WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

EUCALYPTUS CITRIODORA OIL, HYDRATED, CYCLIZED (EC Oil (H/C))

World Health Organization

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DISCLAIMER[1]

WHO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

WHO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may be arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, WHO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

WHO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, WHO does not in any way warrant or represent that any pesticide claimed to comply with a WHO specification actually does so.

[1] This disclaimer applies to all specifications published by WHO.

INTRODUCTION

WHO establishes and publishes specifications[1] for technical material and related formulations of public health pesticides with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 2002, the development of WHO specifications follows the **New Procedure**, described in the "Manual on the development and use of FAO and WHO specifications for chemical pesticides." This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS).

WHO specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 2002 onwards, the publication of WHO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 8 of the above-mentioned manual.

Part Two: The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by WHO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the above-mentioned manual and supported by other information sources. Evaluation reports include the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in chronological order to this report.

WHO specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. WHO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

[1] Publications available on the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products

PART ONE: SPECIFICATIONS

Eucalyptus Citriodora Oil, Hydrated, Cyclized (EC Oil (H/C))

Page

EUCALYPTUS CITRIODORA OIL, HYDRATED, CYCLIZED (EC OIL (H/C)) INFORMATION

ISO common name

Not available

Chemical name(s)

IUPAC

Eucalyptus citriodora oil, hydrated, cyclized or Oil of lemon eucalyptus, hydrated, cyclized is a plant extract containing a mixture of several constituents.

The chemical names for the main constituents are:

Name	IUPAC name
p-Menthane-3,8-diol (PMD)	2-(2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol
Isopulegol	5-methyl-2-(prop-1-en-2-yl)cyclohexan-1-ol
beta-Citronellol	3,7-dimethylocta-6-en-1-ol

CA

Not available

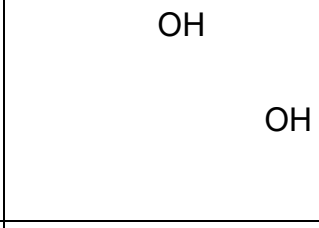
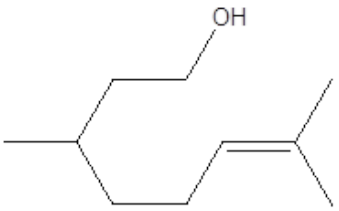
Synonyms

- EC Oil (H/C)
- Oil of Lemon Eucalyptus
- OLE
- Oil of lemon eucalyptus (hydrated, cyclized)
- p-Menthane-3,8-diol (PMD) and related oil of lemon eucalyptus compounds
- PMD rich botanic oil
- p-Menthane diol Rich Botanic Oil
- PMDRBO
- Oil, eucalyptus, E. citriodora, hydrated, cyclized
- Extracts of Lemon Eucalyptus
- Citriodiol (registered trade name)
- Cyclized eucalyptus citriodora leaf/twig oil (INCI name)

Structural formula

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The structural formula for the main constituents are:

Name	Structure
p-Menthane-3,8-diol (PMD)	
Isopulegol	
beta-Citronellol	

Molecular formula

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The molecular formula for the main constituents are:

Name	Molecular Formula
p-Menthane-3,8-diol (PMD)	$C_{10}H_{20}O_2$
Isopulegol	$C_{10}H_{18}O$
beta-Citronellol	$C_{10}H_{20}O$

Relative molecular mass

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The relative molecular mass for the main constituents are:

Name	Relative molecular mass
p-Menthane-3,8-diol (PMD)	172.27
Isopulegol	154.25
beta-Citronellol	156.27

CAS Registry number

1245629-80-4

This CAS No. was assigned for “oils, eucalyptus, E. citriodora, hydrated, cyclized” by the American Chemical Society.

The CAS numbers for the main constituents are:

Name	CAS Number
p-Menthane-3,8-diol (PMD)	42822-86-6
Isopulegol	7786-67-6
beta-Citronellol	106-22-9

CIPAC number

1027

This CIPAC number was assigned for Eucalyptus citriodora oil, hydrated, cyclized. The main constituents have not been assigned a CIPAC number.

EINECS number

800-429-0

Identity tests

GC retention time, mass spectrum (from GC-MS)

EUCALYPTUS CITRIODORA OIL, HYDRATED, CYCLIZED (EC OIL (H/C)) TECHNICAL CONCENTRATE

WHO Specification 1027/TK (January 2026*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (1027/2025). This specification should be applicable to TK produced by this manufacturer, but it is not an endorsement of those products nor a guarantee that they comply with the specification. The specification may not be appropriate for TK produced by other manufacturers. The evaluation report (1027/2025), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of Eucalyptus citriodora oil, hydrated, cyclized (EC Oil (H/C)), in the form of pale yellow to amber liquid containing white crystals at room temperature (Note 1), and shall be free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 Identity tests (1027/TK/M/2, CIPAC/5413, Note 2)

The main constituents of the active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 EC Oil (H/C) content (1027/TK/M/3, CIPAC/5413, Note 2)

The content of the main constituents of the EC Oil (H/C) shall be declared (g/kg) and, when determined, the average measured content of each constituent shall not differ from that declared by more than the following tolerances.

Main constituent	Declared content in g/kg	
	Minimum	Maximum
p-Menthane-3,8-diol (CAS 42822-86-6)	640	800
cis-p-Menthane-3,8-diol (% of total)	55%	70%
trans-p-Menthane-3,8-diol (% of total)	25%	35%
Isopulegol (CAS 7786-67-6)	80	150
beta-Citronellol (106-22-9)	20	110

3 Relevant impurities

3.1 Methyl eugenol (CAS 93-15-2) (Note 3)

Maximum: <1 g/kg.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure

3.2 Limonene (CAS 138-86-3) (Note 4)

Maximum: 10 g/kg.

3.3 Eucalyptol (CAS 470-82-6) (Note 4)

Maximum: 20 g/kg.

Note 1 If the EC Oil (H/C) has been recently homogenised, the crystals may not have had time to form.

Note 2 The capillary gas chromatographic method (CIPAC/5413) for the determination of p-menthane-3,8-diol, isopulegol and beta-citronellol in technical concentrates was accepted as provisional CIPAC method in 2025. Prior to its publication in the next Handbook, the method is available through the CIPAC prepublishment scheme from https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 3 The peer-validated method for determination of the relevant impurity methyl eugenol in EC Oil (H/C) TK is described in Annex 3 to the evaluation, in Part 2 of this document.

Note 4 The peer-validated method for determination of the relevant impurities limonene and eucalyptol in EC Oil (H/C) TK is described in Annex 4 to the evaluation, in Part 2 of this document.

PART TWO: EVALUATION REPORTS

EUCALYPTUS CITRIODORA OIL, HYDRATED, CYCLIZED (EC OIL (H/C))

EUCALYPTUS CITRIODORA OIL, HYDRATED, CYCLIZED (EC OIL (H/C))
FAO/WHO EVALUATION REPORT 1027/2025

Recommendations

The Meeting recommended that the specification for Eucalyptus citriodora oil, hydrated, cyclized TK (EC Oil (H/C)) proposed by Citrefine International Ltd., and as amended, should be adopted by WHO.

Appraisal

The Meeting considered data and information submitted between October 2023 and November 2025 by Citrefine International Ltd. (Citrefine) in support of the development of a new WHO specification for Eucalyptus citriodora oil, hydrated, cyclized (EC Oil (H/C)) technical concentrate (TK). The data submitted met the requirements of the Manual on development and use of FAO and WHO specifications for chemical pesticides (2022, second edition).

Eucalyptus citriodora oil, hydrated, cyclized (EC Oil (H/C)) is a plant extract containing a mixture of several constituents derived solely from *Eucalyptus citriodora* oil with all constituents contributing towards its efficacy as an insect repellent. In the proposer's EC Oil (H/C) TK, the main constituent p-methane-3,8-diol (PMD), which is most closely associated with its efficacy, is present as a mixture of *cis* and *trans* isomers at approximately 700 g/kg (minimum 640 g/kg) of the whole substance. The proposer stated that the main components are those which can be present at ≥ 10 g/kg and collectively make up over 850 g/kg of the substance as a whole.

EC Oil (H/C) has not been evaluated by the FAO/WHO JMPR nor the WHO/IPCS. The EC Oil (H/C) TK from Citrefine International Ltd. has been evaluated and approved as an active ingredient in insect repellents by the US EPA, Australian (APVMA), Health Canada PMRA, Brasilian (Anvisa) and several EU Member State registration authorities. The proposer stated that the confidential data presented to WHO are aligned with those submitted to the European Union under the EU Biocidal Products Regulation (Regulation EU 528/2012). The proposer has provided certificates of registration of EC Oil (H/C) in Australia, USA and Canada.

The Meeting was provided with commercially confidential data on the manufacturing process, GLP 5-batch analysis data on the EC Oil (H/C) TK main constituents and all impurities below or above 1 g/kg and their manufacturing limits in the TK. Data were provided for three different manufacturing sites having the same manufacturing process, the same impurity profile and the same manufacturing limits.

A comprehensive description of the manufacturing process was provided with the information on the plant oil, reagents and catalysts used.

The batches analysed in the 5-batch studies were produced over 2 months in 2020 for the manufacturing site 1, over 2 months in 2014 for the manufacturing site 2 and over 11 months in 2014-2015 for the manufacturing site 3. These batch data reflect oil samples from a wide range of harvests collected over the many years the

proposer has manufactured EC Oil (H/C) for use as an active ingredient in insect repellent products.

Mass balances ranged from 936 g/kg to 969 g/kg for the manufacturing site 1, which is the reference profile, from 912 g/kg to 919 g/kg for the manufacturing site 2 and from 947 g/kg to 968 g/kg for the manufacturing site 3. Mass balances are lower than the threshold of 980 g/kg recommended for a synthetic chemical pesticide TC, but it is nevertheless acceptable for a plant extract TK containing a mixture of several constituents.

The proposer and the Meeting agreed to specify as active ingredients in the WHO specification the main constituents present in the TK at ≥ 100 g/kg which are *p*-Methane-3,8-diol (PMD) (640-800 g/kg) with a *cis:trans* isomer ratio of 55-70%:25-35%, isopulegol (80-150 g/kg) and beta-citronellol (20-110 g/kg). The manufacturing limits for the main constituents and for the PMD isomer ratio, as well for the other constituents and impurities are supported by the 5-batch analysis data and are statistically justified.

The toxicological assessment conducted by the meeting concluded that three impurities are relevant.

Applying the calculation of worst-case possible concentration by an impurity to the toxic hazard of the active ingredient (appendix H of the Manual on FAO and WHO specification for chemical pesticides), **methyl eugenol**, considered as a possible carcinogen by IARC, is a relevant impurity in EC Oil (H/C) TK at ≥ 0.48 g/kg with a maximum acceptable limit of 4.8 g/kg. The manufacturing limit of <1 g/kg is consistent with the 5-batch analysis data and was specified in the WHO specification.

Limonene, considering its potential for skin sensitization, is a relevant impurity in EC Oil (H/C) TK with a maximum acceptable limit of 10 g/kg. The manufacturing limit of 10 g/kg is consistent with the 5-batch analysis data and was specified in the WHO specification.

Applying the calculation of the appendix H of the Manual, **eucalyptol**, classified as a skin sensitizer (Category 1) in Europe, is a relevant impurity in EC Oil (H/C) TK at ≥ 7.0 g/kg with a maximum acceptable limit of 70 g/kg. The manufacturing limit of 20 g/kg is consistent with the 5-batch analysis data and was specified in the WHO specification.

The applicant proposed to include water as a relevant impurity, justifying that water is used in the manufacturing process and removed during the drying step, so water content should be controlled to ensure its complete removal. Water has no effect on the final substance other than potentially reducing the efficacy of the TK due to dilution of the constituents. The Meeting concluded that it is not necessary to include water as a relevant impurity in the WHO specification, as water does not impact the stability of the TK.

The main constituents specified as active ingredient and other minor constituents, including the relevant impurities limonene and eucalyptol, were determined in the three sets of 5 batches of EC Oil (H/C) TK by gas chromatography with flame ionisation detection (GC-FID) using a DB-Wax column (60 m x 0.25 mm i.d., 0.25 µm film thickness) or a DB-5MS columns (30 m x 0.25 mm i.d., 1 µm film thickness) and internal standard calibration. The identity of the constituents and impurities were confirmed by comparison with the retention times of the corresponding

analytical standards and by mass spectrometry (MS). The analytical methods used in the 5-batch analysis studies were adequately validated in term of specificity, linearity of response, accuracy, repeatability and limits of detection and quantification (for impurities). The method validation studies and 5-batch analysis studies were performed according to GLP guidelines.

The capillary gas chromatographic method (1027/TK/M/3, CIPAC/5413) for the determination of the main constituents *p*-menthane-3,8-diol, isopulegol and beta-citronellol in technical concentrates was accepted as a provisional CIPAC method in 2025. The meeting concluded that the in-house methods used in the 5-batch analysis studies are similar to the CIPAC method.

The relevant impurity methyl eugenol was determined in the three sets of 5 batches of EC Oil (H/C) TK by gas chromatography with mass spectrometry detection (GC-MS) using a DB-Wax column (30 m x 0.25 mm i.d., 0.25 µm film thickness) and external standard calibration. The analytical method used in the 5-batch analysis studies was adequately validated in term of specificity, linearity of response, accuracy, repeatability and limits of detection and quantification. The method validation studies and 5-batch analysis studies were performed according to GLP guidelines.

The methods for the main constituents specified as active ingredient and relevant impurities were validated in two different laboratories, meaning that additionally to the CIPAC method for *p*-menthane-3,8-diol, isopulegol and beta-citronellol, methods for relevant impurities methyl eugenol, limonene and eucalyptol are peer-validated and published as annexes to this evaluation report.

The Meeting was provided with data supported by GLP studies on the vapour pressure, melting point, temperature of decomposition, octanol/water partition coefficient, solubility in water and organic solvents, hydrolysis and photolysis characteristics and dissociation characteristics for EC Oil (H/C). Physical- chemical properties were determined for the mixture (e.g. melting point, temperature of decomposition, dissociation characteristics and solubility in organic solvents) and in some cases for the individual compounds (e.g. vapor pressure, solubility in water, octanol/water partition coefficient). Test methods for determination of physical-chemical properties of the active ingredient were OECD or EEC or CIPAC test methods.

The Meeting was provided with summary data supported by studies on the acute toxicology profile, the sub-acute to chronic toxicology profile, the mutagenicity profile and the ecotoxicology profile of EC Oil (H/C) TK.

Supporting Information
for
Evaluation Report 1027/2025

Uses

Eucalyptus citriodora oil, hydrated cyclized or EC Oil (H/C) is a biocidal active substance used in the formulation of personal insect repellents. It is used in repellent products to protect against a variety of biting insects, including but not limited to mosquitoes and ticks, which carry and transmit malaria, West Nile virus, Zika virus, dengue fever, chikungunya and Lyme disease, among others.

Identity of the active ingredient

ISO common name

Not available

Chemical name(s)

IUPAC

Eucalyptus citriodora oil, hydrated cyclized or Oil of lemon eucalyptus, hydrated, cyclized is a plant extract containing a mixture of several constituents.

The chemical names for the main constituents are:

Name	IUPAC name
p-Menthane-3,8-diol (PMD)	2-(2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol
Isopulegol	5-methyl-2-(prop-1-en-2-yl)cyclohexan-1-ol
beta-Citronellol	3,7-dimethylocta-6-en-1-ol

CA

Not available

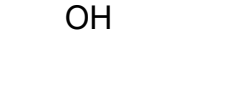
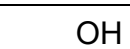
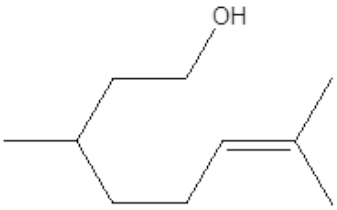
Synonyms

- EC Oil (H/C)
- Oil of lemon eucalyptus
- OLE
- Oil of lemon eucalyptus (hydrated, cyclized)
- p-Menthane-3,8-diol (PMD) and related oil of lemon eucalyptus compounds
- PMD rich botanic oil
- p-Menthane diol rich botanic oil
- PMDRBO
- Oil, eucalyptus, E. citriodora, hydrated, cyclized
- Extracts of lemon eucalyptus
- Citriodiol (registered trade name)
- Cyclized eucalyptus citriodora leaf/twig oil (INCI name)

Structural formula

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The structural formula for the main constituents are:

Name	**Structure**
p-Menthane-3,8-diol (PMD)	
Isopulegol	
beta-Citronellol	

Molecular formula

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The molecular formula for the main constituents are:

Name	Molecular formula
p-Menthane-3,8-diol (PMD)	$C_{10}H_{20}O_2$
Isopulegol	$C_{10}H_{18}O$
beta-Citronellol	$C_{10}H_{20}O$

Relative molecular mass

EC Oil (H/C) is a plant extract containing a mixture of several constituents.

The relative molecular mass for the main constituents are:

Name	Relative molecular mass
p-Menthane-3,8-diol (PMD)	172.27
Isopulegol	154.25
beta-Citronellol	156.27

CAS Registry number

1245629-80-4

This CAS No. was assigned for “oils, eucalyptus, E. citriodora, hydrated, cyclized” by the American Chemical Society.

The CAS numbers for the main constituents are:

Name	CAS number
p-Menthane-3,8-diol (PMD)	42822-86-6
Isopulegol	7786-67-6
beta-Citronellol	106-22-9

CIPAC number

1027

This CIPAC number was assigned for Eucalyptus citriodora oil, hydrated, cyclized. The main constituents have not been assigned a CIPAC number.

EINECS number

800-429-0

Identity tests

GC retention time, mass spectrum (from GC-MS)

Physico-chemical properties of EC Oil (H/C)

Table 1. Physico-chemical properties of pure EC Oil (H/C)

Physical and chemical properties of EC Oil (H/C) are given below when data are available. Tests were conducted on EC Oil (H/C) unless stated otherwise. For some data, information on the majority constituent of EC Oil (H/C), i.e. PMD (ca. 70% of EC Oil (H/C)), is presented. Alternatively, data on the three main components (PMD, isopulegol and citronellol), which make up ≥ 85% w/w of EC Oil (H/C), are included.

Parameter	Value(s) and conditions	Purity %	Method reference (and technique if the reference gives more than one)	Study number
Vapour pressure	It is not technically feasible to determine the vapour pressure of EC Oil (H/C) as a mixture of multiple components. Here, the vapour pressure of the three main EC Oil (H/C) components were measured via gas saturation or ebulliometry.			
	***cis*-PMD** 0.05 Pa at 25.0°C 0.04 Pa at 20.0°C	99.3%	EEC A4 Gas saturation	CIL-110
	Isopulegol 62 Pa at 20°C 87 Pa at 25°C	≥ 98.0%	EEC A4 Ebulliometry	CIL-110
	beta-Citronellol 0.10 Pa at 20°C 0.19 Pa at 25°C	96.6%	EEC A4 Ebulliometry	CIL-110
Melting point	As a complex mixture of many components, the provision of a single value or range of melting point for EC Oil (H/C) is not appropriate. EC Oil (H/C) is a liquid containing crystals at ambient room temperature. At 60°C crystals will have dissolved and will recrystallize on cooling. A pour point of -12°C	100%	ASTM Method D97-09; Pour point determined as described in OECD 102	CIL-111
Temperature of decomposition	Thermally stable and stable under air and nitrogen when heated to a temperature of 150ºC at 103.750 kPa. No endothermic/exothermic behaviour was observed.	100%	OECD 113	CIL-112
Solubility in water	***cis*-PMD**: 5251 mg/L (25°C) **Isopulegol**: 2634 mg/L (25°C) **Citronellol**: 285 mg/L (25°C)	99.35% 100.0% 95.6%	EEC A.6	CIL-113

Octanol/water partition coefficient	***cis*-PMD**: log Kow 1.8 (25°C) **Isopulegol**: log Kow 2.52 (25°C) **Citronellol**: log Kow 3.29 (25°C)	99.35% 100.0% 95.6%	EEC A.8	CIL-113
Hydrolysis characteristics	Not determined as EC Oil (H/C) and its main components are hydrolytically stable (extraction from plant material undertaken by steam distillation)	Not applicable	Waiver	Not available
Photolysis characteristics	Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites: It is not expected to be a major process for removal of EC Oil (H/C) and its main components from environmental compartments. The components of EC Oil (H/C) do not absorb visible light and many do not absorb strongly in the UV spectra, so photo-transformation in water is unlikely.	Not applicable	Waiver	Not available
Dissociation characteristics	None of the components of EC Oil (H/C) have functional groups capable of dissociation at pH levels expected under natural environmental conditions. pKa values range from 16-18 so significant dissociation will not occur until above pH 14.	Not stated; assumed 100%	Reasoned statement acc. to OPPTS 830.7370	CIL-114
Solubility in organic solvents	n-hexane: >250 g/l, 20°C acetone: >250 g/l, 20°C	>99.8%	CIPAC MT181	CIL-112

Table 2. Chemical composition and properties of EC Oil (H/C) technical concentrate (TK)

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by WHO. The mass balance average is 94% for the constituents determined in the 5-batch analysis data.
Declared active ingredients content: - p-Menthane-3,8-diol - Isopulegol - beta-Citronellol	 640 to 800 g/kg 80 to 150 g/kg 20 to 110 gkg
Relevant impurities ≥ 1 g/kg and maximum limits for them	Limonene: 10 g/kg Eucalyptol: 20 g/kg
Relevant impurities **<** 1 g/kg and maximum limits for them	Methyl eugenol: <1 g/kg
Stabilisers or other additives and maximum limits for them	None

Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature of the TK	The provision of a single value or range of melting point for EC Oil (H/C) is not appropriate. A temperature of 50°C-60°C is required to homogenise the product into a liquid due to its semi-solid nature at ambient temperatures.	100%	Not Applicable	Not Applicable
Solubility in organic solvents	n-hexane: >250 g/l, 20°C acetone: >250 g/l, 20°C methanol: >250 g/l, 20°C ethyl acetate: >250 g/l, 20 °C	100%	CIPAC MT 181	CIL-112 and CIL-144

Hazard summary

EC Oil (H/C) has not been evaluated by the FAO/WHO JMPR nor the WHO/IPCS.

Formulations and co-formulated active ingredients

The main formulation types available are emulsion, oil in water (EW), suspension concentrate (SC) and other liquids to be applied undiluted (AL), formulated as insect repellents for use by the general public.

EC Oil (H/C) is not co-formulated with other pesticides.

These formulations are registered and sold in many countries throughout the world.

Methods of analysis and testing

The analytical method for the active ingredient (including identity tests) is TM1951-1. The EC Oil (H/C) is determined by capillary gas chromatography using flame ionisation detection (GC-FID) with internal and external standardisation and was externally validated in a GLP study. This is the method used for the analysis of the batches of the manufacturing site 1.

The batches of the manufacturing sites 2 and 3 were analysed with method M821, which is a similar method to TM1951-1 and has also been externally validated according to GLP standards. This method has been superseded by TM1951-1.

Methyl eugenol is analysed in the batches of the manufacturing site 1 using the method TM1952-2 by capillary gas chromatography with mass spectrometry detection (GC-MS) using selective ion monitoring with external standardisation. This method is equivalent to M822, which was used in the batches of the manufacturing sites 2 and 3. Both methods have been externally validated according to GLP standards.

TM1951-1 is suitable for all constituents of EC Oil (H/C) aside from methyl eugenol, which due to the low level of this constituent, is analysed using TM1952-2.

The method for the main constituents specified as active ingredient (p-menthane-3,8-diol, isopulegol and beta-citronellol) was accepted as a provisional CIPAC method in 2025. The methods for the relevant impurities methyl eugenol, limonene and eucalyptol were peer-validated and are published as annexes to this evaluation report.

Test methods for determination of physico-chemical properties of the technical active ingredient were performed in accordance with the guidelines listed in the testing section.

Annex 1: Hazard Summary Provided by the Proposer

Notes:

(i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary tables below were derived from EC Oil (H/C) TK having impurity profiles similar to that referred to in Table 2 above.

(ii) Unless otherwise stated, the data below were all conducted on Eucalyptus Citriodora Oil, Hydrated, Cyclized (EC Oil (H/C) TK as manufactured by the proposer, starting from source oil as described in the manufacturing process.

(iii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table 3. Toxicology profile of the EC Oil (H/C) technical concentrate, based on acute toxicity, irritation and sensitization

Species	Test	Purity % Note[3]	Guideline, duration, doses and conditions	Result	Study number[4]
Rat, Sprague-Dawley, Male & Female	Acute oral	100%	FIFRA section 81-1 Acute oral toxicity, OECD No. 401 and EU method B1 of 92/69/EEC	LD_{50} > 2000 mg/kg bw/d	CIL-117
Rat, Sprague-Dawley, Male & Female	Acute dermal	100%	EPA Guideline 81-2 Acute dermal toxicity, OECD 402 and method B3 of 84/449/EEC	LD_{50} > 2000 mg/kg bw/d	CIL-118
Rat, Sprague-Dawley, Male & Female	Acute inhalation (limit dose test)	50% EC Oil (H/C) end use product; EC Oil (H/C) 100% purity	EPA/FIFRA Guideline 81-3 Subdivision M 152-14 for biochemical pest control agents (Acute inhalation toxicity)	LC_{50} ≥ 2.06 mg/L (equivalent to ≥ 1.03 mg/L for EC Oil (H/C)) (maximum obtainable concentration)	CIL-119
Rabbits, New Zealand white, Male & Female	Skin irritation	100%	EPA Guideline 81-5 Acute dermal irritation, OECD 404 and EU method B4 of 84/449/EEC	Some irritation but not sufficient for classification	CIL-120
Rabbits, New Zealand white, Male & Female	Eye irritation	100%	EPA Guideline 81-4 Acute eye irritation, OECD 405 and method B5 of 92/69/EEC	Irritant, Eye Irrit. Cat 2	CIL-121
Guinea Pigs, Dunkin Hartley, Female	Skin sensitisation	100%	EPA Section 81 – 6 Dermal sensitization study and OECD 406	Maximisation Test – Negative; not a skin sensitizer	CIL-122
Guinea Pigs, Albino Dunkin Hartley, Female	Phototoxicity	100%	No guideline available	No phototoxic potential	CIL-123

[3] Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.
[4] Note: Study numbers appearing in the data tables must match study numbers in the references section at the end of the document.

Table 4. Toxicology profile of the EC Oil (H/C) technical concentrate based on repeated administration (subacute to chronic)

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
Rat, Sprague Dawley: Crl:CD (SD)IGS BR VAF/Plus®, Female	14-day repeated dermal dose toxicity study in rats	100%	EPA Guideline OPPTS 870.3200 21/28 day dermal toxicity Dose Level: 0, 3000 and 5000 mg/kg bw/d Semi-occlusive application in mineral oil, 6 hr/d, 7d/week	NOAEL could not be determined LOAEL = 3000 mg/kg bw/d 5000 mg/kg bw/day: clinical signs of toxicity, Erythema, desquamation, lacrimation, ocular/nasal discharge, inactivity, hypoactivity, tremors, hunched posture and abnormal gait. *Mortality* One mortality, one further animal killed *in extremis.* *Organ weights* ↑ Liver weight (10% relative) **3000 mg/kg** bw *Clinical signs* Erythema, desquamation, lacrimation, nasal and ocular discharge,	CIL-124

[5] Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.
[6] Note: Study numbers appearing in the data tables must match study numbers in the references section at the end of the document.

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
				inactivity, hypoactivity, hunched posture	
Rat, Crl:CD (SD)IGS BR VAF/Plus®, Male & Female	28-day dermal toxicity study (limit dose)	100%	EPA Guideline OPPTS 870.3200 21/28 day dermal toxicity; OECD 410. Dose Level: 0, 1000 mg/kg bw/d Semi-occlusive application in mineral oil 6 hr/d, 7d/week	NOAEL (systemic) > 1000 mg/kg bw/d LOAEL (systemic) ≥1000 mg/kg bw/day NOAEL (local) = 1,000 mg/kg bw/d for dermal effects LOAEL (local) – could not be established for dermal effects. *Dermal effects at application site* Erythema and flaking of the skin. Hyperplasia and hyperkeratosis of the epidermis. Hyperplasia of the sebaceous glands. Dermal inflammatory cell infiltration	CIL-125
Sprague-Dawley Rats, Crl:CD(SD), Male & Female	Rat 14/28 day dermal toxicity (preliminary study)	EC Oil (H/C) was 90% of TA with 100% purity. Remaining	Non-guideline study Dose Level per group (corrected for specific gravity of the test article): 0, 2838, 3784, 3311, 3075, & 2838 mg/kg bw/d Test article was identical to test article from dermal absorption study in rat	No relevant systemic effects observed. Not appropriate to derive a NOAEL from this preliminary study	CIL-126

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
		10% white mineral oil	(Study No. CIL-128). White mineral oil included to reduce dermal irritation.		
Sprague-Dawley Rats, Crl:CD(SD), Male & Female	90-d repeat dose dermal toxicity in the rat	EC Oil (H/C) was 90% of TA with 100% purity. Remaining 10% white mineral oil	OECD TG 411 = Dose Level: 2500, 3000 & 3500 mg/kg bw/d Test article was identical to test article from dermal absorption study in rat (CIL-128). White mineral oil included to reduce dermal irritation.	NOAEL: >3500 mg/kg bw/d LOAEL = not established <u>Local</u> **3500 mg/kg bw/day (top dose)** Occasional erythema, eschar-formation and/or oedema at the dermal test site was considered of no toxicological significance since this tended to be transient, did not progress and was also seen in some controls. These changes were likely to be a result of the daily treatment. <u>Systemic</u> **3500 mg/kg bw/day (top dose)** Although there were effects noted on bodyweight gains during dosing, typical of a	CIL-127

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
				dermal repeat dose study the final (Week 13) absolute bodyweights were only slightly lower than controls in all treatment groups, the values were all >94% of control values. The observed effects on motor activity are considered secondary to the local effects and the resultant stress.	
Crl:CD (SD) rats	*In vitro* percutaneous absorption of radio labelled material through human and rat split-thickness skin	EC Oil (H/C) was 90% of TA with 100% purity. Remaining 10% white mineral oil	OECD 428: Skin Absorption: *In Vitro* Method (2004); OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004); European Commission Guidance Document on Dermal Absorption – Sanco/222/2000/Rev.7 (19 March 2004); Scientific Opinion on Dermal Absorption (EFSA Journal, 2012; 10(4): 2665). 6 hrs semi-occluded exposure period; Receptor fluid aliquots were collected prior to dosing and at 1, 2, 4, 6, 8, 12 and 24 hours post-dose application	16% dermal absorption value is the rate for unformulated active ingredient	CIL-128

Species	**Test**	**Purity % Note[5]**	**Guideline, duration, doses and conditions**	**Result with recapitulation of findings at the LOAEL dose**	**Study number [6]**
Sprague Dawley Rats, Crl:CD(SD), Male & Female	A 90-Day Oral (Dietary) Toxicity with a 28-Day Recovery Period	100%	OECD 408, and U.S. EPA OPPTS 870.3100 Dose Levels: 0, 350, 500 & 600 mg/kg bw/d	NOAEL = 600 mg/kg bw/d LOAEL = not established No adverse effects at the top dose (↑ Relative liver weight; 18.5% in males and 25.2% in females). The effects seen on the liver were considered adaptive as not hepatotoxic and were shown to be reversible during the recovery period. This conclusion is supported by an enzyme induction analysis of liver samples which demonstrated an adaptive, non-adverse, reversible response by the liver, associated with high doses of certain chemical classes.	CIL-129
Rat, Crl:CD (SD) IGS BR/VAF Plus®, Female	Oral (gavage) dosage range-finding developmental toxicity study in rats	100%	US EPA OPPTS 870.3700; study is also consistent with OECD 414 Dose Level: 0, 100, 300 and 1000 mg/kg	NOAEL = 300 mg/kg LOAEL = 1,000 mg/kg bw/d *Clinical signs-dams*	CIL-130

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
				Ataxia, decreased activity, impaired/lost righting reflex. *Liver weight-dams* ↑Absolute (26%) and relative (30%) liver weight. *Histopathology-dams* Centrilobular hepatocellular hypertrophy *Developmental effects* No developmental effects	
Rat, Crl:CD (SD) IGS BR/VAF Plus®, Female	Dermal developmental toxicity study in rat	100%	EPA OPPTS 870.3700; OECD 414 Dose Level: 0, 1000 mg/kg	NOAEL = 1000 mg/kg LOAEL ≥ 1000 mg/kg No systemic effects observed *Developmental effects* No developmental effects	CIL-131
Rat, Crl:CD (SD), Female	An oral gavage peri-and post-natal reproduction/developmental toxicity screening study in rat	100%	Non guideline - dose range finding study. Female animals only were treated to determine maximum tolerated doses	NOAEL ≥ 500 mg/kg bw/d LOAEL ≥ 1000 mg/kg bw/d	CIL-132

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
			of test substance on dams and their offspring.	*Clinical signs-dams* Dermal irritation – erythema, oedema and flaking of the skin at the application site No systemic effects observed *Developmental effects* No developmental effects	
Rat, CD® [Crl:CD® (SD)], Male & Female	Oral dietary two-generation reproduction study in rats	100%	OPPTS 870.3800; OECD 416 Dose Level: 0,1000, 3000 and 7500 ppm in diet	NOAEL (reproductive) ≥ 7500 ppm (512 mg/kg bw/d) LOAEL (reproductive): >7500 ppm (512 mg/kg bw/d) NOAEL (parental and neonatal toxicity): 3,000 ppm (207 mg/kg bw/d) LOAEL (parental and neonatal toxicity): =7500 ppm (512 mg/kg bw/d) *Organ weight*	CIL-133

Species	Test	Purity % Note[5]	Guideline, duration, doses and conditions	Result with recapitulation of findings at the LOAEL dose	Study number [6]
				↑ P_0 liver weight - absolute (♂ 11%, ♀14%), relative (♂ 13%, ♀17%) ↑ F_1 liver weight - absolute (♂ 8%, ♀22%), relative (♂ 12%, ♀19%) *Histopathology* Hepatocellular hypertrophy and bile duct hyperplasia. *Developmental effects* No developmental effects *Effects on fertility* No effects on fertility	

Table 5. Mutagenicity profile of the EC Oil (H/C) technical material based on *in vitro* and *in vivo* tests

Species	Test	Purity % Note[7]	Guideline, duration, doses and conditions	Result	Study number[8]
Salmonella Typhimurium strains and Escherichia coli strain WP2uvrA	Bacterial reverse mutation assay "Ames test"	100%	OECD 471 and Method B14 in 92/69/EEC *S.typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 1538 Expt 1: 0, 8, 40, 200, 1000 and 5000 µg/plate Expt 2: 0. 312.5, 625, 1250, 2500, 5000 µg/plate Both tests with and without metabolic activation.	Non-mutagenic No significant increases in revertant colonies for any strain, at any dose level with or without metabolic activation. No toxicity to any strains of S. Typhimurium; decrease in revertant frequency at high concentrations.	CIL-134
Cultured human lymphocytes	In vitro mammalian chromosomal aberration assay	100%	OECD 473 and Method B10 of Commission Directive 92/69/EEC Expt 1: 215.38, 430.75. 861.5 and 1723 µg/ml Expt 2: 53.84, 107.69 (-S9), 161.53 (-S9), 215.38, 430.75, 861.5 (+S9) and 1292.25 (+S9) µg/ml 4 hr exposure/20 hr incubation with metabolic activation	Non-mutagenic No significant increases in chromosomal aberrations and polyploidy with and without metabolic activation. Cytotoxicity (decreased mitotic index) observed at mid to high concentrations without metabolic activation and at high concentrations with metabolic activation.	CIL-135

[7] Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.
[8] Note: Study numbers appearing in the data tables must match study numbers in the references section at the end of the document.

Species	Test	Purity % Note[7]	Guideline, duration, doses and conditions	Result	Study number[8]
L5178Y mouse lymphoma cells	Gene mutation in mammalian cells in vitro L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay with oil of lemon eucalyptus GLP, Unpublished	100%	US EPA FIFRA guideline 84-2 Expt 1, -S9: 50, 75, 100, 150, 175, 200 and 210 µg/mL Expt 1, +S9: 50, 75, 100, 150, 175, 200, 250, 275, 300 and 325 µg/mL Expt 2, -S9: : 50, 75, 100, 150 and 225 µg/mL Expt 2, +S9: 250, 275, 300, 310, 320, 330, 340 and 350 µg/mL	Non-mutagenic None of the concentrations tested induced a mutation frequency above background with or without metabolic activation. In both assays, cytotoxicity ranged from none at the lowest concentration to moderate or high at the highest concentration with and without activation.	CIL-136
Mouse – CD-1(ICR)BR	In vivo mammalian cytogenetics (bone marrow micronuclei)	100%	OECD Guideline 474 0, 250, 500 and 1000 mg/kg b i.p. in corn oil Bone marrow harvested at 24 hr for two lowest dose groups, and 24 and 48 hr for 1000 mg/kg bw group.	Non-mutagenic No statistically significant increases in bone marrow micronuclei at any dose level and timepoint. ≥ 500 mg/kg bw: Prostration, laboured breathing, ataxia, hypoactivity; 1000 mg/kg bw: 8/18 deaths. No cytoxicity to bone marrow at any dose (no change in PCE/NCEf).	CIL-137

Table 6. Ecotoxicology profile of the EC Oil (H/C) technical material

Species	Test	Purity % Note[9]	Guideline, duration, doses and conditions	Result	Study number[10]
Daphnia magna	Acute toxicity (48-hr)	100%	OECD TG No. 202 Loading rates: 0, 0.316, 1.00, 3.16, 10.0, 31.6, 100 mg/L	EC_{50} = 25.5 mg/L (nom) not acutely toxic to *Daphnia magna*	CIL-138
Daphnia magna	Chronic toxicity (21-d)	100%	OECD Guideline 211 for Testing of Chemicals (2012) Loading rates: 0.64, 1.60, 4.00, 10.0, 25.00 mg/L	NOEC = 4.0 mg/L (nom) EC_{10} = 8.7 mg/L (nom)	CIL-139
Fish: *Danio rerio*	Acute (96-hr)	100%	OECD Test Guideline 203, "Fish, Acute Toxicity Test". 0-100mg/L	LC_{50} >35 mg/L (M.m) not acutely toxic to zebra fish	CIL-140
Algae: *(R. subcapitata)*	72-hr inhibition	100%	OECD Test Guideline 201 "Algae, Growth Inhibition Test" 2.2-101mg/L	EC_{50} = 37 mg/L (M.m) NOEC = 11.0 mg/L (M.m)	CIL-141
Algae: *(P. subcapitata)*	72-hr inhibition	100%	OECD Guideline 201 for Testing of Chemicals and Council Regulation (EC) No. 761/2009 Method C.3	EC_{50} = 69.9 mg/L (nom) NOEC = 25 mg/L (nom)	CIL-142

[9] Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.
[10] Note: Study numbers appearing in the data tables must match study numbers in the references section at the end of the document.

Annex 2: References

References Section 1. Confidential data (sorted by study number)

Study Number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
CIL-100	Maher, M.	2018a	Analytical Method Validation for Analysis of Samples Containing Eucalyptus Citriodora Oil, Hydrated, Cyclized and Eucalyptus Citriodora Oil and Citronellal, Hydrated, Cyclized by GC Using Flame Ionisation Detection (GC-FID) for the Determination of Ten Constituents Intertek Pharmaceutical Services Study No. 1340088 GLP, Unpublished
CIL-101	Wood, M.	2018	Analysis of Samples Containing Eucalyptus Citriodora Oil, Hydrated, Cyclized by Gas Chromatography Using Flame Ionisation Detection – For the Detection and Quantification of Ten Compounds Intertek Pharmaceutical Services TM-1951 Unpublished
CIL-102	Maher, M.	2020	Analytical Validation for the Determination of a Minor Component Present at <0.1% in Eucalyptus citriodora oil, hydrated, cyclized and Citronellal rich oil, hydrated, cyclized by Gas Chromatography - Mass Spectrometry (GC-MS) Intertek Phamaceutical Services Study No. 1340087 GLP, Unpublished
CIL-103	Wood, M.	2018	Determination of a minor component at <0.1% TM 1952 By Gas Chromatography Using Mass Spectrometry Detection Intertek Pharmaceutical Services TM-1952 Unpublished
CIL-104	Kelly, K.	2014a	Validation of Analytical Method M821: Analysis of PMD Rich Botanic Oil by Gas Chromatography Using Flame Ionisation Detection GC Laboratories Ltd Report No: J 20000 GLP, Unpublished
CIL-105	Kelly, K.	2015a	Validation of Analytical Method M821/B: Analysis of PMD Rich Botanic Oil by Gas Chromatography Using Flame Ionisation Detection for the Determination of Limonene GC Laboratories Ltd Report No: J 20162 GLP, Unpublished
CIL-106	Kelly, K.	2014c	Validation of Analytical Method M822: Determination of Impurity by Gas Chromatography Using Mass Spectrometry Detection GC Laboratories Ltd Report No: J 20007 GLP, Unpublished

CIL-107	Maher, M.	2021	Citriodiol - 5 Batch Analysis Intertek Pharmaceutical Services Study No. 1348493 GLP, Unpublished
CIL-108	Kelly, K.	2014b	5 Batch Analysis of Citriodiol CG Laboratories Ltd. Report No: J20006 GLP; Unpublished
CIL-109	Kelly, K.	2015b	Analysis of Five Batches of Citriodiol, (p-Menthane-3,8-diol Rich Botanic Oil (PMDRBO)) Technical Material CG Laboratories Ltd. Report No: J20160 GLP; Unpublished
CIL-148	Parker, G.	2024	Mass Spectra Data of the Two Largest Constituents of EC Oil (H/C) Citrefine International Ltd. Unpublished

References Section 2. Non-confidential data (sorted by study number)

Study Number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
CIL-110	Sudworth, J.	2015	Determination of Vapour Pressure of cis-p-Menthane-3,8-diol (cis-PMD), p-Menthane-3,8-diol-Citronellal Acetal (PMD Acetal Isomers), Isopulegol and beta-Citronellol. Amendment 1. Intertek Pharmaceutical Services Manchester Study No: 1334317 GLP, unpublished
CIL-111	Whitsel, M.	2010	Melting and Pour Point Analysis of PMDRBO MPI Research, Inc. Report No. 1792-004 GLP, Unpublished
CIL-112	White, D.F. and Mullee D.M.	2006	Citriodiol: determination of general physico-chemical properties SafePharm Laboratories Ltd. Report No. 0640/0013 GLP, Unpublished
CIL-113	O'Connor, Y.	2018b	Water Solubility and Partition Coefficient of Selected Constituents of EC Oil (H/C) Intertek Pharmaceutical Services Manchester Study No. 1342392 GLP, Unpublished
CIL-114	Brookman, D.J. and Curry, K.K.	1998	Extract of lemon eucalyptus: physical and chemical characteristics data volume Technology Sciences Group Inc. Report No. WPC-9802 GLP, Unpublished
CIL-115	Bates, G.	2016	Analysis of PMD Rich Botanical Oils for pH and Flash Point Testing GC Laboratories Ltd. Report Number J20380 GLP, Unpublished

CIL-116	Bee, T.	2016	Autoflammability of Liquids Testing on a Sample of PMD Rich Botanic Oil (Citriodiol) Chilworth Technology Ltd. Report No. GLP3016000225R1V1/2016 GLP, Unpublished
CIL-117	Driscoll, A.	1994a	PMD-07: Acute oral toxicity test in the rat, Derby, UK Report No. 640/1 GLP, Unpublished
CIL-118	Driscoll, A.	1994b	PMD-07: Acute dermal toxicity (limit test) in the rat Report No. 640/2 GLP, Unpublished
CIL-119	Kukulinski, M.	1998	Repel Aerosol: Acute Inhalation Toxicity Study Report No. 98-0193-3 GLP, Unpublished
CIL-120	Driscoll, A.	1994c	PMD-07: Acute dermal irritation test in the rabbit Report No. 640/3 GLP, Unpublished
CIL-121	Driscoll, A.	1994d	PMD-07: Acute eye irritation test in the rabbit Report No. 640/4 GLP, Unpublished
CIL-122	Driscoll, A.	1994f	PMD-07: Magnusson and Kligman maximisation study in the Guinea Pig Report No. 640/5 GLP, Unpublished
CIL-123	Driscoll, A.	1994e	PMD-07: Determination of the phototoxic potential in the Guinea-pig by topical application Report No. 640/6A GLP, Unpublished
CIL-124	Moore, G.	2001	14-day repeated dermal dose toxicity study in rats with Oil of Lemon Eucalyptus Report No. 10315 GLP, Unpublished
CIL-125	Parker, R.	2000a	28-day dermal toxicity study with Oil of Lemon Eucalyptus in rats Report No. ARGUS 720-004 GLP, Unpublished
CIL-126	Coleman, D.	2018a	EC Oil (H/C): Preliminary Toxicity Study by Dermal Administration to Sprague-Dawley Rats for 2 or 4 Weeks Study No. GH02PD Non-GLP, Unpublished
CIL-127	Coleman, D.	2018b	EC Oil (H/C): Toxicity Study by Dermal Administration to Sprague-Dawley Rats for 13 Weeks Study No. BX73MJ GLP, Unpublished
CIL-128	Blackstock, E.	2017a	EC Oil (H/C): The In Vitro Percutaneous Absorption of Radiolabelled Eucalyptus Citriodora Oil, Hydrated, Cyclized in Five Test Preparations Through Human and Rat Split-Thickness Skin Charles River Laboratories Edinburgh Ltd. Test Facility Study No. 799498, Report No. 38688 GLP, Unpublished
CIL-129	Lambert, E.	2016	A 90-Day Oral (Dietary) Toxicity Study of p-menthane-3,8-diol Rich Botanic Oil (PMDRBO) in Sprague Dawley Rats with a 28-Day Recovery Period Report No. WIL-225503 GLP, Unpublished

CIL-130	Trenton, N.	2001	Oral (gavage) dosage developmental toxicity study of Oil of Lemon Eucalyptus (OLE) in rats Report No. 720-006 GLP, Unpublished
CIL-131	Parker, R.	2000b	Dermal developmental toxicity study of oil of lemon eucalyptus in rats Report No. 720-005 GLP, Unpublished
CIL-132	Schroeder, R.	2010	PMDRBO (p-methane diol rich botanic oil): An oral gavage peri-and post-natal reproduction/developmental toxicity screening study in rat Report No. 1792-005 GLP, Unpublished
CIL-133	Schroeder, R.	2012	PMDRBO: An oral dietary two-generation reproduction study in rats Report No. 1792-003 GLP, Unpublished
CIL-134	Thompson, P.	1994	PMD-07: Reverse mutation assay “Ames test” using Salmonella typhimurium Report No. 640/8 GLP, Unpublished
CIL-135	Wright, N.	2002	Citriodiol: Chromosome aberration test in human lymphocytes in vitro Report No. 640/010 GLP, Unpublished
CIL-136	Cifone, M.A.	2000	L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay with oil of lemon eucalyptus Report No. 20901-0-431 OECD GLP, Unpublished
CIL-137	Myhr, B.	2000	In vivo mouse micronucleus assay with oil of lemon eucalyptus Report No. 20901-0-455OECD GLP, Unpublished
CIL-138	Klix, V.	2017a	Citriodiol – Acute Immobilization Test to *Daphnia magna*, Semi-static, 48 Hours, in a Closed System without Headspace Noack Laboratorien GmbH Report No. 160419TR/DAI17100 GLP, Unpublished
CIL-139	Klix, V.	2017b	Eucalyptus citriodora oil, hydrated, cyclized *Daphnia magna* Reproduction Test, Semi-static, 21 Days Noack Laboratorien GmbH Report No. 160419TR/DRE17211 GLP, Unpublished
CIL-140	Sijoln, A.	2005	Acute Aquatic toxicity assessment of Citriodiol from MASTA, Toxicon AB, Report No. 072-C/05 GLP, unpublished
CIL-141	Sijoln, A.	2005	Citriodiol: Algae Growth Inhibition Test Toxicon AB Report No. 072-A/05 GLP, unpublished

CIL-142	Klix, V.	2016	Citriodiol – Alga, Growth Inhibition Test with *pseudokirchneriella subcapitata*, 72 Hours Noack Laboratorien GmbH Report No. 160419TR/SPO17100 GLP, Unpublished
CIL-143	Kania, I.	2024	Report Title: MT181 Solubility in organic solvents Report No. CIL240401 Citrefine International Ltd. Unpublished

Annex 3: Determination of Methyl Eugenol in EC Oil (H/C) TK

The following method should be used to determine the content of methyl eugenol contained within Eucalyptus citriodora oil, hydrated, cyclized.

1. Apparatus: Gas chromatograph fitted with a mass spectrometer

2. Substances used:
Methyl eugenol - suitable reference standard of known purity
Methanol analytical grade (e.g. Sigma-Aldrich 34885)

3. Chromatographic conditions:
Column: Solgelwax (30m x 0.25mm x 0.25µm film thickness) or equivalent
Initial oven temperature: 50°C, hold for 2 minutes
Final oven temperature: 255°C
Temperature ramp: 15°C/min to 240°C, then 50°C/min to 255°C hold for 30 minutes
Injection temperature: 250°C
Detector temperature: 260°C
Injection volume: 2µL
Column flow rate: 1ml/min
Gas: Helium
Split Ratio: 20 :1
Run time: 45 minutes

Autosampler Parameters
Wash vials are to be filled with dilution solvent (section 4.b)
Pre-injection solvent flush: 3
Post-injection solvent flush: 3
Fill speed: High
Inject speed: High

Mass spectrometer parameters

Solvent delay: 3 minutes
Selective Ions: M/z 147,151,163 & 178
NOTE: results are calculated using m/z 178
MS Source temperature: 230°C
MS Quad Temperature: 150°C

4. Preparation of standards and samples

NOTE: Volumes may be scaled as required; however, final (nominal) concentrations must remain the same.

4.a Preparation of Eucalyptus Citriodora oil, Hydrated, Cyclized

Warm the sample in an oven set to 55°C for at least 2 hours, shaking the sample to ensure that any crystals have dissolved and the solution is homogenous. If crystals are still present continue to heat and mix until fully dissolved.

4.b Preparations of the 1000ppm stock solution

Accurately weigh 100mg (± 10% of the nominal weight) of methyl eugenol into a 100ml volumetric flask. Make to volume with methanol and mix well by inversion.

4.c Preparation of linearity solutions

Table A3.1. Volumes Required of 1000ppm Stock to Prepare Calibration Curves

Standard reference	Std A	Std B	Std C	Std D	Std E	Std F	Std G
Flask size (ml)	10	10	10	10	10	10	10
Volume of 1000ppm stock (ml)	5	4	3	2	1	0.5	0.25
Nominal methyl eugenol concentration (mg/ml)	0.5	0.4	0.3	0.2	0.1	0.05	0.025

All dilutions use methanol as dilution solvent. Further dilutions can be performed as necessary.

4.d Preparation of samples

Accurately weigh 3000mg (± 10% of the nominal weight) of homogenous sample (see section 4.a) into a tared 10ml volumetric flask. Add by pipette 5ml of methanol dilution solvent. Sonicate for 5 minutes, shake by inversion. Make to the required volume with dilution solvent.

5. Calculations

The calibration curve for each analyte has the form y=mx+c, or:

$$\text{Avg Peak Area}_{\text{Analyte}} = \text{Gradient} \times \text{Analyte Concentration} + \text{Intercept}$$

Using five consecutive standard concentrations chosen so that the response of the analyte is in the mid-range of the calibration curve, create a calibration graph plotting component peak area for the standards (y) against mg/ml (x). Correct the mg/ml for standard purity and calculate both the gradient and intercept of the line.

The amount of analyte present in the sample solution is determined as follows:

$$\text{Component Concentration (mg/mL)} = \frac{\text{Avg Peak Area}_{\text{Analyte}} - \text{Intercept}}{\text{Gradient}}$$

This can be converted to the units %w/w by dividing by the amount of sample analysed:

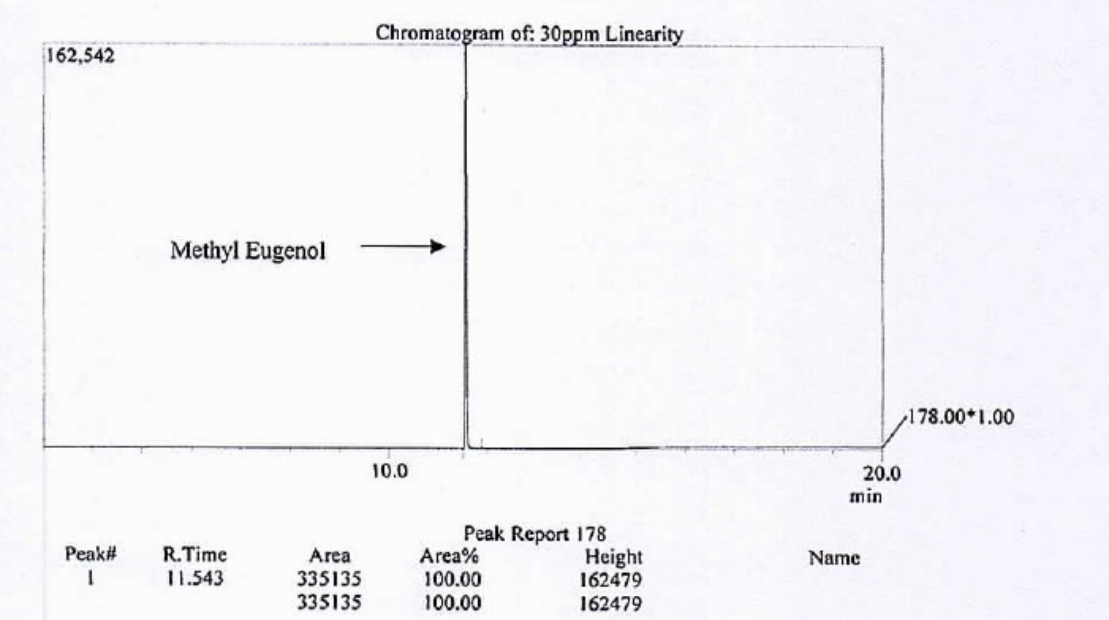
$$\text{Component Percent (\%w/w)} = \frac{\text{Component Concentration (mg/mL)}}{\text{Sample Concentration (mg/mL)}} \times 100$$

Where the sample concentration is:

$$\text{Sample Concentration (mg/mL)} = \frac{\text{Mass of the Sample (mg)}}{\text{Capacity of the Volumetric Flask (mL)}}$$

6. Example Chromatography

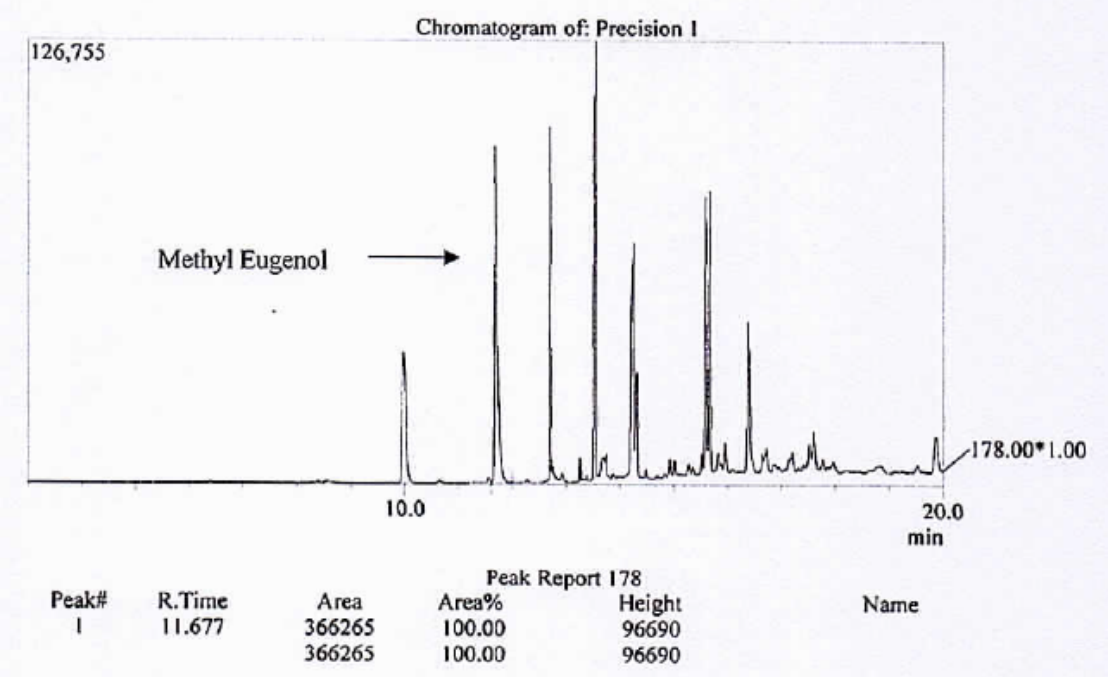
Fig. A3.1. 30ppm methyl eugenol linearity calibration standard, SIM Scan at m/z 178. Methyl eugenol elutes at 11.543 minutes.



Peak Report 178

Peak#	R.Time	Area	Area%	Height	Name
1	11.543	335135	100.00	162479	
		335135	100.00	162479	

Fig. A3.2. EC Oil(H/C) example chromatogram, SIM Scan at m/z 178. Methyl eugenol elutes at 11.677 minutes.



Peak Report 178

Peak#	R.Time	Area	Area%	Height	Name
1	11.677	366265	100.00	96690	
		366265	100.00	96690	

Annex 4: Determination of Limonene and Eucalyptol in EC Oil (H/C) TK

The following method should be used to determine the content of limonene and eucalyptol contained within Eucalyptus citriodora oil, hydrated, cyclized (EC Oil (H/C)).

1. Apparatus: Gas chromatograph fitted with a flame ionisation detector

2. Substances used:
Eucalyptol - suitable reference standard of known purity
(R)-(+)-Limonene - suitable reference standard of known purity
n-Tridecane (e.g. Sigma-Aldrich 91490)
Methanol analytical grade (e.g. Sigma-Aldrich 34885)
Isopropyl alcohol analytical grade (e.g. Sigma-Aldrich 278475)

3. Chromatographic conditions:
Column: Stabilwax, 60m x 0.25mm, 0.25µm film or equivalent.
Initial oven temperature: 90°C, hold for 0.5 minutes
Final oven temperature: 250°C
Temperature ramp: 20°C/min to 150°C, hold for 2 minutes then 5°C/min to 250°C, hold for 5 minutes
Injection temperature: 250°C
Detector temperature: 300°C
Injection volume: 1µL
Column flow rate: 1ml/min
FID Makeup Flow: 25.0ml/min
FID Fuel (H_2) Flow: 30.0ml/min
FID Air flow: 400ml/min
Gas: Helium
Column Pressure mode: Constant flow
Split Ratio: 10 : 1
Run time: 30.5 minutes

Autosampler parameters:
Wash vials are to be filled with dilution solvent (section 4.b)
Pre-injection solvent flush: 3
Post-injection solvent flush: 3
Fill speed: High
Inject speed: High

4. Preparation of standards and samples
NOTE: Volumes may be scaled as required; however, final (nominal) concentrations must remain the same.

4. a Preparation of Eucalyptus citriodora oil, hydrated, cyclized
Warm the sample in an oven set to 55°C for at least 2 hours, shaking the sample to ensure that any crystals have dissolved and the solution remains homogenous. If crystals are still present, continue to heat and mix until fully dissolved.

4. b Preparation of dilution solvent

Transfer 500ml of analytical grade methanol to a 1L glass bottle. Add 500ml of analytical grade isopropyl alcohol. Mix well by inversion. Different volumes can be prepared depending on the amount required, ensuring the ratio is 50:50 v/v.

4. c Preparation of the internal standard

Accurately weigh 5g ± 0.5g of *n*-tridecane into a tared 500ml volumetric flask and make up to the required volume with dilution solvent. Different volumes can be prepared depending on the amount required, ensuring the concentration is 10mg/mL.

4. d Preparations of the stock solutions

Prepare a mixed stock at the concentrations listed in Table A4.2. Accurately weigh the appropriate amount (± 10% of the nominal weight) of each standard into a 100ml volumetric flask. Make to volume with dilution solvent and mix well by inversion.

Table A4.1. Weights and nominal concentrations of analytes in mixed stock 1

Analytical Standard	Amount Weighed into 100mL Volumetric Flask (mg)	Nominal Conc (mg/mL)
Eucalyptol	56	0.560
(R)-(+)-Limonene	19	0.190

4. e Preparation of linearity solutions

Table A4.2. Stock quantities required for calibration curves

Standard Reference	Std A	Std B	Std C	Std D	Std E	Std F	Std F2	Std G	Std H
Flask Size (ml)	10	10	10	10	10	10	10	10	10
Volume of Stock 1 (ml)	2	1.6	1.2	0.8	0.4	0.2	0.2	X	X
Volume of Std F2 (ml)	X	X	X	X	X	X	X	5	2
Volume of Internal Standard (ml)	0.05	0.05	0.05	0.05	0.05	0.05	X	0.05	0.05
Nominal Eucalyptol Concentration	0.112	0.090	0.067	0.045	0.022	0.011	0.011	0.006	0.002
Nominal Limonene Concentration	0.038	0.030	0.023	0.015	0.008	0.004	0.004	0.002	0.001

All dilutions use dilution solvent.

4. d Preparation of samples

Weigh precisely 250mg of homogenous sample (see section 4.a) into a tared 50ml volumetric flask. Add by pipette 0.25ml of the internal standard solution and 20ml of dilution solvent. Sonicate for 5 minutes, shake by inversion. Make to the volume with dilution solvent. To check for tridecane interference prepare as above but without Internal standard solution (see section 6).

5. Calculations

The calibration curve for each analyte has the form y=mx+c, or:

$$\frac{\text{Avg Peak Area}_{\text{Analyte}}}{\text{Avg Peak Area}_{\text{IS}}} = \text{Gradient} \times \text{Analyte Concentration} + \text{Intercept}$$

First calculate the component peak area ratio, which is the ratio of the peak areas of the analyte and the internal standard for standards and samples.

$$\text{Component Peak Area Ratio} = \frac{\text{Peak Area}_{\text{Analyte}}}{\text{Peak Area}_{\text{IS}}}$$

Using five consecutive standard concentrations, chosen so that the response of the analyte is in the mid-range of the calibration curve, create a calibration graph plotting component area ratio for the standards (y) against mg/ml (x). Correct the mg/ml for standard purity and calculate the gradient and intercept of the line

The amount of analyte present in the sample solution is determined as follows:

$$\text{Component Amount (mg/mL)} = \frac{\text{Component Peak Area Ratio} - \text{Intercept}}{\text{Gradient}}$$

This can be converted to the units %w/w by dividing by the concentration of the analysed sample:

$$\text{Component Amount (\%w/w)} = \frac{\text{Component Concentration (mg/mL)}}{\text{Sample Concentration (mg/mL)}} \times 100$$

Where the sample concentration is:

$$\text{Sample Concentration (mg/mL)} = \frac{\text{Mass of the Sample (mg)}}{\text{Capacity of the Volumetric Flask (mL)}}$$

6. Tridecane correction

NOTE: There is a potential for a small interference in the tridecane internal standard peak in EC Oil (H/C) samples. This can be tested by comparison of the chromatography of a sample solution prepared without tridecane internal standard and an injection of dilute internal standard (0.05ml in 10ml of dilution solvent). If there is no observed interference at the retention time of tridecane then the following calculation is not required.

If interference is observed in a sample prepared without internal standard at the retention time of tridecane, the following calculation is required to correct the peak area of tridecane and calculate sample amounts.

Prepare and analyse six un-spiked samples, then assess the peak area of the interference at the retention time of tridecane. Weight adjust the area by dividing

the peak area by the sample amount in mg/ml. Calculate the average of the weight adjusted peak areas.

This is the matrix correction factor (MCF).

$$\text{MCF} = \frac{\text{Peak Area}_{\text{Interference}}}{\text{Amount of Sample (mg/mL)}}$$

The average MCF of each batch of Eucalyptus citriodora oil, hydrated, cyclized is calculated by determining the MCF of each un-spiked sample. The average MCF will be used going forward.

From two samples spiked with internal standard, calculate the amount of the tridecane peak that is due to the matrix. The matrix correction factor calculated above is multiplied by the sample amount in mg/mL. This is the matrix peak blank area (MPBA).

$$\text{MPBA} = \text{Avg MCF} \times \text{Amount of Sample Containing Internal Std (mg/mL)}$$

The matrix peak blank area is subtracted from the internal standard peak area to give the corrected internal standard peak. The corrected internal standard peak is then used to calculate the sample amounts.

$$\text{Corrected Peak Area}_{\text{IS}} = \text{Sample Peak Area}_{\text{IS}} - \text{MPBA}$$

For each component, divide the peak area by the corrected internal standard peak area to give the normalised component peak area.

$$\text{Normalised Component Peak Area Ratio} = \frac{\text{Peak Area}_{\text{Analyte}}}{\text{Corrected Peak Area}_{\text{IS}}}$$

The normalised component peak area is the used in the following equation to calculate the component amount in sample (mg/ml).

$$\text{Component Concentration (mg/mL)} = \frac{\text{Normalised Component Peak Area Ratio} - \text{Intercept}}{\text{Gradient}}$$

This can be converted to the units %w/w by dividing by the concentration of the analysed sample:

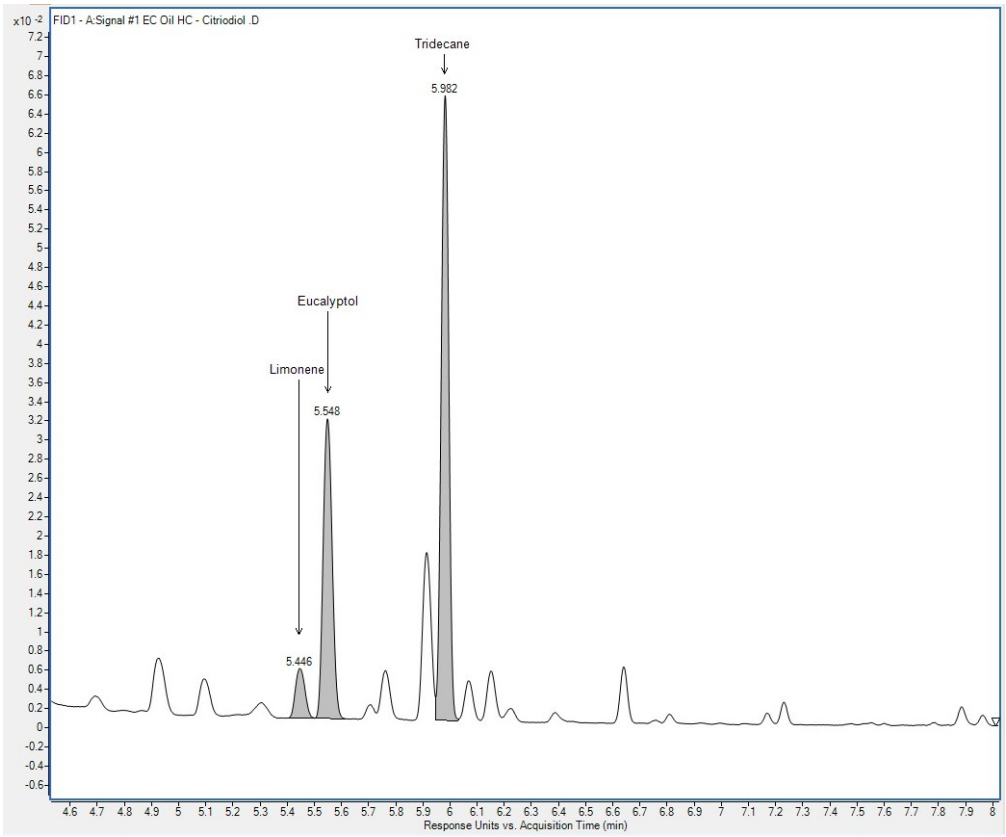
$$\text{Component Percent (\%w/w)} = \frac{\text{Component Concentration (mg/mL)}}{\text{Sample Concentration (mg/mL)}} \times 100$$

Where the sample concentration is:

$$\text{Sample Concentration (mg/mL)} = \frac{\text{Mass of the Sample (mg)}}{\text{Capacity of the Volumetric Flask (mL)}}$$

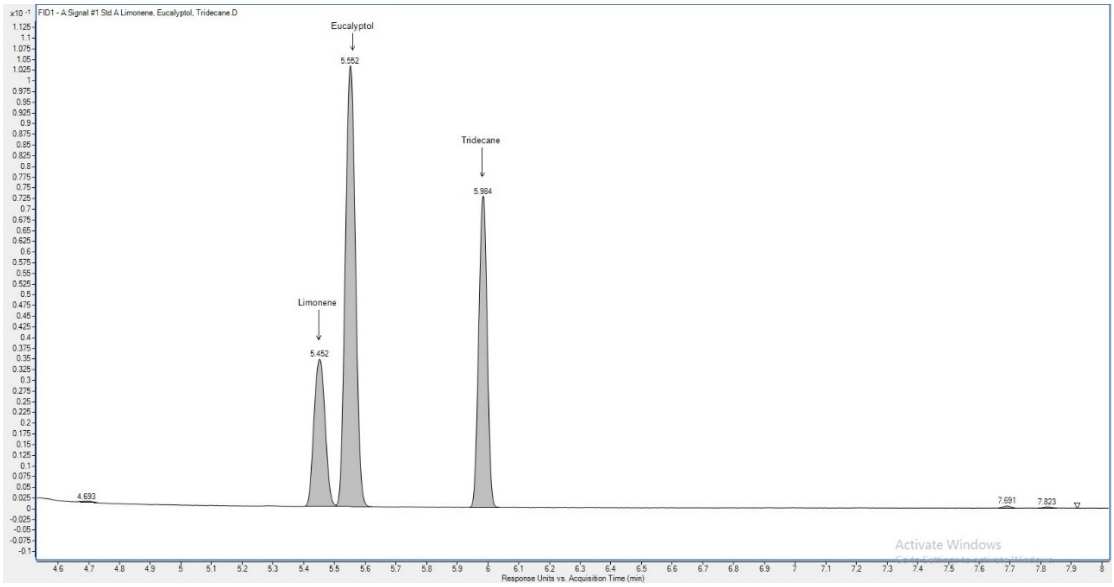
7. Example chromatography

Fig. A4.1. Example zoomed chromatogram of EC Oil (H/C) 4.5-8 mins.



Limonene elutes at 5.4 minutes; Eucalyptol elutes at 5.5 minutes and Tridecane at 6.0 minutes.

Fig. 2. Example zoomed chromatogram of Calibration Standard A 4.5-8 mins.



Limonene elutes at 5.4 minutes; Eucalyptol elutes at 5.5 minutes and Tridecane at 6.0 minutes.