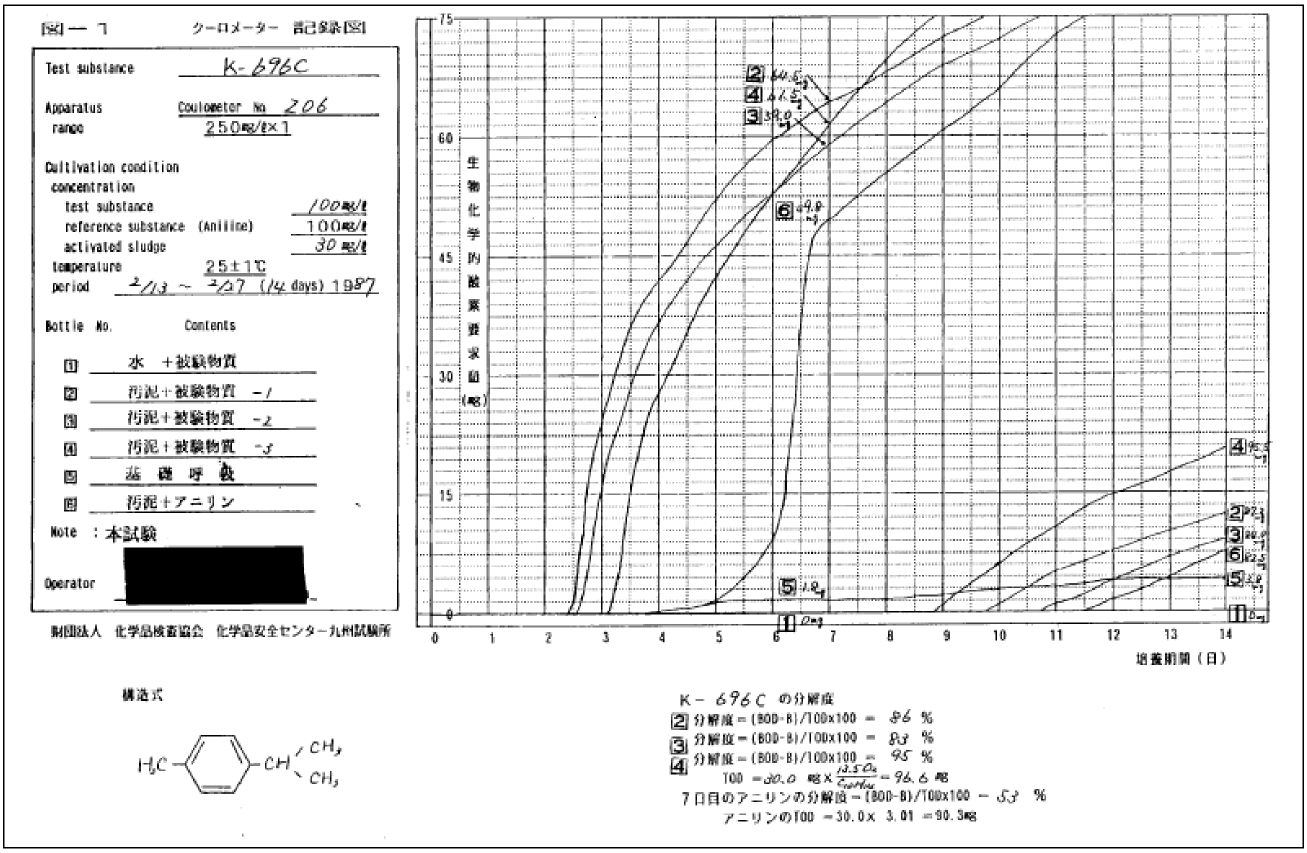
13.2 Raw data, records, etc.

Results of analyses, measurements and observations generated in the study and other raw data such as laboratory notebooks which were used for the development of final report, test protocol, records of inspection, reference materials, etc. are stored along with the final report in the archive of the laboratory for the period specified in paragraph 32 of the above Ordinance.

14 Major apparatuses, instruments, reagents, etc. used in the tests

Not translated as not relevant for reliability assessment

Page 19



8.2.2 NITE – ALGAL GROWTH INHIBITION STUDY

Summary

Test sponsor

Environment Agency

Title

The degradation test by microorganisms of the test substance K-696C

Test number

No. 10051

Test method

The study was carried out in compliance with the OECD chemicals test guideline 201 "algae growth inhibition test" (1984).

1) Test substance: p-cymene
2) The culture method: shaking culture method (100rpm)
3) Exposure period: 72 hours
4) Number of stations: 1 concentration groups triplicate + analytical test incubator (total of four)
5) Test organisms: Selenastrum capricornium (ATCC22662 shares)
6) Initial cell concentration: about 1 × 104 cells/mL
7) Test water: 100 ml/L series (OECD medium)
8) Test temperature: 23 ± 2 ° C
9) Lighting: continuous illumination (4 in the vicinity of the flask liquid surface, 000 ~ 5, 0001x)
10) Test concentrations: Control, solvent control, 1.0, 2 2, 4 6, 10, 22, 46 and 100 mg/L
11) Analysis of the test substance in solution: GC-MS (at start and end of exposure)

Results

Since the measured concentration of the test substance exceeded the nominal test concentrations by more than ±20%, the following results were calculated using measured concentrations.

1) 50% growth inhibitory concentration by comparing the area under the growth curve (EbC50) and maximum no-effect concentration (NOEC)

   EbC50 (0-72hr): 3.7 mg/L, 95% C.I: 3.1 ~ 4.2 mg/L (linear regression analysis)

   NOEC (area method 0 - 72hr): 0.51 mg/L (Dunnett's multiple comparison method)

2) 50% growth inhibitory concentration by comparing the ratio of the growth rate (ErC50) and maximum no-effect concentration (NOEC)

   ErC50 (24-48hr): 5.4 mg/L, 95% C.I: 5.1 ~ 5.8 mg/L (linear regression analysis)

   NOEC (velocity method 24-48hr): 1.3 mg/L (Dunnett's multiple comparison method)

   ErC50 (24-72hr): 6.7 mg/L, 95% C.I: 5.6 ~ 6.8 mg/L (linear regression analysis)

   NOEC (velocity method 24-72hr): 2.7 mg/L (Dunnett's multiple comparison method

8.2.3 NITE – DAPHNIA REPRODUCTION STUDY

Summary

Test sponsor

Environment Agency

Title

The water flea (*Daphnia magna*) breeding inhibition test with p-cymene

Test number

No. 10053

Test method

The study was conducted in compliance with OECD Chemical Test Guideline 211 "reproductive test with Daphnia magna" (adopted in September 1998).

1) Test substance: p-cymene
2) Exposure method: semi-static (every 48 hours total exchange of solution)
3) Exposure period: 21 days
4) Number of stations: 1 concentration group, 10 replicates
5) Test organisms: *Daphnia magna* (*Daphnia magna*)
6) Biological number: 10 animals per concentration group
7) Test volume: 80 mL per replicate
8) Test water temperature: 20 ± 1 ℃
9) Lighting: room light (1, 2001x or less), 16 h light / 8 hours dark
10) Feed: *Chlorella vulgaris*
11) Feeding amount: about 0.15 mg C / daphnia / day
12) Test concentration: control, solvent control, 0.010, 0.022, 0.046, 0.10, 0.22, 0.46 & 1.0 mg/L
13) Analysis of the test substance in solution: GC-MS (before and after renewal at start, and subsequently before and after renewal after 2, 4, 6, 8 14, and 16 days)

Results

The detection limit is 1.0 mg/L. The tested concentrations below 1.0 mg/L cannot be measured. The following results are expressed in nominal concentrations:

1) Parent daphnia of median lethal concentration (LC50)
   LC50 (21days): 1.0 mg/L or more
2) 50% breeding inhibitory concentration (EC50)
   EC50 (21days): 1.0 mg/L or more
3) Maximum no-effect concentration (NOEC)
   NOEC (21days): 0.46 mg/L (Dunnett's multiple comparison method)
4) Minimum effect concentration (LOEC)
   LOEC (21days): 1.0 mg/L (Dunnett's multiple comparison method)

8.2.4 NITE – FISH ACUTE TOXICITY STUDY

Summary

Test sponsor

Environment Agency

Title

Medaka (*Oryzias latipes*) acute toxicity test with p-cymene

Test number

No. 10054

Test method

The study was conducted in compliance with OECD Chemical Test Guideline 203 "fish acute toxicity test " (1992).

1) Test substance: p-cymene
2) Exposure method: flow-through (using the serial dilution device using a metering pump)
3) Exposure period: 96 hours
4) Number of stations: 1 concentration group, 1 station
5) Test organisms: Medaka (*Oryzias latipes*)
6) Test fish number: 10 fish per concentration group
7) Test volume: about 9 L
8) Flow rate and renewal rate: 50 mL/min, about 8 times/day
9) Test water temperature: 24 ± 2 °C
10) Lighting: room light, 16-hour light / 8-h dark
11) Feeding: No feeding
12) Aeration: None
13) Test concentration: Control, solvent control, 1.0, 1.8, 3.2, 5.6 and 10 mg/L
14) Analysis of the test substance in solution: GC-MS (exposure at the start and after 48 hours)

Results

Since the measured concentration of the test substance exceeded the nominal test concentrations by more than ±20%, the following results were calculated using measured concentrations

1) Median lethal concentration (LC50)
   LC50 (96hr): 2.0 mg/L (geometric mean)
2) 0% mortality highest concentration (96hr): 1.2 mg/L
3) 100% mortality lowest concentration (96hr): 3.4 mg/L

8.2.5 NITE – FISH EARLY LIFE STAGE TOXICITY STUDY

Summary

Early life stage toxicity test with p-cymene using killifish (*Oryzias latipes*).

Study used 60 fertilized eggs per group. 5 nominal test concentrations [2.00, 1.00, 0.500, 0.250 and 0.125 mg/L (ratio of 2.0)], and a solvent control were included. The control group was subdivided (4 stations per test group). The water temperature was 24±1 ℃. The duration was 40 days (hatching after 31 days) with continuous exposure to test solution using flow-through design. During the test the following was monitored:, hatching numbers and time to hatch, developmental abnormalities, survival after hatching, toxic symptoms, body weight and body length of surviving fry. These were indicators to determine the effect of the test substance. In addition, the test substance concentration was measured in the test solution and the water quality was monitored.

The test substance concentration in the measured test solution was 58.4 to 80.0% of the nominal concentrations, and exceeded the range of ± 20% of nominal. Therefore, the following test results were calculated based on the measured concentrations.

For killifish exposed to p-cymene no significant effects were observed on the indicators of the embryonic stage (hatching rate, hatching days and developmental abnormalities rate) in the treated versus control groups. In the larval and juvenile fish life, significant effect on survival and growth after hatching was observed in the 1.44 mg /L treatment (length and weight), significant toxicity symptoms were also observed.

Based on the results obtained in the present study for killifish exposed to p-cymene, the LOEC (minimum effect concentration) is 1.44 mg/L, and the NOEC (maximum no-effect concentration) was 0.690 mg/L.

8.3 ANNEX III

8.3.1 QSAR model reporting format ACUTE DAPHNID

	QMRF Title: **ECOlogical Structure Activity Relationship (ECOSAR), Acute Daphnid 48-hour LC_{50}- Neutral Organics**
	Printing Date: **Oct 1, 2015**

1. QSAR identifier

1.1. QSAR identifier (title):

ECOlogical Structure Activity Relationship (ECOSAR), Acute Daphnid 48-hour LC_{50}- Neutral Organics.

Please note: The (Q)SAR under evaluation is one on many available in the ECOSAR Program (see section 1.3). The evaluation, statistics and data presented are only applicable to the acute daphnid 48-hour LC_{50} (Q)SAR and no other (Q)SARs available within the program.

1.2. Other related models:

1.3. Software coding the model:

ECOSAR™ Version 1.11 (Sept 2012): The Ecological Structure Activity Relationships (ECOSAR) Class Program estimates the aquatic toxicity of industrial chemicals. The program estimates acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms to fish, aquatic invertebrates, and green algae, and has limited SARs for other salt water and terrestrial species, where data were available.
ECOSAR is included in the EPI (Estimation Programs Interface) Suite which is a window based suite of physical/chemical property, environmental fate and ecotoxicity models.

2. General information

2.1. Date of QMRF:

1 October 2015

2.2. QMRF author(s) and contact details:

Bureau REACH
The National Institute of Public Health and the Environment (RIVM)
The Netherlands
Email: bureau- reach@rivm.nl

2.3. Date of QMRF update(s):

Not known

2.4. QMRF update(s):

Not known

2.5. Model developer(s) and contact details:

Kelly E. Mayo-Bean Risk Assessment Division (7403M), 1200 Pennsylvania Ave, N.W., Washington, DC 20460-0001 202-564-7662 mayo.kelly@epa.gov

Gordon G. Cash Risk Assessment Division (7403), U.S. Environmental Protection Agency, 1200 Pennsylvania Avenue, NW, Washington, DC 20460-0001 Phone: 202-564-8923 cash.gordon@epa.gov

2.6. Date of model development and/or publication:
September 2012

2.7. Reference(s) to main scientific papers and/or software package:
ECOSAR v 1.11 Methodology Document for the ECOlogical Structure Activity Relationship Model (ECOSAR) Class program. Estimating toxicity of industrials chemicals to aquatic organisms using ECOSAR (Ecological structure activity relationship) class program. MS-Windows Version 1.11.Mayo-Bean K, Moran K, Meylan B, Ranslow P. May 2012. PFD document available in the ECOSAR help menu.
ECOSAR v 1.11 Operation Manual for the ECOlogical Structure Activity Relationship Model (ECOSAR) Class program. Estimating toxicity of industrials chemicals to aquatic organisms using ECOSAR (Ecological structure activity relationship) class program. MS-Windows Version 1.11.Mayo-Bean K, Moran K, Nabholz JV, Meylan E, Howard PH. March 2012. PFD document available in the ECOSAR help menu.
EPISuite (Version 4.1.1) program is publically available at: http://www2.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

2.8. Availability of information about the model:
The model is non-proprietary but some of the information within the predictive system is confidential business information (CBI) collected by EPA under the New Chemicals Programs and is therefore restricted from being revealed.

2.9. Availability of another QMRF for exactly the same model:
Not known.

3. Defining the endpoint - OECD Principle 1

3.1. Species:
Daphnia magna

3.2. Endpoint:
Ecotoxic effects, Short-term toxicity to invertebrates (freshwater)

3.3. Comment on endpoint:

3.4. Endpoint units:
LC50 values are presented in mg/L

3.5. Dependent variable:
Log 48-hour LC50
"LC_{50}" means that experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

3.6. Experimental protocol(s):
OECD TG 202: Daphnia sp., Acute Immobolisation Test
OPPTS 850.1010: Aquatic Invertebrates Acute Toxicity Test, Freshwater Daphnids
40CFR.797.1300: Daphnid Acute Toxicity Test

3.7. Endpoint data quality and variability:
The data used for ECOSAR development undergo an extensive data validation step to ensure appropriateness for inclusion in the model. ECOSAR study criteria articulate that the toxicity should be measured at pH levels between 6 and 8 (replicating environmental conditions), the total organic carbon content should not exceed 2 mg/L, the water hardness should be less than 150 mg/L CaCO3, results should be adjusted to, or measured at, 100% active ingredient, and measured test concentrations maintained at greater than 80% of nominal concentrations.

4. Defining the algorithm - OECD Principle 2

4.1. Type of model:
Regression based QSAR

4.2. Explicit algorithm:
Log Toxicity (mmol/L) = -0.8580(log Kow) + 1.3848

4.3. Descriptors in the model:
To estimate the toxicity of aquatic organisms, the low Kow and MW are required.
Log Kow: Log of octanol/water partition coefficient (no units)
MW: Molecular weight. The LC50 predictions from the equation are presented in millimoles per liter (mmol/L). ECOSAR then converts the LC50 from mmol/L to mg/L, by multiplying value by molecular weight of the compound.

4.4. Descriptor selection:
The use of log Kow and MW to predict acute toxicity was determined experimentally through experience in US EPA, OPPT New Chemical Program and a need to derive the simplest approach for calculating acute toxicity to Daphnia.

4.5. Algorithm and descriptor generation:
To estimate LogKow, ECOSAR uses the KOWWIN v1.68 program from the EPISuite model. The underlying predictive methodology is described in the reference listed below: Meylan, WM; Howard, P. (1995) Atom/Fragment Contribution Method for Estimating Octanol-Water Partition Coefficients. J Pharm Sci 84: 83-92.
ECOSAR will accept user entered Log Kow.

4.6. Software name and version for descriptor generation:

KOWWIN v1.67

4.7. Chemicals/Descriptors ratio:
76 (152 chemicals / 2 descriptors)

5. Defining the applicability domain - OECD Principle 3

5.1. Description of the applicability domain of the model:
ECOSAR cannot be used for all chemical substances. The intended domain is organic chemicals.

Class definition
ECOSAR derives toxicity values for three general types of chemicals: neutral organics, organics with excess toxicity and surfactant (Surface-Active) organic chemicals. The (Q)SAR under evaluation, acute daphnid 48-hour LC_{50} falls under the neutral organics class.

LogKow

In general, when the logKow is less than or equal to 5.0 for daphnid, ECOSAR provides reliable quantitative (numeric) toxicity estimates for acute effects. However, the method may be used to estimate toxic effects equal to "no-toxic-effect-at-saturation or "*" for chemicals exceeding logKow values of 5. Therefore, the domain of the model is much larger than the values covered in the regression equation and covers all logKow ranges.

Molecular weight:

The molecular weight may also be considered to determine the absorption cutoff limit for aquatic organisms. Compounds with a molecular weight of greater than 1000 g/mol are considered too large to present any significant toxicity.

Water solubility

If the predicted toxicity exceeds the water solubility, no acute toxicity is expected to be observed in the absence of an organic carrier solvent.

5.2. Method used to assess the applicability domain:

Assess if substances properties fall within the limits of applicability of the model mentioned in sections 5.1 and 5.4.

5.3. Software name and version for applicability domain assessment:

Not applicable

5.4. Limits of applicability:

Maximum logKow: 5.0
Maximum MW: 1000

If the log Kow value is greater than 5.0, or if the compound is solid and the LC50 exceeds the water solubility by 10X, no effects at saturation are predicted.

6. Internal validation - OECD Principle 4

6.1. Availability of the training set:

Yes, however some information of the information contained in the training set is confidential business information (CBI) collected by EPA under the New Chemicals Program and is therefore restricted from being revealed. For these substances the name and CAS numbers are not revealed but data on the descriptors and dependent variables are available in the QSAR equation document.

6.2. Available information for the training set:

CAS RN: Yes Chemical Name: Yes

Smiles: No

Formula: No

INChI: No

MOL file: No

Data table for the neutral organics - training set is available in the ECOSAR User Guide accessible via the Help tab.

6.3. Data for each descriptor variable for the training set:

MW, log Kow (CLogP) log Kow (EPI), log Kow (M)

6.4. Data for the dependent variable for the training set:

Daphnia 48-h LC50 (mg/L) and Log Daphnia 48-h LC50 (mmol/L)

6.5. Other information about the training set:

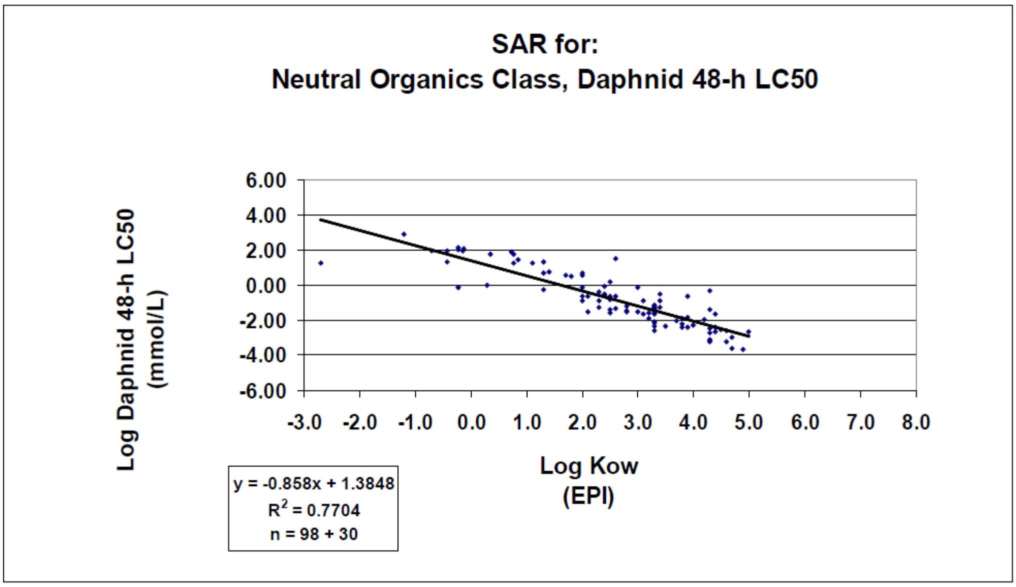
References for (measured Kow) and References (Daphnia 48-h LC50) are provided.

6.6. Pre-processing of data before modelling:

Not applicable

6.7. Statistics for goodness-of-fit:

The correlation (r^2) for neutral organics Daphnid 48-hour (Q) SAR equals 0.7704 obtained from standard statistical regression software.



The number of chemicals in the training set is represented by N = x + y where 'x' equals the number of studies used in the actual equation development and 'y' equals 1) logKow cut-off and/or SAR data not included in regression equation.

6.8. Robustness - Statistics obtained by leave-one-out cross-validation:
Not applicable

6.9. Robustness - Statistics obtained by leave-many-out cross-validation
Not applicable

6.10. Robustness - Statistics obtained by Y-scrambling:
Not applicable

6.11. Robustness - Statistics obtained by bootstrap:
Not applicable

6.12. Robustness - Statistics obtained by other methods:
Not applicable

7. External validation - OECD Principle 4

7.1. Availability of the external validation set:
See section 7.9

7.2. Available information for the external validation set:
See section 7.9

7.3. Data for each descriptor variable for the external validation set:
See section 7.9

7.4. Data for the dependent variable for the external validation set:
See section 7.9

7.5. Other information about the external validation set:
See section 7.9

7.6. Experimental design of test set:

See section 7.9

7.7. Predictivity - Statistics obtained by external validation:

See section 7.9

7.8. Predictivity - Assessment of the external validation set:

See section 7.9

7.9. Comments on the external validation of the model:

All available valid data were used by U.S. EPA/OPPT in development of the (Q)SARs within ECOSAR. Subsequent validation studies have been completed by multiple stakeholders. A list of supporting validation exercise performed in conjunction with EPA and other stakeholders on the ECOSOR model are listed below.

• External Peer Reviews
An independent peer review of ECOSAR was conducted as part of the development of the Organization for Economic Cooperation and Development’s (OECD) guidance, The Principles for Establishing the Status of Development and Validation of (Quantitative)
Structure-Activity Relationships [(Q)SARs] (OECD, 2004a).

• Participation in US-European Union Validation Exercise
EPA participated with the European Union in a large-scale verification study of ECOSAR to compare SAR predictions with the results of data from testing. That study (OECD 1994; U.S.EPA 1994) found our methods to be accurate 60-90% of the time depending on the endpoint assessed.

• International Collaboration in Development of Effective Predictive Tools
ECOSAR was included in OECD’s Report on the Regulatory Uses and Applications in OECD Member Countries of (Q)SAR Models in the Assessment of New and Existing Chemicals (OECD, 2006). Subsequently, the OECD solicited EPA to include ECOSAR into the OECD QSAR Application Toolbox, which was developed starting in 2006. Inclusion in the OECD toolbox
requires specific documentation, validation and acceptability criteria and subjects ECOSAR to international use, review, providing a means for receiving additional and on-going input for improvements. In an evaluation of a number of predictive tools used to profile chemicals and group them together based on similar toxicity, ECOSAR was the top performer.
[http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html#Additional_information_on_the_QSARs_Application_Toolbox]

8. Providing a mechanistic interpretation - OECD Principle 5

8.1. Mechanistic basis of the model:

Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug induced loss of conscience (general anesthesia). The octanol/water partition coefficient (Kow) is the major physical-chemical attribute correlating a chemical structure to toxic effect for nonreactive neutral organic chemicals. The most frequently used relationship is the logarithm of the Kow value versus the median toxicity (LC50 and EC50) value. This general narcosis is often referred to baseline toxicity.
The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfides.

8.2. A priori or a posteriori mechanistic interpretation:

8.3. Other information about the mechanistic interpretation:

9. Miscellaneous information

9.1. Comments:

9.2. Bibliography:

References Neutral Organics (Q)SAR

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Peer-Reviewed Publications Related to Validation, Verification and Performance of the ECOSAR Program

Book Chapters or Reports

OECD (Organization for Economic Cooperation and Development). (2006) Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationships [(Q)SAR] Models in the Assessment of New and Existing Chemicals. Organization for Economic Cooperation and Development, Paris; ENV/JM/MONO(2006)25.

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OECD (Organization for Economic Cooperation and Development). (2004c) Comparison of SIDS Test Data with (Q)SAR Predictions for Acute Aquatic Toxicity, Biodegradability and Mutagenicity on Organic Chemicals Discussed at SIAM 11-18. Organization for Economic Cooperation and Development, Paris; ENV/JM/TG(2004)26.

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Lynch, DG; Macek, G; Nabholz, J; et al. (1994) Ecological Risk Assessment Case Study: Assessing the Ecological Risks of a New Chemical Under the Toxic Substances Control Act. In: A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective, Volume II. Washington, DC: Risk Assessment Forum, Office of Research and Development, U.S. Environmental Protection Agency, pp. 1-1 to 1-B4.

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Kaiser, KL; Deardon J; Klein W; et al. (1999) Short Communication: A Note of Caution to Users of ECOSAR. Water Qual Res J Can 34:179-182.

Abstracts

Chun, J; Nabholz, J; Wilson, M. (2002) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT/SAR Prediction on PPG Polymers. Society of Environmental Toxicology and Chemistry Annual Meeting, Salt Lake City, UT.

Chun, J; Nabholz, J; Wilson, M. (2001) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT SAR Predictions on PPG Polymers. Society of Toxicology Annual Meeting, San Francisco, CA..

9.3. Supporting information:
Training set(s) Test set(s) Supporting information

10. Summary (JRC QSAR Model Database)

10.1. QMRF number:

10.2. Publication date:

10.3. Keywords:

10.4. Comments:

8.3.2 QSAR PREDICTION reporting format ACUTE DAPHNID

QSAR Prediction Reporting Format (QPRF):

***p*-cymene, Acute toxicity to Daphnid**

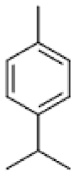
1. Substance

1.1 CAS number:
99-87-6

1.2 EC number:
202-796-7

1.3 Chemical name:
Substance name: 1-isopropyl-4-methylbenzene
Synonym: *p*-cymene

1.4 Structural formula:



p-Cymene

1.4 Structural formula:
SMILES: c(ccc(c1)C)(c1)C(C)C

2. General information

2.1 Date of QPRF:
23 November 2015

2.2 QPRF author and contact details:
Bureau REACH
The National Institute of Public Health and the Environment (RIVM)
The Netherlands
Email: bureau- reach@rivm.nl

3. Prediction

3.1 Endpoint (OECD Principle 1)
a. Endpoint:
Short-term toxicity to Daphnia 48-hour

b. Dependent variable:
Log 48-hour LC5

3.2 Algorithm (OECD Principle 2)
a. Model or submodel name:
ECOSAR

b. Model version:
Version 1.11 (Sept 2012)

c. Reference to QMRF:
Title: ECOlogical Structure Activity Relationship (ECOSAR), Acute Daphnid 48-hour LC50 –Neutral Organics.
Date: 1 October 2015
Author: Bureau REACH, The Netherlands

d. Predicted value (model result):
Daphnid 48-hour LC50 = 0.988 mg/L

e. Predicted value (comments):
Acute aquatic toxicity for crustacea
The predicted value will be compared to the criteria for classifying and categorizing a substance as "hazardous to the aquatic environment" as summarized in Table 4.1.0 (a) of the CLP Annex I:

Acute (short-term) aquatic hazard **Acute Category 1**	Note 1
96 hr LC50 (for fish) 48 hr EC (for crustacea) 72 or 96 hr (for algae or other aquatic plants)	≤ 1 mg/l and/or ≤ 1 mg/l and/or ≤ 1 mg/l. Note 2

Note 1: When classifying substances as Acute Category 1 and/or Chronic Category 1 it is necessary at the same time to indicate an appropriate M-factor (see table 4.1.3).
Note 2: Classification shall be based on the ErC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no ErC50 is recorded, classification shall be based on the lowest EC50 available.

f. Input for prediction:
See section 1.5

g. Descriptor values:
Log Kow (user entered): 4.1 (measured value)
Water solubility (user entered): 23.35 mg/L (measured value)

Applicability domain (OECD principle 3)
h. Domains:
The applicability domain criteria are fulfilled.

Descriptor domain
p-cymene: log Kow = 4.1 and molecular weight = 134.22
LogKow is less than maximum Log Kow 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50) and the molecular weight is less than the maximum of 1000.

Structural fragment domain and mechanism domain
Class definition: Neutral organic class
Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug-induced loss of conscience (general anesthesia). This general narcosis is often referred to as baseline toxicity (Franks and Lieb 1990, Veith and Broderius 1990). The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfides.

The structural formula of p-cymene (section 1.4) shows that it is an aromatic hydrocarbon and therefore falls within the neutral organic class as defined in ECOSAR.

i. Structural analogues:
j. Considerations on structural analogues:
Data tables on the neutral organics - training set is available (see related - QMRF).

3.3 The uncertainty of the prediction (OECD principle 4)

Model performance:
y = 0.857x + 1.2695 ($R2 = 0.7712$)
n = 115 + 37

3.4 The chemical and biological mechanisms according to the model underpinning the predicted result (OECD principle 5)

Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug induced loss of conscience (general anesthesia).

The octanol/water partition coefficient (Kow) is the major physical-chemical attribute correlating a chemical structure to toxic effect for nonreactive neutral organic chemicals. The most frequently used relationship is the logarithm of the Kow value versus the median toxicity (LC50 and EC50) value. This general narcosis is often referred to baseline toxicity.

The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfides.

4. Adequacy (Optional)

4.1 Regulatory purpose:

The present prediction will be used for classification and labelling purposes as required by Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

4.2 Approach for regulatory interpretation of the model result:

The predicted result (numeric value) is presented in the format directly useable for the intended regulatory purpose

4.3 Outcome:

The estimated daphnid 48-hr LC 50 of 0.988 mg/L is below the threshold value of 1 mg/L. Based on this information p-cymene would meet the criteria for classification for Aquatic Acute Category 1.

4.4 Conclusion:

Considering the above, the predicted result can be considered adequate for the regulatory conclusion described in 4.1.

8.3.3 QSAR model reporting format CHRONIC DAPHNID

QMRF	
	QMRF Title: **ECOlogical Structure Activity Relationship (ECOSAR), Daphnid Chronic Toxicity Value (ChV) – Neutral Organics**
	Printing Date: **Oct 1, 2015**

1. QSAR identifier

1.1. QSAR identifier (title):

ECOlogical Structure Activity Relationship (ECOSAR), Daphnid chronic toxicity value - Neutral Organics

Please note: The (Q)SAR under evaluation is one on many available in the ECOSAR Program (see section 1.3). The evaluation, statistics and data presented are only applicable to long term toxicity (Q)SAR for daphnid and no other (Q)SARs available within the program.

1.2. Other related models:

1.3. Software coding the model:

ECOSAR™ Version 1.11 (Sept 2012): The Ecological Structure Activity Relationships (ECOSAR) Class Program estimates the aquatic toxicity of industrial chemicals. The program estimates acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms to fish, aquatic invertebrates, and green algae, and has limited SARs for other salt water and terrestrial species, where data were available.

ECOSAR is included in the EPI (Estimation Programs Interface) Suite which is a window based suite of physical/chemical property, environmental fate and ecotoxicity models.

2. General information

2.1. Date of QMRF:

1 October 2015

2.2. QMRF author(s) and contact details:

Bureau REACH
The National Institute of Public Health and the Environment (RIVM)
The Netherlands
Email: bureau- reach@rivm.nl

2.3. Date of QMRF update(s):

Not known

2.4. QMRF update(s):

Not known

2.5. Model developer(s) and contact details:

Kelly E. Mayo-Bean Risk Assessment Division (7403M), 1200 Pennsylvania Ave, N.W., Washington, DC 20460-0001 202-564-7662 mayo.kelly@epa.gov

Gordon G. Cash Risk Assessment Division (7403), U.S. Environmental Protection Agency, 1200 Pennsylvania Avenue, NW, Washington, DC 20460-0001 Phone: 202-564-8923 cash.gordon@epa.gov

2.6. Date of model development and/or publication:
September 2012

2.7. Reference(s) to main scientific papers and/or software package:
ECOSAR v 1.11 Methodology Document for the ECOlogical Structure Activity Relationship Model (ECOSAR) Class program. Estimating toxicity of industrials chemicals to aquatic organisms using ECOSAR (Ecological structure activity relationship) class program. MS-Windows Version 1.11.Mayo-Bean K, Moran K, Meylan B, Ranslow P. May 2012. PFD document available in the ECOSAR help menu.

ECOSAR v 1.11 Operation Manual for the ECOlogical Structure Activity Relationship Model (ECOSAR) Class program. Estimating toxicity of industrials chemicals to aquatic organisms using ECOSAR (Ecological structure activity relationship) class program. MS-Windows Version 1.11.Mayo-Bean K, Moran K, Nabholz JV, Meylan E, Howard PH. March 2012. PFD document available in the ECOSAR help menu.

EPISuite (Version 4.1.1) program is publically available at: http://www2.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

2.8. Availability of information about the model:
The model is non-proprietary but some of the information within the predictive system is confidential business information (CBI) collected by EPA under the New Chemicals Programs and is therefore restricted from being revealed.

2.9. Availability of another QMRF for exactly the same model:
Not known.

3. Defining the endpoint - OECD Principle 1

3.1. Species:
Daphnia magna

3.2. Endpoint:
Ecotoxic effects, long-term toxicity to invertebrates (freshwater)

3.3. Comment on endpoint:
Regulatory endpoint: No observed effect level (NOEC)

ECOSAR: Chronic toxicity (long-term exposure) is assessed using Chronic (ChV) values. The ChV is defined as the geometric mean between lowest observed level (LOEC) and no observed effect level (NOEC) from the study. The ChV value is converted to a NOEC by:
NOEC = ChV/√2.

3.4. Endpoint units:
ChV values are presented in mg/L

3.5. Dependent variable:
Log 16-d ChV.

3.6. Experimental protocol(s):
OPPTS 850.1300, EPA OTS 797.1300 Daphnid Chronic Toxicity test
OECD TG 211/EU Method C.20: Daphnia magna reproduction test (similar or equivalent to)

3.7. Endpoint data quality and variability:
The data used for ECOSAR development undergo an extensive data validation step to ensure appropriateness for inclusion in the model. ECOSAR study criteria articulate that the toxicity should be measured at pH levels between 6 and 8 (replicating environmental conditions), the total organic carbon content should not exceed 2 mg/L, the water hardness should be less than 150 mg/L CaCO3, results should be adjusted to, or

measured at, 100% active ingredient, and measured test concentrations maintained at greater than 80% of nominal concentrations.

4. Defining the algorithm - OECD Principle 2

4.1. Type of model:
Regression based QSAR

4.2. Explicit algorithm:
Log 16-d ChV (mmol/L) = - 0.7469 log Kow + 0.1961

4.3. Descriptors in the model:
To estimate the toxicity of aquatic organisms, the low Kow and MW are required.
Log Kow: Log of octanol/water partition coefficient (no units)
MW: Molecular weight. The LC50 predictions from the equation are presented in millimoles per liter (mmol/L). ECOSAR then converts the LC50 from mmol/L to mg/L, by multiplying value by molecular weight of the compound.

4.4. Descriptor selection:
The use of log Kow and MW to predict toxicity was determined experimentally through experience in US EPA, OPPT New Chemical Program.

4.5. Algorithm and descriptor generation:
To estimate LogKow, ECOSAR uses the KOWWIN v1.68 program from the EPISuite model.

The underlying predictive methodology is described in the reference listed below: Meylan, WM; Howard, P. (1995) Atom/Fragment Contribution Method for Estimating Octanol-Water Partition Coefficients. J Pharm Sci 84: 83-92.

ECOSAR will accept user entered Log Kow.

4.6. Software name and version for descriptor generation:
KOWWIN v1.67

4.7. Chemicals/Descriptors ratio:
15 (30 chemicals/ 2 descriptors))

5. Defining the applicability domain - OECD Principle 3

5.1. Description of the applicability domain of the model:
ECOSAR cannot be used for all chemical substances. The intended domain is organic chemicals.

Class definition
ECOSAR derives toxicity values for three general types of chemicals: neutral organics, organics with excess toxicity and surfactant (Surface-Active) organic chemicals. The (Q)SAR under evaluation falls under the neutral organics class.

LogKow

In general, when the logKow is less than or equal to 8.0 for daphnid, ECOSAR provides reliable quantitative (numeric) toxicity estimates for chronic effects.

Molecular weight:

The molecular weight may also be considered to determine the absorption cutoff limit for aquatic organisms. Compounds with a molecular weight of greater than 1000 g/mol are considered too large to present any significant toxicity.

Water solubility
If the predicted toxicity exceeds the water solubility, no acute toxicity is expected to be observed in the absence of an organic carrier solvent.

5.2. Method used to assess the applicability domain:
Assess if substances properties fall within the limits of applicability of the model mentioned in sections 5.1 and 5.4.

5.3. Software name and version for applicability domain assessment:
Not applicable

5.4. Limits of applicability:
Maximum logKow: 8.0
Maximum MW: 1000

If the log Kow value is greater than 8.0, or if the compound is solid and the ChV exceeds the water solubility effects at saturation are predicted.

6. Internal validation - OECD Principle 4

6.1. Availability of the training set:
Yes, however some information of the information contained in the training set is confidential business information (CBI) collected by EPA under the New Chemicals Program and is therefore restricted from being revealed. For these substances the name and CAS numbers are not revealed but data on the descriptors and dependent variables are available in the QSAR equation document.

6.2. Available information for the training set:
CAS RN: Yes
Chemical Name: Yes
Smiles: No
Formula: No
INChI: No
MOL file: No

Data table for the neutral organics - training set is available in the ECOSAR User Guide accessible via the Help tab.

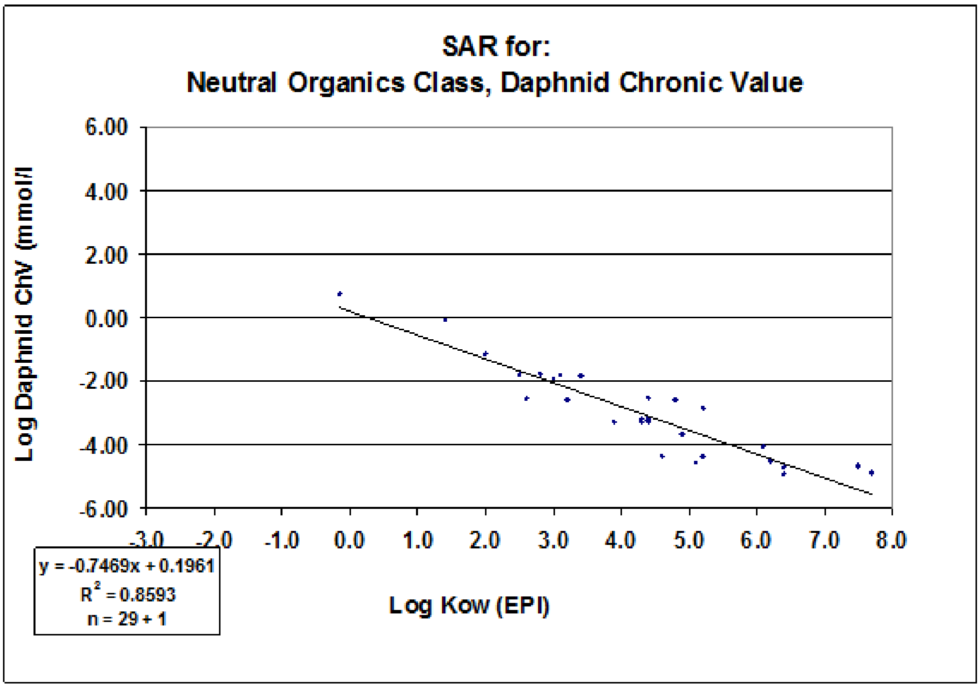
6.3. Data for each descriptor variable for the training set:
MW, log Kow (CLogP) log Kow (EPI), log Kow (M)

6.4. Data for the dependent variable for the training set:
Daphnid ChV (mg/L) and Log Daphnid ChV (mmol/L)

6.5. Other information about the training set:
References for (measured Kow) and References (Daphnid ChV) are provided.

6.6. Pre-processing of data before modelling:
Not applicable

6.7. Statistics for goodness-of-fit:
The correlation (r^2) for neutral organics Daphnid ChV (Q)SAR equals 0.8593 obtained from standard statistical regression software.



The number of chemicals in the training set is represented by N = x + y where 'x' equals the number of studies used in the actual equation development and 'y' equals 1) logKow cut-off and/or 2) SAR data not included in regression equation.

6.8. Robustness - Statistics obtained by leave-one-out cross-validation:
Not applicable

6.9. Robustness - Statistics obtained by leave-many-out cross-validation
Not applicable

6.10. Robustness - Statistics obtained by Y-scrambling:
Not applicable

6.11. Robustness - Statistics obtained by bootstrap:
Not applicable

6.12. Robustness - Statistics obtained by other methods:
Not applicable

7. External validation - OECD Principle 4

7.1. Availability of the external validation set:
See section 7.9

7.2. Available information for the external validation set:
See section 7.9

7.3. Data for each descriptor variable for the external validation set:
See section 7.9

7.4. Data for the dependent variable for the external validation set:
See section 7.9

7.5. Other information about the external validation set:

See section 7.9

7.6. Experimental design of test set:
See section 7.9

7.7. Predictivity - Statistics obtained by external validation:
See section 7.9

7.8. Predictivity - Assessment of the external validation set:
See section 7.9

7.9. Comments on the external validation of the model:
All available valid data were used by U.S. EPA/OPPT in development of the (Q)SARs within ECOSAR. Subsequent validation studies have been completed by multiple stakeholders. A list of supporting validation exercise performed in conjunction with EPA and other stakeholders on the ECOSOR model are listed below.

• External Peer Reviews
An independent peer review of ECOSAR was conducted as part of the development of the Organization for Economic Cooperation and Development’s (OECD) guidance, The Principles for Establishing the Status of Development and Validation of (Quantitative)
Structure-Activity Relationships [(Q)SARs] (OECD, 2004a).

• Participation in US-European Union Validation Exercise
EPA participated with the European Union in a large-scale verification study of ECOSAR to compare SAR predictions with the results of data from testing. That study (OECD 1994; U.S.EPA 1994) found our methods to be accurate 60-90% of the time depending on the endpoint assessed.

• International Collaboration in Development of Effective Predictive Tools
ECOSAR was included in OECD’s Report on the Regulatory Uses and Applications in OECD Member Countries of (Q)SAR Models in the Assessment of New and Existing Chemicals (OECD, 2006). Subsequently, the OECD solicited EPA to include ECOSAR into the OECD QSAR Application Toolbox, which was developed starting in 2006. Inclusion in the OECD toolbox
requires specific documentation, validation and acceptability criteria and subjects ECOSAR to international use, review, providing a means for receiving additional and on-going input for improvements. In an evaluation of a number of predictive tools used to profile chemicals and group them together based on similar toxicity, ECOSAR was the top performer.
[http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html#Additional_information_on_the_QSARs_Application_Toolbox]

8. Providing a mechanistic interpretation - OECD Principle 5

8.1. Mechanistic basis of the model:
Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug induced loss of conscience (general anesthesia). The octanol/water partition coefficient (Kow) is the major physical-chemical attribute correlating a chemical structure to toxic effect for nonreactive neutral organic chemicals. The most frequently used relationship is the logarithm of the Kow value versus the median toxicity (LC50 and EC50) value. This general narcosis is often referred to baseline toxicity.
The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfide.

8.2. A priori or a posteriori mechanistic interpretation:

8.3. Other information about the mechanistic interpretation:

9. Miscellaneous information

9.1. Comments:

9.2. Bibliography:

References Neutral Organics (Q)SAR

Hermans J, Canton H, Janssen P, and De Jong R. 1984. Quantitative structure-activity relations of mixtures of chemicals with anesthetic potency: Acute lethal and sublethal toxicity to Daphnia magna Toxicology 5: 143-154.

Niederlehner BR, Cairns J, Smith EP. 1998. Modeling acute and chronic toxicity of nonpolar narcosis mixtures to Ceriodaphnia dubia. Ecotoxicology and Environmental Safety. 39:136-146.

Oris et al. 1991. Environ. Toxicol. Chem. 10, 217-224.

Savino JF, Tanabe LL. 1989. Sublethal effects of Phenanthrene, Nicotine, and Pinane on Daphn Contam. Toxicol. 42(5):778-784.

SIAR. 2004. SIDS Initial Assessment Report. SIAR for the 3rd SIAM. Undecylbenzene. Sponsor Available at EPA docket.

Tong Z, Huailian Z, Hongjun J. 1996. Chronic toxicity of acrylonitrile and acetonitrile to Daphnia magna toxicity tests. Bull. Environ. Contam. Toxicol. 57(4)655-659.

U.S. Environmental Protection Agency (USEPA). 2006. Database of environmental toxicity data for Notifications (PMN). Washington DC: Risk Assessment Division (RAD), OPPT, USEPA, 1400 Pe (Unpublished test data.)

U.S. Environmental Protection Agency (USEPA). 2006. Database of environmental toxicity data for the “Toxic Substance Control Act” (TSCA). Public Law 94-469, 90 Stat. 2003, October 11, 1976. USEPA, 1400 Pennsylvania Ave., N.W.

Wong et al.. 2001. Development of a freshwater aquatic toxicity database for ambient water quality tertiary-butyl ether. Environmental Toxicology and Chemistry. 20(5):1125-1132.

Peer-Reviewed Publications Related to Validation, Verification and Performance of the ECOSAR Program

Book Chapters or Reports

OECD (Organization for Economic Cooperation and Development). (2006) Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationships [(Q)SAR] Models in the Assessment of New and Existing Chemicals. Organization for Economic Cooperation and Development, Paris; ENV/JM/MONO(2006)25.

Eriksson, L; Johansson, E; Wold S. (1997) Quantitative Structure-Activity Relationship Model Validation. In: Chen, F; Schuurmann, G; eds. Quantitative Structure-Activity Relationships in Environmental Sciences - VII. Pensacola, FL: SETAC Press, pp. 381-397.

OECD (Organization for Economic Cooperation and Development). (2004a) The Principles for Establishing the Status of Development and Validation of (Quantitative) Structure-Activity Relationships [(Q)SARs]. Organization for Economic Cooperation and Development, Paris; ENV/JM/TG(2004)27.

OECD (Organization for Economic Cooperation and Development). (2004b) Annex 6: ECOSAR. In: Annexes to the Report on the Principles for Establishing the Status of Development and Validation of (Quantitative) Structure-Activity Relationships [(Q)SARs]; ENV/JM/TG(2004)27/ANN.

OECD (Organization for Economic Cooperation and Development). (2004c) Comparison of SIDS Test Data with (Q)SAR Predictions for Acute Aquatic Toxicity, Biodegradability and Mutagenicity on Organic Chemicals Discussed at SIAM 11-18. Organization for Economic Cooperation and Development, Paris; ENV/JM/TG(2004)26.

Posthumus, R; Sloof, W. (2001) Implementation of QSARS in Ecotoxicological Risk Assessments. Research for Man and Environment/National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands; RIVM report 601516003.

Zeeman, M; Rodier, D; Nabholz, J. (1999) Ecological Risks of a New Industrial Chemical Under TSCA. In: Ecological Risk Assessment in the Federal Government. U.S. White House, National Science & Technology Council, Committee on Environment & Natural Resources (CENR), Washington, DC; CENR/5-99/001, pp. 2-1 to 2-30.

Kaiser, KL; Niculescu, S; Mckinnon ,M. (1997) On Simple Linear Regression, Multiple Linear Regression, and Elementary Probabilistic Neural Network with Gaussian Kernel’s Performance in Modeling Toxicity Values to Fathead Minnow Based on Microtox Data, Octanol/Water Partition Coefficient, and Various Structural Descriptors for a 419-Compound Dataset. In: Chen, F; Schuurmann, G; eds. Quantitative Structure-Activity Relationships in Environmental Sciences-VII, Pensacola, FL: SETAC Press, pp. 285-297.

OECD (Organization for Economic Cooperation and Development). (1994) US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). OECD Environment Monographs No. 88. Organization for Economic Cooperation and Development, Paris, France; OECD/GD(94)28.

U.S. EPA (Environmental Protection Agency). (1994) US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). U.S. Environmental 22 Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; EPA 743-R-94-001.

OECD (Organization for Economic Cooperation and Development). (1994) U.S. EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). OECD Environmental Monographs No. 88. Organization for Economic Cooperation and Development, Paris, France; OECD/GD(94)28.

Lynch, DG; Macek, G; Nabholz, J; et al. (1994) Ecological Risk Assessment Case Study: Assessing the Ecological Risks of a New Chemical Under the Toxic Substances Control Act. In: A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective, Volume II. Washington, DC: Risk Assessment Forum, Office of Research and Development, U.S. Environmental Protection Agency, pp. 1-1 to 1-B4.

Nabholz, JV; Clements, R; Zeeman, M; et al. (1993) Validation of Structure Activity Relationships used by the Office of Pollution Prevention and Toxics for the Environmental Hazard Assessment of Industrial Chemicals. In: Gorsuch J; Dwyer F; Ingersoll C, et al.; eds. Environmental Toxicology and Risk Assessment: 2nd Volume. Philadelphia: American Society for Testing and Materials, pp. 571-590.

<u>Scientific Journal Articles</u>

Reuschenbach, P; Silvania, M; Dammannb, M; et al. (2008) ECOSAR Model Performance with a Large Test Set of Industrial Chemicals. Chemosphere 71(10):1986-1995.

Tunkel, J; Mayo, K; Austin, C; et al. (2005) Practical Considerations of the Use of Predictive Methods for Regulatory Purposes. Environ Sci Technol 39:2188-2199.

Öberg, T. (2004) A QSAR for Baseline Toxicity: Validation, Domain of Application, and Prediction. Chem Res Toxicol 7 (12):1630-1637.

Moore, D; Breton, R; MacDonald, D. (2003) A Comparison of Model Performance for Six QSAR Packages that Predict Acute Toxicity to Fish. Environ Toxicol Chem 22(8):1799-1809.

Cronin, M; Walker, J; Jaworska, J; et al. (2003) Use of QSARs in International Decision-Making Frameworks to Predict Ecologic Effects and Environmental Fate of Chemical Substances. Environ Health Perspect 111(10):1376-1390.

Hulzebos, EM; Posthumus, R. (2003) (Q)SARs: Gatekeepers Against Risk on Chemicals? SAR QSAR Environ Res 14: 285-316.

Kaiser, KL; Deardon J; Klein W; et al. (1999) Short Communication: A Note of Caution to Users of ECOSAR. Water Qual Res J Can 34:179-182.

Abstracts

Chun, J; Nabholz, J; Wilson, M. (2002) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT/SAR Prediction on PPG Polymers. Society of Environmental Toxicology and Chemistry Annual Meeting, Salt Lake City, UT.

Chun, J; Nabholz, J; Wilson, M. (2001) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT SAR Predictions on PPG Polymers. Society of Toxicology Annual Meeting, San Francisco, CA..

9.3. Supporting information:
Training set(s) Test set(s) Supporting information

10. Summary (JRC QSAR Model Database)

10.1. QMRF number:
10.2. Publication date:
10.3. Keywords:
10.4. Comments:

8.3.4 QSAR PREDICTION reporting format CHRONIC DAPHNID

QSAR Prediction Reporting Format (QPRF):

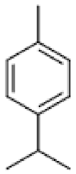
***p*-cymene, Chronic toxicity to Daphnid**

1. Substance

1.1 CAS number:
99-87-6

1.2 EC number:
202-796-7

1.3 Chemical name:
Substance name: 1-isopropyl-4-methylbenzene
Synonym: *p*-cymene

1.4 Structural formula:


p-Cymene

1.4 Structural formula:
SMILES: c(ccc(c1)C)(c1)C(C)C

2. General information

2.1 Date of QPRF:
23 November 2015

2.2 QPRF author and contact details:
Bureau REACH
The National Institute of Public Health and the Environment (RIVM)
The Netherlands
Email: bureau- reach@rivm.nl

3. Prediction

3.1 Endpoint (OECD Principle 1)
a. Endpoint:
Long -term toxicity to Daphnia 48-hour

b. Dependent variable:
Log 16-d ChV

3.2 Algorithm (OECD Principle 2)
a. Model or submodel name:
ECOSAR

b. Model version:

Version 1.11 (Sept 2012)

c. Reference to QMRF:

Title: ECOlogical Structure Activity Relationship (ECOSAR), Acute Daphnid 48-hour LC50 –Neutral Organics.
Date: 1 October 2015
Author: Bureau REACH, The Netherlands

d. Predicted value (model result):

Daphnid ChV = 0.165 mg/L

NOEC = ChV/$\sqrt{2}$ = 0.165 mg/L/1.41 = 0.117 mg/L

NOEC = 0.117 mg/L

e. Predicted value (comments):

Chronic aquatic toxicity for crustacea
The predicted NOEC value for p-cymene will be compared to the criteria for classifying and categorizing a substance as "hazardous to the aquatic environment" as summarized in Table 4.1.0 (b) of the CLP Annex I:

f. Input for prediction:

See section 1.5

g. Descriptor values:

Log Kow (user entered): 4.1 (measured value)
Water solubility (user entered): 23.35 mg/L (measured value)

Applicability domain (OECD principle 3)

h. Domains:

The applicability domain criteria are fulfilled.

Descriptor domain
p-cymene: log Kow = 4.1 and molecular weight = 134.22
LogKow is less than maximum Log Kow 8.0 for chronic effects and the molecular weight is less than the maximum of 1000.

Structural fragment domain and mechanism domain
Class definition: Neutral organic class
Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug-induced loss of conscience (general anesthesia). This general narcosis is often referred to as baseline toxicity (Franks and Lieb 1990, Veith and Broderius 1990). The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfides.

The structural formula of p-cymene (section 1.4) shows that it is an aromatic hydrocarbon and therefore falls within the neutral organic class as defined in ECOSAR.

i. Structural analogues:

j. Considerations on structural analogues:

Data tables on the neutral organics - training set is available (see related - QMRF).

3.3 The uncertainty of the prediction (OECD principle 4)

Model performance:
y = - 0.7469 log Kow + 0.1961 (R2 = 0.8593)
n = 29 + 1

3.4 The chemical and biological mechanisms according to the model underpinning the predicted result (OECD principle 5)

Neutral organic chemicals are nonionizable and nonreactive and act via simple nonpolar narcosis generally thought of as a reversible, drug induced loss of conscience (general anesthesia).

The octanol/water partition coefficient (Kow) is the major physical-chemical attribute correlating a chemical structure to toxic effect for nonreactive neutral organic chemicals. The most frequently used relationship is the logarithm of the Kow value versus the median toxicity (LC50 and EC50) value. This general narcosis is often referred to baseline toxicity.

The types of chemicals that are known to present general narcosis include, but are not limited to, alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, cyanates, sulfides, and disulfides.

4. Adequacy (Optional)

4.1 Regulatory purpose:

The present prediction will be used for classification and labelling purposes as required by Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

4.2 Approach for regulatory interpretation of the model result:

The ChV value is converted to a NOEC by: NOEC = ChV/√2.

ChV = 0.165 mg/L

NOEC = ChV/√2 = 0.165 mg/L/1.41 = 0.117 mg/L

NOEC = 0.117 mg/L

4.3 Outcome:

The estimated daphnid NOEC for p-cymene is 0.117 mg/L..

4.4 Conclusion:

Considering the above, the predicted result can be considered adequate for the regulatory conclusion described in 4.1.