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Short review

RIFM fragrance ingredient safety assessment, Ethyl 2,3,6-trimethylcyclohexyl carbonate, CAS Registry Number 93981-50-1

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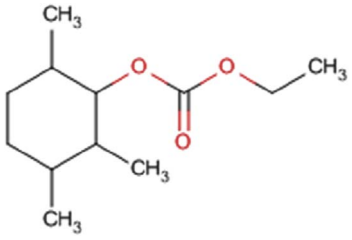
CAS Registry Number: 93981-50-1



Abbreviation list:

2-Box Model - a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM model - The Creme RIFM model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017) compared to a deterministic aggregate approach.
DEREK - Derek nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union

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GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC- No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC- Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test.
TTC - Threshold of Toxicological Concern
UV/Vis Spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WOE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe under the limits described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015) which should be referred to for clarifications.

Each endpoint discussed in this safety assessment reviews the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a two-digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (i.e., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria such as, acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative end-point value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM guidance relevant to human health and environmental protection

Summary: The use of this material under current conditions is supported by existing information.

The material (ethyl 2,3,6-trimethylcyclohexyl carbonate) was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that ethyl 2,3,6-trimethylcyclohexyl carbonate is not genotoxic. Data from the read across analog 2-*tert*-butylcyclohexyl acetate (CAS# 88-41-5) show that ethyl 2,3,6-trimethylcyclohexyl carbonate does not have skin sensitization potential. The repeated dose, developmental and reproductive and local respiratory toxicity endpoint reviews were completed using the TTC for a Cramer Class I material (0.03, 0.03 mg/kg/day and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint review was completed based on UV spectra and data on the target material; ethyl 2,3,6-trimethylcyclohexyl carbonate was not phototoxic/photoallergenic. The environmental endpoints were evaluated and ethyl 2,3,6-trimethylcyclohexyl carbonate was not found to be PBT as per IFRA environmental standards and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC) are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2016a,b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below TTC.
Developmental and Reproductive Toxicity: No NOAEL available. Exposure is below TTC.
Skin Sensitization: Not a sensitization concern. (RIFM, 2002)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic (UV Spectra, RIFM DB; RIFM, 1981a,b)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 3% (OECD 301D) (RIFM, 2013a,b,c,d)
Bioaccumulation: Screening Level: 294 L/kg (US EPA, 2012)
Ecotoxicity: Screening Level: Fish LC50: 3.199 mg/L (Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-Level: PEC/PNEC (North America and Europe) < 1 (Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 3.199 mg/L (Salvito et al., 2002)
RIFM PNEC is: 0.003199 μg/L
- **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: not applicable; cleared at screening level

1. Identification

1. **Chemical Name:** Ethyl 2,3,6-trimethylcyclohexyl carbonate
2. **CAS Registry Number:** 93981-50-1
3. **Synonyms:** Carbonic acid, ethyl 2,3,6-trimethylcyclohexyl ester; ethyl 2,3,6-trimethylcyclohexyl carbonate; rholiate
4. **Molecular Formula:** $C_{12}H_{22}O_2$
5. **Molecular Weight:** 214.05
6. **RIFM Number:** 5546

2. Physical data

1. **Boiling Point:** 240.6 C [RIFM, 2013c], 271.27 °C [EPI Suite]
2. **Flash Point:** 100 °C [RIFM, 2013d], 100 °C [GHS]
3. **Log K_{OW}:** 4.25 [EPI Suite]
4. **Melting Point:** −21.42 °C [EPI Suite]
5. **Water Solubility:** 8.707 mg/L [EPI Suite]
6. **Specific Gravity:** 0.96000 to 0.97000 @ 25.00 °C*
7. **Vapor Pressure:** 0.00861 mm Hg @ 25 °C [EPI Suite], 0.00528 mmHg @ 20 °C [EPI Suite 4.0]
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1}·cm^{-1})
9. **Appearance/Organoleptic:** A colorless clear oily liquid with a medium floral, warm, dark, red rose, leather, metallic odor while at 10% or less in dipropylene glycol.*

*http://www.thegoodscentscompany.com/data/rw1013531.html#toorgano, retrieved 12/3/2015.

3. Fragrance exposure

1. **Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA, 2011)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.015% (RIFM, 2016c)
3. **Inhalation Exposure*:** 0.00028 mg/kg/day or 0.016 mg/day (RIFM, 2016c)
4. **Total Systemic Exposure**:** 0.00067 mg/kg/day (RIFM, 2016c)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; Safford et al., 2015 and Safford et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 4. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015 and Safford et al., 2017).

4. Derivation of systemic absorption

1. **Dermal:** Assumed 100%
2. **Oral:** Assumed 100%
3. **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low (Expert Judgment)

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
I*	III	II

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was also determined using expert judgment based on the Cramer decision tree (Cramer et al., 1976). See Appendix below for further details.

2. **Analogs Selected:**
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Developmental and Reproductive Toxicity:** None
   d. **Skin Sensitization:** 2-*tert*-Butylcyclohexyl acetate (CAS# 88-41-5)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. **Read-across Justification:** See Appendix below

6. Metabolism

Not considered for this risk assessment.

6.1. Natural occurrence (discrete chemical) or composition (NCS)

Ethyl 2,3,6-trimethylcyclohexyl carbonate is not reported to occur in food by the VCF*.

*VCF Volatile Compounds in Food: database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. [eds]. – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database, contains information on published volatile compounds which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

7. IFRA standard

None.

8. REACH dossier

Pre-registered for 2010, no dossier available as of 6/30/2017.

9. Summary

9.1. Human health endpoint summaries

9.1.1. Genotoxicity

Based on the existing data, ethyl 2,3,6-trimethylcyclohexyl carbonate does not present a concern for genetic toxicity.

9.1.2. Risk assessment

Ethyl 2,3,6-trimethylcyclohexyl carbonate was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2013a). The mutagenic activity of ethyl 2,3,6-trimethylcyclohexyl carbonate has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation/preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with ethyl 2,3,6-trimethylcyclohexyl carbonate in DMSO (dimethyl sulfoxide) at concentrations up

to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9; except for TA1535 where a small statically significant increase was observed in absence of S9 but was not considered to be biologically relevant since this increase was observed at toxic doses (RIFM, 2016a). Under the conditions of the study, ethyl 2,3,6-trimethylcyclohexyl carbonate was not mutagenic in the Ames test.

The clastogenic activity of ethyl 2,3,6-trimethylcyclohexyl carbonate was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with ethyl 2,3,6-trimethylcyclohexyl carbonate in solvent DMSO (dimethyl sulfoxide) at concentrations up to 125 μg/ml in the presence and absence of metabolic activation (S9) at the 4-h and 24-h timepoints. Ethyl 2,3,6-trimethylcyclohexyl carbonate did not induce binucleated cells with micronuclei when tested up to the maximum dose in either non-activated or S9 activated test systems (RIFM, 2016b). Under the conditions of the study, ethyl 2,3,6-trimethylcyclohexyl carbonate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, ethyl 2,3,6-trimethylcyclohexyl carbonate does not present a concern for genotoxic potential.

Additional References: RIFM, 2013a.

Literature Search and Risk Assessment Completed on: 01/11/2017.

9.1.3. Repeated dose toxicity

There are insufficient repeated dose toxicity data on ethyl 2,3,6-trimethylcyclohexyl carbonate or any read across materials evaluated. The total systemic exposure to ethyl 2,3,6-trimethylcyclohexyl carbonate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

9.1.4. Risk assessment

There are no repeated dose toxicity data on ethyl 2,3,6-trimethylcyclohexyl carbonate or any read across materials evaluated that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to ethyl 2,3,6-trimethylcyclohexyl carbonate (0.67 μg/kg bw/day) is below the TTC (30 μg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 01/12/2017.

9.1.5. Developmental and reproductive toxicity

There are insufficient developmental and reproductive toxicity data on ethyl 2,3,6-trimethylcyclohexyl carbonate or any read across materials evaluated. The total systemic exposure to ethyl 2,3,6-trimethylcyclohexyl carbonate is below the TTC for the developmental and reproductive toxicity endpoints of a Cramer Class I material at the current level of use.

9.1.6. Risk assessment

There are no developmental or reproductive toxicity data on ethyl 2,3,6-trimethylcyclohexyl carbonate or any read across materials evaluated that can be used to support the developmental or reproductive toxicity endpoints. The total systemic exposure to ethyl 2,3,6-trimethylcyclohexyl carbonate (0.67 μg/kg bw/day) is below the TTC (30 μg/kg bw/day) for the developmental and reproductive toxicity endpoints of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 01/12/2017.

9.1.7. Skin sensitization

Based on the existing data on ethyl 2,3,6-trimethylcyclohexyl carbonate and read across analog 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5), ethyl 2,3,6-trimethylcyclohexyl carbonate does not present a concern for skin sensitization.

9.1.8. Risk assessment

Based on existing data on ethyl 2,3,6-trimethylcyclohexyl carbonate data and read across analog 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5; see Section 5), ethyl 2,3,6-trimethylcyclohexyl carbonate does not present a concern for skin sensitization. The chemical structure of these materials indicates that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v3.4). In a Buehler guinea pig sensitization study, no reactions were observed with ethyl 2,3,6-trimethylcyclohexyl carbonate (RIFM, 1981a). Similarly, in another guinea pig sensitization study no reactions were reported for 2-*tert*-butylcyclohexyl acetate (RIFM, 1972). Additionally, no reactions indicative of skin sensitization were observed in human studies with 2-*tert*-butylcyclohexyl acetate (RIFM, 1976, 2002, 1964).

Additional References: None.

Literature Search and Risk Assessment Completed on: 1/25/17.

9.1.9. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra and available *in vivo* data, ethyl 2,3,6-trimethylcyclohexyl carbonate would not present a concern for phototoxicity or photoallergenicity.

9.1.10. Risk assessment

UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. Corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity, 1000 L $mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009). In phototoxicity and photoallergenicity studies conducted in guinea pigs, topical application of 1.5% ethyl 2,3,6-trimethylcyclohexyl carbonate did not result in phototoxic or photoallergenic reactions, though the studies are of limited value due to inappropriate UV dose (RIFM, 1981b,c). Based on lack of absorbance and the available *in vivo* study data, ethyl 2,3,6-trimethylcyclohexyl carbonate would not be expected to present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 04/17/17.

9.1.11. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, ethyl 2,3,6-trimethylcyclohexyl carbonate, exposure level is below the Cramer Class I TTC value for inhalation exposure local effects.

9.1.12. Risk assessment

There are no inhalation data available on ethyl 2,3,6-trimethylcyclohexyl carbonate. Based on the Creme RIFM model, the inhalation exposure is 0.016 mg/day. This exposure is 87.5 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed on: 12/13/2016.

10. Environmental endpoint summary

10.1. Screening-level assessment

A screening level risk assessment of ethyl 2,3,6-trimethylcyclohexyl carbonate was performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of

screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor as discussed in Salvito et al. (2002). At Tier 2, the model ECOSAR (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this safety assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. Following the RIFM Environmental Framework, ethyl 2,3,6-trimethylcyclohexyl carbonate was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening level PEC/PNEC < 1).

A screening-level hazard assessment using EPISUITE ver 4.1 did not identify ethyl 2,3,6-trimethylcyclohexyl carbonate as either being possibly persistent nor bioaccumulative based on its structure and physical-chemical properties. This screening level hazard assessment is a weight of evidence review of a material's physical-chemical properties, available data on environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies) and fish bioaccumulation, and review of model outputs (e.g., USEPA's BIOWIN and BCFBAF found in EPISUITE ver.4.1). Specific key data on biodegradation and fate and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.1.1. Risk assessment

Based on current Volume of Use (2011), ethyl 2,3,6-trimethylcyclohexyl carbonate does not present a risk to the aquatic compartment in the screening level assessment.

10.1.1.1. Biodegradation. RIFM, 2013b: Ready biodegradability of the test material was evaluated according to the OECD 301D method. Under the conditions of the study, biodegradation of 3% was observed after 28 days.

10.1.1.2. Ecotoxicity. No data available.

10.1.1.3. Other available data. Ethyl 2,3,6-trimethylcyclohexyl carbonate has been pre-registered for REACH with no additional data at this time.

11. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in µg/L).

Endpoints used to calculate PNEC are underlined.

Exposure information and PEC calculation (following RIFM Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.25	4.25
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

Based on available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.003199 µg/L. The revised PEC/PNECs for EU and NA: not applicable; cleared at screening level and, therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed on: 1/16/2017.

12. Literature search*

- **RIFM database:** target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** http://tools.niehs.nih.gov/ntp_tox/index.cfm
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PUBMED:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** (http://monographs.iarc.fr)
- **OECD SIDS:** http://www.chem.unep.ch/irptc/sids/oecdsids/sidspub.html
- **EPA Actor:** http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=0EF5C212B7906229F477472A9A4D05B7
- **US EPA HPVIS:** http://www.epa.gov/hpv/hpvis/index.html
- **US EPA Robust Summary:** http://cfpub.epa.gov/hpv-s/
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base:** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com/webhp?tab=ww&ei=KMSoUpiQK-arsQS324GwBg&ved=0CBQQ1S4

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list.

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	3.199 mg/l			1,000,000	0.003199 µg/L	

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2017.11.012.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.11.012.

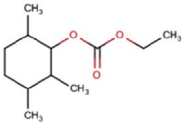
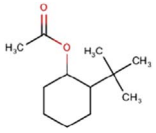
Appendix

Read across justification

Methods

The read across analog was identified following the strategy for structuring and reporting a read across prediction of toxicity described in Schultz et al. (2015) and is consistent with the guidance provided by OECD on the reporting of defined approached used within Integrated Approaches for Testing and Assessment or IATA (OECD, 2015) and the European Chemical Agency (ECHA) read across assessment framework or RAAF (ECHA, 2016).

- In essence, materials were first clustered based on their structure similarity. In the second step, data availability and data quality on the selected cluster was examined. Finally, appropriate read across analog from the cluster were confirmed by using expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physicochemical properties of the target substance and the read across analog were calculated using EPI Suite™ v4.11 developed by US EPA (US EPA, 2012).
- J_{max} were calculated using RIFM skin absorption model (SAM), and the parameters were calculated using consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts and oncologic classification were generated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- Developmental toxicity and skin sensitization were estimated using CAESAR v.2.1.7 and 2.1.6, respectively (Cassano et al., 2010).
- Protein binding was estimated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- The major metabolites for the target and read across analogs were determined and evaluated using OECD QSAR Toolbox (v3.4) (OECD, 2012).

	Target material	Read across material
Principal Name	Ethyl 2,3,6-trimethylcyclohexyl carbonate	2-*tert*-Butylcyclohexyl acetate
CAS No.	93981-50-1	88-41-5
Structure		
Similarity (Tanimoto score)		0.61
Read across endpoint		• Skin sensitization
Molecular Formula	$C_{12}H_{22}O_2$	$C_{12}H_{22}O_2$
Molecular Weight	214.31	198.31
Melting Point (°C, EPISUITE)	−21.42	10.93
Boiling Point (°C, EPISUITE)	271.27	232.55
Vapor Pressure (Pa @ 25 °C, EPISUITE)	1.15	7.1
Log Kow (KOWWIN v1.68 in EPISUITE)	4.25	4.2
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	8.707	7.462
J_{max} (mg/cm^2/h, SAM)	30.807	17.080
Henry's Law (Pa·m^3/mol, Bond Method, EPISUITE)	3.50E-003	9.90E-004
Skin Sensitization		
Protein binding by OASIS v1.4	• No alert found	• No alert found
Protein binding by OECD	• No alert found	• Acylation
Protein binding potency	• Not possible to classify	• Not possible to classify
Protein binding alerts for skin sensitization by OASIS v1.1	• No alert found	• No alert found
Skin Sensitization model (CAESAR) (version 2.1.6)	• Sensitizer (low reliability)	• Sensitizer (good reliability)
Metabolism		
OECD QSAR Toolbox (3.4) Rat liver S9 metabolism simulator	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on the target material ethyl 2,3,6-trimethylcyclohexyl carbonate (CAS # 93981-50-1). Hence *in silico* evaluation was conducted by determining read across analogs for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5) was identified as a read across material with data for its

respective toxicological endpoints.

- Metabolism

Metabolism of the target substance was not considered for the risk assessment and therefore metabolism data were not reviewed, except where it may pertain as described in specific endpoint sections above. Metabolism of the target material ethyl 2,3,6-trimethylcyclohexyl carbonate (CAS # 93981-50-1) and the read across material 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5) was predicted using the rat liver S9 Metabolism Simulator (OECD QSAR Toolbox v3.4) (See table above). The target material as well as the read across analog are predicted to be metabolized to 2,3,6-trimethylcyclohexanol (CAS # 58210-03-0) and 2-*tert*-butylcyclohexanol (CAS # 13491-79-7), respectively. The target substance will produce formic acid (CAS # 64-18-6) and ethanol (CAS # 64-17-5) as additional metabolic products, while the read across analog will produce acetic acid (CAS # 64-19-7). The cyclic aliphatic alcohols and organic acids produced in the metabolism of the read across analog as well as the target substance are structurally similar. Ethanol is the only additional metabolic product produced by the target substance. Ethanol does not show any structural alerts for the skin sensitization endpoint. Hence, 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5) can be use as read across for the target material ethyl 2,3,6-trimethylcyclohexyl carbonate (CAS # 93981-50-1). The read across analog materials was out of domain for *in vitro* rat S9 simulator (OASIS TIMES v2.27.19). However, based on expert judgement, the model's domain exclusion was overridden and a justification is provided.

Conclusion/Rationale

- 2-*tert*-butylcyclohexyl acetate (CAS # 88-41-5) could be used as a structurally similar read across analog for the target material ethyl 2,3,6-trimethylcyclohexyl carbonate (CAS # 93981-50-1) for the skin senzitization endpoint.
  - The read across analog belongs to the structural class of aliphatic esters with a cyclic alcohol portion while the target belongs to the class of aliphatic carbonates with a structurally similar cyclic extended fragment on the alcohol portion.
  - The target substance and the read across analog share an alkyl substituted cyclohexanol substructure common among them.
  - The key difference between the target substance and the read across analog is that, the target is an ethyl carbonate whereas, the read across analog is an acetate ester.
  - Similarity between the target substance and the read across analog is indicated by the Tanimoto score in the above table. The Tanimoto score is mainly driven by the alkyl substituted cyclohexyl fragment. The differences in the structure which are responsible for a Tanimoto score < 1 are not relevant from a toxicological endpoint perspective.
  - The target substance and the read across analog have similar physical chemical properties. Any differences in the physical chemical properties of the target substance and the read across analog are estimated to be toxicologically insignificant for the skin senzitization endpoint.
  - Structural alerts for the skin senzitization endpoint are consistent between the target substance and the read across analog as seen in the table above. The read across analog shows a structural alert for protein binding by OECD QSAR Toolbox v3.4. Other protein binding models do not show any alert for the target substance or the read across analog. The CAESAR model predicts the target and the read across analog to be sensitizers. The data described in the skin sensitization section above demonstrates that the read across analog is not a concern for skin sensitization. Hence the prediction is superseded by the data.
  - The target substance and the read across analog are expected to be metabolized similarly as shown by the metabolism simulator.
  - The structural alerts for the skin senzitization endpoint are consistent between the metabolites of the read across analog and the target substance.

Explanation for Cramer Class

Q1. Normal constituent of the body? No;
Q2. Contains functional groups associated with enhanced toxicity? No;
Q3. Contains elements other than C, H, O, N, divalent S? No;
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No;
Q6. Benzene derivative with certain substituents? No;
Q7. Heterocyclic? No;
Q16. Common terpene? Yes – Class I Low

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renkers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the research institute for fragrance materials, inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of cramer classification between toxtree, the OECD QSAR toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. Chem. Central J. 4 (Suppl. 1), S4.

Cramer, G.M., Ford, R.A., Hall, R.L., 1976. Estimation of toxic hazard—a decision tree approach. Food Cosmet. Toxicol. 16 (3), 255–276.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

ECHA, 2016. Read-across Assessment Framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.

IFRA (International Fragrance Association), 2011. Volume of Use Survey, February 2011.

OECD, 2012. The OECD QSAR Toolbox. v. 3.4. Retrieved from. http://www.qsartoolbox.org/.

OECD, 2015. Guidance Document on the Reporting of Integrated Approaches to Testing and Assessment (IATA). ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.

RIFM (Research Institute for Fragrance Materials, Inc.), 1964. Repeated Insult Patch Test of 2-tert-butylcyclohexyl Acetate. Unpublished report from International Flavor and Fragrances. RIFM report number 53338. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 1972. Human Patch Test, Guinea Pig Skin Sensitization, and Rabbit Eye Irritation Studies with 2-tert-butylcyclohexyl

Acetate (Agrumex). [Report in German] Unpublished report from Symrise. RIFM report number 55996. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1797. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1981a. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate) in Peanut Oil: Delayed Contact Hypersensitivity Modified by Buehler. Unpublished report from Dragoco Gerberding and Co. GmbH. RIFM report number 37825. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1981b. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate) 1.5% in Peanut Oil: Experimental Investigation on Photosensitizing Effect in Guinea-pigs. Unpublished report from Dragoco Gerberding and Co. GmbH. RIFM report number 37826. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1981c. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate) 1.5% in Peanut Oil: Experimental Investigation on Phototoxic Effect in Guinea-pigs. Unpublished report from Dragoco Gerberding and Co. GmbH. RIFM report number 37827. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2002. Repeat Insult Patch Test of 2-tert-butylcyclohexyl Acetate. Unpublished report from International Flavor and Fragrances. RIFM report number 53340. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013a. Report on the Testing of Ethyl 2,3,6-trimethylcyclohexyl Carbonate in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM report number 66079. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013b. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate): Ready Biodegradability - Closed Bottle Test. Unpublished report from Symrise. RIFM report number 66410. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013c. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate): Boiling Point. Unpublished report from Symrise. RIFM report number 66411. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013d. Ethyl 2,3,6-trimethylcyclohexyl Carbonate (Rholiate): Flash Point. Unpublished report from Symrise. RIFM report number 66414. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016a. Ethyl 2,3,6-trimethylcyclohexyl Carbonate: Reverse Mutation Assay 'Ames Test' Using Salmonella typhimurium and *Escherichia coli*. RIFM report number 70476. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016b. Ethyl 2,3,6-trimethylcyclohexyl Carbonate: Micronucleus Test in Human Lymphocytes *in vitro*. RIFM report number 70846. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials), 2016c. Use Level Survey, March 2016.
Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., et al., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.
Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., et al., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T.D., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.
Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74 (12), 164–176.
US EPA, 2012. Estimation Programs Interface Suite™ for Microsoft® Windows. v. 4.11. United States Environmental Protection Agency, Washington, DC, USA.