Food and Chemical Toxicology 163 (2022) 113041

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

RIFM fragrance ingredient safety assessment, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, CAS Registry Number 67801-38-1

A.M. Api [a], D. Belsito [b], D. Botelho [a], M. Bruze [c], G.A. Burton Jr. [d], J. Buschmann [e], M.A. Cancellieri [a], M.L. Dagli [f], M. Date [a], W. Dekant [g], C. Deodhar [a], A.D. Fryer [h], L. Jones [a], K. Joshi [a], M. Kumar [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], D.C. Liebler [i], H. Moustakas [a], M. Na [a], T.M. Penning [j], G. Ritacco [a], J. Romine [a], N. Sadekar [a], T.W. Schultz [k], D. Selechnik [a], F. Siddiqi [a], I.G. Sipes [l], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [m]

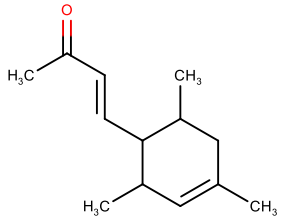
[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel for Fragrance Safety, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel for Fragrance Safety, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member Expert Panel for Fragrance Safety, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member Expert Panel for Fragrance Safety, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member Expert Panel for Fragrance Safety, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member Expert Panel for Fragrance Safety, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member Expert Panel for Fragrance Safety, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of Expert Panel for Fragrance Safety, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member Expert Panel for Fragrance Safety, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN 37996- 4500, USA*
[l] *Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member Expert Panel for Fragrance Safety, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman, Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 111021. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrance materialsafetyresource.elsevier.com.

Name: 4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one CAS Registry Number: 67801-38-1



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* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

https://doi.org/10.1016/j.fct.2022.113041
Received 16 November 2021; Accepted 14 April 2022
Available online 20 April 2022

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Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
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., 2017; Safford et al., 2015a, 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed 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
NOEL - No Observed Effect Level
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
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
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 as 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 includes 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 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., 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 endpoint 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 with guidance relevant to human health and environmental protection.

Summary: The existing information supports the use of this material as described in this safety assessment.

4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog methyl ionone (mixture of isomers) (CAS # 1335-46-2) show that 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is not expected to be genotoxic. Data on analog β-ionone (CAS # 14901-07-6) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and local respiratory toxicity endpoints. Data on analog (E)-β-ionone (CAS # 79-77-6) provide a calculated MOE >100 for the reproductive toxicity endpoint. Data from analog β-irone (CAS # 79-70-9) provided a No Expected Sensitization Induction Level (NESIL) of 1700 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic.	(RIFM, 1999; RIFM, 2000a; RIFM, 2000b)
Repeated Dose Toxicity: NOAEL = 10 mg/kg/day.	RIFM, (1983)
Reproductive Toxicity: Developmental toxicity: NOAEL = 50 mg/kg/day. Fertility: NOAEL = 719.6 mg/kg/day.	(RIFM, 2014b; RIFM, 2004d)
Skin Sensitization: NESIL = 1700 μg/cm^2.	RIFM, (2015b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: NOAEC = 7.9 mg/m^3.	RIFM, (2013a)

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Screening-level: 2.75 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

(*continued on next page*)

(*continued*)

Bioaccumulation:	
Screening-level: 299.5 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 2.804 mg/L	(RIFM Framework; 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	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 2.804 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.002804 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** 4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one
2. **CAS Registry Number:** 67801-38-1
3. **Synonyms:** 3-Buten-2-one, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-; Iritone; 4-(2,4,6-Trimethylcyclohex-3-en-1-yl)but-3-en-2-one; 4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one
4. **Molecular Formula:** $C_{13}H_{20}O$
5. **Molecular Weight:** 192.3
6. **RIFM Number:** 1037
7. **Stereochemistry:** Isomer not specified. One geometric center and a total of 2 stereoisomers possible.

2. Physical data

1. **Boiling Point:** 263.5 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System)
3. **Log K_{OW}:** 4.26 (EPI Suite)
4. **Melting Point**: 30.17 °C (EPI Suite)
5. **Water Solubility:** 11.06 mg/L (EPI Suite)
6. **Specific Gravity:** 0.921 (RIFM)
7. **Vapor Pressure:** 0.0116 mm Hg at 20 °C (EPI Suite v4.0), 0.006 mm Hg 20 °C (Fragrance Materials Association), 0.02 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient (68 L mol^{-1} • cm^{-1}, condition not specified) is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** A pale straw-colored, slightly viscous liquid

3. Volume of use (worldwide band)

1. <1 metric ton per year (IFRA, 2015)

4. EXPOSURE TO FRAGRANCE INGREDIENT (CREME RIFM AGGREGATE EXPOSURE MODEL v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.31% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00048 mg/kg/day or 0.030 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0044 mg/kg/day (RIFM, 2019)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. 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 (RIFM, 2015a; Safford, 2015; Safford, 2017; Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. *Cramer Classification*

Class I, Low

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
I	I	I

6.2.

Analogs Selected

a. **Genotoxicity:** Methyl ionone (mixture of isomers) (CAS # 1335-46-2)
b. **Repeated Dose Toxicity:** β-ionone (CAS # 14901-07-6)
c. **Reproductive Toxicity:** (E)-β-Ionone (CAS # 79-77-6)
d. **Skin Sensitization:** β-Irone (CAS # 79-70-9)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** β-Ionone (CAS # 14901-07-6)
g. **Environmental Toxicity:** None

3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References:
None.

8. Natural occurrence

4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is not reported to occur in foods 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 containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 11/10/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.038
2	Products applied to the axillae	0.039
3	Products applied to the face/body using fingertips	0.11
4	Products related to fine fragrances	0.73
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.18
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.038
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.038
5D	Baby cream, oil, talc	0.013
6	Products with oral and lip exposure	0.038
7	Products applied to the hair with some hand contact	0.038
8	Products with significant ano-genital exposure (tampon)	0.013
9	Products with body and hand exposure, primarily rinse-off (bar soap)	1.4
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.038
10B	Aerosol air freshener	0.038
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.013
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	24

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, the basis was the reference dose of 0.10 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 1700 μg/cm^2.
[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.4.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) and negative for genotoxicity, with and without metabolic activation (RIFM, 2013b). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no data assessing the mutagenicity of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Read-across material methyl ionone (mixture of isomers) (CAS # 1335-46-2; see Section VI) 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with methyl ionone (mixture of isomers) in dimethyl sulfoxide (DMSO) 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 (RIFM, 1999). Under the conditions of the study, methyl ionone (mixture of isomers) was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one; however, read-across can be made to methyl ionone (mixture of isomers; CAS # 1335-46-2; see Section VI). The clastogenic activity of methyl ionone (mixture of isomers) was evaluated using Chinese hamster ovary (CHO) cells in the presence and absence of S9 metabolic activation at doses up to 50 μg/mL. Cells were exposed for 4 and 20 h without S9 and for 4 h with the S9-activation system. No biologically significant structural chromosome aberrations were observed in the 4-h treatments in the absence or presence of S9 activation. However, statistically significant increases in structural chromosome aberrations were observed at concentrations of 12.5 and 25 μg/mL in the 20-h treatment without S9. This result was not dose-responsive (RIFM, 2000a). Based on these results, methyl ionone (mixture of isomers) was concluded to be positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations *in vitro*. These *in vitro* effects do not translate *in vivo* as demonstrated in a mouse micronucleus test where groups of male and female ICR mice were administered up to 1850 mg/kg of methyl ionone (mixture of isomers) in corn oil via intraperitoneal injection. Methyl ionone (mixture of isomers) did not induce a significant increase in micronucleated polychromatic erythrocytes in female mice at any of the tested doses; the only statistically significant response observed was at 925 mg/kg in male animals. However, these increases were well with in historical control range, and no dose response was observed. Therefore, these increases were not considered to be biologically relevant (RIFM, 2000b). Furthermore, the Expert Panel for Fragrance Safety previously reviewed the ionone materials and concluded that the ionones do not possess significant *in vivo* mutagenic or genotoxic potential under the intended conditions of use as fragrance ingredients (Belsito et al., 2007).

Taken together, this information indicates that 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one does not present a concern for genetic toxicity.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/10/21.

11.1.2. Repeated dose toxicity

The MOE for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Read-across material β-ionone (CAS # 14901-07-6; see Section VI) has sufficient repeated dose toxicity data. A 90-day dietary GLP study was conducted in Sprague Dawley rats. Groups of 15 rats/sex/group were fed diets

containing test material, β-ionone at concentrations of 10 or 100 mg/kg/day for 90 days, while the control group consisted of 30 rats/sex. The NOAEL was considered to be 10 mg/kg/day, based on reduced weight gain, food consumption, serum glucose concentration, increased water intake, and mild renal changes (RIFM, 1983). In another study, isomer α-ionone (CAS # 127-41-3; see Section VI) was administered to groups of 15 Sprague Dawley rats/sex/group via diet at daily intake values of 10 or 100 mg/kg/day. The NOAEL was considered to be 10 mg/kg/day, based on reduced weight gain, food consumption, serum glucose concentration, and mild renal changes (RIFM, 1983). Another isomer, (E)-β-ionone (CAS # 79-77-6; see Section VI), has an OECD/GLP 408 dietary 90-day subchronic toxicity study conducted in rats. Groups of 10 rats/sex/dose were fed diets containing 0, 100, 1000, or 10000 ppm (equivalent to 0, 7.1, 71.8, and 719.6 mg/kg/day for males and 0, 8.2, 83.0, and 801.0 mg/kg/day for females) of test material, (E)-β-ionone, for 3 months. Test material-related alterations in clinical chemistry and urinalysis, along with increases in the liver and kidney weights with associated histopathological changes, were observed in the high-dose animals. Decreased thyroxine was observed in males, as well as higher degrees of severity of altered colloid in the thyroid gland of males and a high incidence with higher gradings of altered colloid in females of the high-dose group. The mid-dose animals were reported to have increased liver weights among both sexes, with associated histopathological alterations in the liver, which were considered to be adaptive and not an adverse effect. There was also an increase in urinary casts and urinary transitional epithelial cells and higher amounts of α-2u-globulin in tubular epithelial cells of the kidneys in mid-dose males. The kidney and urinary findings in males were considered to be related to α-2u-globulin nephropathy, which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman & Caudill, 1992; Lehman-McKeeman et al., 1990). Thus, the NOAEL was considered to be 1000 ppm or 71.8 and 83.0 mg/kg/day for males and females, respectively (RIFM, 2004d). The most conservative NOAEL of 10 mg/kg/day was considered for the repeated dose toxicity endpoint.

The 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one MOE for the repeated dose toxicity endpoint can be calculated by dividing the β-ionone NOAEL in mg/kg/day by the total systemic exposure to 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, 10/0.0044 or 2273.

In addition, the total systemic exposure to 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (4.4 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020b) and a reference dose (RfD) of 0.10 mg/kg/day.

11.1.2.1.1. Derivation of RfD. The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The RfD for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 10 mg/kg/day by the uncertainty factor, 100 = 0.10 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/16/21.

11.1.3. Reproductive toxicity

The MOE for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no developmental toxicity data on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Read-across material (E)-β-ionone (CAS # 79-77-6; see Section VI) has sufficient developmental toxicity data. An OECD/GLP 414 gavage developmental toxicity study was conducted in Wistar rats. Groups of 25 pregnant female rats/dose were administered via gavage daily the test material, (E)-β-ionone, at doses of 0, 25, 100, or 400 mg/kg/day in an olive oil vehicle on days 6–19 post-coitum. High-dose females exhibited significantly reduced food consumption on days 6–8 post-coitum, significantly reduced bodyweight gain on days 8–10 post-coitum (29% below control), and increased liver weights (29% above control). Thereafter, food uptake and weight gains of these animals reached or even exceeded control values on the days following exposure. The corrected bodyweight gain of the high-dose dams was 17% below the controls, without attaining statistical significance. The increased liver weights, which extended to mid-dose females (9% above the controls), were not considered to be adverse but as adaptive effects of metabolism. There were no effects on gestational parameters and no adverse signs of developmental toxicity up to 400 mg/kg/day, the highest dose tested. The most conservative NOAEL for maternal toxicity was considered to be 100 mg/kg/day, based on a decrease in bodyweight gain in high-dose dams. The NOAEL for developmental toxicity was considered to be 400 mg/kg/day, the highest dose tested (RIFM, 2004c).

In another study, an OECD/GLP 414 dietary developmental toxicity study was conducted in New Zealand White rabbits. Groups of 22 mated female rabbits/dose were fed diets formulated to provide a target dose of 0, 50, 200, or 1000 mg/kg/day of the test material (E)-β-ionone daily on days 6–29 post-coitum. Rabbits fed 1000 mg/kg/day showed severely reduced food intake after the introduction of the test diet, and as no recovery occurred up to day 10 post-coitum, these rabbits were removed from the study without further examination. An additional group of 22 mated females that was fed a diet at a target dose of 17 mg/kg/day was added to the study. The average compound intake was 0, 16, 50, or 160 mg/kg/day over the treatment period. At 160 mg/kg/day, reduced food consumption, reduced body weights, lower bodyweight gain, and/or bodyweight loss were observed. Fetal body weights were slightly lower at 160 mg/kg/day, which reached statistical significance for male pups only; this was considered to be secondary to the reduced food intake and markedly decreased bodyweight gain of the dams. The incidence of unossified metacarpals and/or metatarsals and unossified sternebrae were slightly higher in the fetuses at 160 mg/kg/day, which were at or just outside of the historical control values. This finding was considered to be related to the slightly lower fetal weights observed at this dose. There were no toxicologically relevant teratogenic effects on viability, litter size, sex ratio, or fetal morphological findings up the highest dose tested. Thus the NOAEL for maternal and developmental toxicity was considered to be 50 mg/kg/day, based on reduced fetal body weight and increased incidences of 2 ossification parameters at 160 mg/kg/day (RIFM, 2014b). The most conservative NOAEL of 50 mg/kg/day was considered for the developmental toxicity endpoint. **The 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one MOE for the developmental toxicity endpoint can be calculated by dividing the (E)-β-ionone NOAEL in mg/kg/day by the total systemic exposure to 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, 50/0.0044 or 11364.**

There are no fertility data on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Read-across material (E)-β-ionone (CAS # 79-77-6; see Section VI) has an OECD/GLP 408 dietary 90-day subchronic toxicity study. Groups of 10 rats/sex/dose were fed diets containing 0, 100, 1000, or 10000 ppm (equivalent to 0, 7.1, 71.8 and 719.6 mg/kg/day for males and 0, 8.2, 83.0, and 801.0 mg/kg/day for females) of test material, (E)-β-ionone. In addition to the systemic toxicity parameters, the thyroid hormones, estrous cycling, sperm parameters, reproductive organ weights, and histopathology (pituitary gland, adrenal glands, thyroid glands, parathyroid glands, oviducts/uterus/vagina, prostate gland, seminal vesicles, female mammary gland, testis, epididymis) were also evaluated. There were no toxicologically significant effects observed on the reproductive parameters up to the highest dose of 10000 ppm (719.6

Table 1
Data Summary for β-irone as read-across for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one.

LLNA Weighted Mean EC3 Value μg/cm^2 [No. Studies]	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2
900 [1]	Moderate	1772	NA	NA	1700

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

[a] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

and 801.0 mg/kg/day for males and females, respectively). Thus, the NOAEL for fertility was considered to be 10000 ppm or 719.6 mg/kg/day, the highest dose tested (RIFM, 2004d). **Therefore, the 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one MOE for the fertility endpoint can be calculated by dividing the (E)-β-ionone NOAEL in mg/kg/day by the total systemic exposure to 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, 719.6/0.0044 or 163545.**

In addition, the total systemic exposure to 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (4.4 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental and fertility endpoints of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/08/21.

11.1.4. Skin sensitization

Based on the existing data and read-across analog β-irone (CAS # 79-70-9) 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is considered a skin sensitizer with a defined NESIL of 1700 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Based on the existing data and read-across to β-irone (CAS # 79-70-9; see Section VI), 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is considered a skin sensitizer. The chemical structure of these materials indicate that they would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), β-irone was found to be sensitizing with an EC3 value of 3 6% or 900 μg/cm^2 (RIFM, 2013c). In a Confirmation of No Induction in Humans test (CNIH) (limited details were given) and a human maximization test, no reactions indicative of sensitization were reported with 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (RIFM, 1978; RIFM, 1964). In a CNIH with 1772 μg/cm^2 of read-across material β-irone in 1:3 ethanol:diethyl phthalate, no reactions indicative of sensitization were observed in any of the 96 volunteers (RIFM, 2015b).

Based on the weight of evidence (WoE) from structural analysis, human studies, and data on read-across analog β-irone, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is a moderate sensitizer with a WoE NESIL of 1700 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020b) and a reference dose of 0.10 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/28/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis absorbance spectra, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one does not present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of significant absorbance in the critical range, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficient (68 L mol^{-1} • cm^{-1}) is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/01/21.

11.1.6. Local respiratory toxicity

There are no inhalation data available on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one; however, in an acute, 2-week inhalation study for the analog β-ionone (CAS # 14901-07-6; see Section VI), a NOAEC of 7.9 mg/m^3 was reported (RIFM, 2013a).

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In an acute, 2-week inhalation study conducted in rats, a NOAEC of 1 ppm (7.9 mg/m^3) was reported for β-ionone (RIFM, 2013a). Test material-related microscopic findings were noted in nasal levels II, III, IV, V, and VI and included olfactory epithelial degeneration, olfactory nerve bundle degeneration (males only), inflammatory exudate or cell debris, respiratory epithelial hyperplasia, transitional epithelial hyperplasia, and subacute inflammation at the middle and highest concentrations (79 mg/m^3 and 790 mg/m^3). The NOAEC was determined to be 7.9 mg/m^3 (1 ppm), the lowest dose given.

This NOAEC expressed in mg/kg lung weight/day is:

- (7.9 mg/m^3) × (1 m^3/1000 L) = 0.0079 mg/L
- Minute ventilation of 0.17 L/min for a Sprague Dawley rat × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 61.2 L/day
- (0.0079 mg/L) × (61.2 L/day) = 0.48 mg/day
- (0.48 mg/day)/(0.0016 kg lung weight of rat*) = 300 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.030 mg/day—this value was derived from the concentration survey data in the Creme RIFM exposure model (RIFM, 2015a; Safford, 2015). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 0.046 mg/kg lung weight/day resulting in a MOE of 6521.7 (i.e., [300 mg/kg lung weight/day]/[0.046 mg/kg lung weight/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to inter-species and intra-species variation, the material exposure by inhalation at 0.030 mg/day is deemed to be safe

under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: "Comparative Physiology and Anatomy," subsection, "Comparative Airway Anatomy."

Additional References: UGCM (1997); Pinching and Doving, 1974; Buchbauer et al., 1993; RIFM, 2003b; RIFM, 2003c; Rogers et al., 2003a; RIFM, 2003d; RIFM, 2003a; RIFM, 2004a; RIFM, 2004b; Isola et al., 2004a; Rogers et al., 2005; Vethanayagam et al., 2013; RIFM, 2014a.

Literature Search and Risk Assessment Completed On: 03/12/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one 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 EPI Suite v4.11 (US EPA, 2012a) did not identify 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one presents no risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. No data available.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. 4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one has been pre-registered for REACH with no additional data at this time.

11.2.3. 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	4.26	4.26
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 additional assessment is necessary.

The RIFM PNEC is 0.002804 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 03/08/21.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	2.804			1000000	0.002804	

- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 11/10/21.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113041.

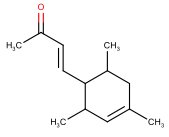
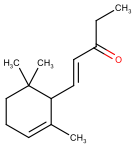
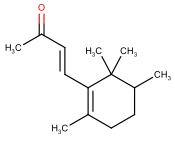
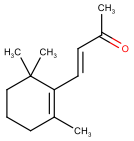
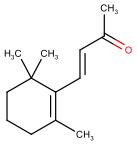
Appendix

Read-across justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (Schultz et al., 2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material	Read-across Material	Read-across Material
Principal Name	4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Methyl ionone (mixture of isomers)	β-Irone	β-Ionone	(E)-β-Ionone
CAS No.	67801-38-1	1335-46-2	79-70-9	14901-07-6	79-77-6
Structure					
Similarity (Tanimoto Score)		0.77	0.68	0.67	0.67

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	Target Material	Read-across Material	Read-across Material	Read-across Material	Read-across Material
Endpoint		• Genotoxicity	• Skin sensitization	• Repeated dose toxicity • Local respiratory toxicity	• Reproductive toxicity
Molecular Formula	$C_{13}H_{20}O$	$C_{14}H_{22}O$	$C_{14}H_{22}O$	$C_{13}H_{20}O$	$C_{13}H_{20}O$
Molecular Weight	192.302	206.329	206.329	192.302	192.302
Melting Point (°C, EPI Suite)	30.17	53.53	59.38	52.45	52.45
Boiling Point (°C, EPI Suite)	263.50	276.02	274.64	262.93	262.93
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.67E+00	8.68E-01	1.65E+00	7.20E+00	7.20E+00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.11E+01	3.33E+00	2.98E+00	1.69E+02	1.69E+02
Log KOW	4.26	4.7[1]	4.84	3.84	3.84
J_{max} (μg/cm^2/h, SAM)	1.42	0.48	0.44	16.14	16.14
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.83E+01	2.43E+01	2.34E+01	8.20E+00	8.20E+00
Genotoxicity					
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	No alert found			
DNA Binding (OECD QSAR Toolbox v4.2)	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β-unsaturated ketones	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β-unsaturated ketones			
Carcinogenicity (ISS)	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity			
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found			
***In Vitro* Mutagenicity (Ames, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls			
***In Vivo* Mutagenicity (Micronucleus, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls			
Oncologic Classification	Reactive Ketone Reactive Functional Groups	Reactive Ketone Reactive Functional Groups			
Repeated Dose Toxicity					
Repeated Dose (HESS)	Not categorized			Vitamin A (Hepatotoxicity) Alert	
Reproductive Toxicity					
ER Binding (OECD QSAR Toolbox v4.2)	Non-binder, without OH or NH2 group				Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (low reliability)				Toxicant (low reliability)
Skin Sensitization					
Protein Binding (OASIS v1.1)	Michael addition|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ α,β-Carbonyl compounds with polarized double bonds		Michael addition|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ α,β-Carbonyl compounds with polarized double bonds		
Protein Binding (OECD)	Michael addition|Michael addition ≫ Polarized Alkenes|Michael addition ≫ Polarized Alkenes ≫ Polarized alkene - ketones		Michael addition|Michael addition ≫ Polarized Alkenes|Michael addition ≫ Polarized Alkenes ≫ Polarized alkene - ketones		
Protein Binding Potency	Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Substituted 1-Alken-3-ones (MA)		Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Alkenes and cycloalkenes (AN)|Moderately reactive (GSH) ≫ Substituted 1-Alken-3-ones (MA)		
Protein Binding Alerts for Skin	Michael Addition|Michael Addition ≫ Michael addition on		Michael Addition|Michael Addition ≫ Michael addition on		

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(*continued*)

	Target Material	Read-across Material	Read-across Material	Read-across Material	Read-across Material
Sensitization (OASIS v1.1)	conjugated systems with electron-withdrawing group|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ α,β-Carbonyl compounds with polarized double bonds		conjugated systems with electron-withdrawing group|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ α,β-Carbonyl compounds with polarized double bonds		
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Michael Acceptor identified		Alert for Michael Acceptor identified		
Metabolism					
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3	See Supplemental Data 4	See Supplemental Data 5

[1] RIFM, 2006.

Summary

There are insufficient toxicity data on the 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (CAS # 67801-38-1). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, methyl ionone (mixture of isomers) (CAS # 1335-46-2), (E)-β-ionone (CAS # 79-77-6), β-ionone (CAS # 14901-07-6), and β-irone (CAS # 79-70-9) were identified as read-across materials with data for their respective toxicity endpoints.

Conclusions

- Methyl ionone (mixture of isomers) (CAS # 1335-46-2) was used as a read-across analog for the target material 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (CAS # 67801-38-1) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of cyclic aliphatic ketones.
  - o The target material and the read-across analog share a cyclohexene ring with methyl substitutions and aliphatic ketone fragment.
  - o The key difference between the target material and the read-across analog is that the read-across analog and the target differ in methyl substitutions on cyclohexene ring. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have an equal or greater potential for toxicity as compared to the target.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o They were predicted for J_{max}, which estimates skin absorption. The J_{max} values translate to ≤80% skin absorption for the target material ≤40% absorption for the read-across analog. While percentage skin absorption estimated from J_{max} values indicate exposure to the substance, they do not represent hazard or toxicity parameters. Therefore, the J_{max} of the target material and the appropriate read-across analog material are not used directly in comparing substance hazard or toxicity. However, these parameters provide context to assess the impact of bioavailability on toxicity comparisons between the individual materials.
  - o According to the QSAR OECD Toolbox (v4.2), structural alerts for genotoxicity endpoint are consistent between the target material and the read-across analog.
  - o The target material and the read-across analog have carcinogenicity alert by the ISS model. Both substances also have an *in vivo* and *in vitro* mutagenicity alert and a DNA binding alert by OECD. Furthermore, the target material and read-across analog are also classified as containing reactive ketone functional groups. This shows that the read-across analog is predicted to have comparable reactivity with the target material. The data described in the genotoxicity section shows that the read-across analog does not pose a concern for genetic toxicity. Therefore, the alert will be superseded by the availability of the data.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the genotoxicity endpoint are consistent between the metabolites of the read-across analog and the target material.
  - o The structural differences between the target material and the read-across analog do not affect consideration of the genotoxicity endpoint.
- (E)-β-Ionone (CAS # 79-77-6) was used as a read-across analog for the target material 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (CAS # 67801-38-1) for the reproductive toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of cyclic aliphatic ketones.
  - o The target material and the read-across analog share a cyclohexene ring with methyl substitutions and aliphatic ketone fragment.
  - o The key difference between the target material and the read-across analog is that the read-across analog and the target differ in methyl substitutions on cyclohexene ring. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target material.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.

- According to the QSAR OECD Toolbox (v4.2), structural alerts for the reproductive toxicity endpoint are consistent between the target material and the read-across analog.
- The read-across analog and the target material are predicted to be toxicants by the CAESAR model for developmental toxicity. The data described in the reproductive toxicity section above shows that the read-across analog has an adequate MOE at the current level of use. Therefore, the alert will be superseded by the availability of the data. The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for reproductive toxicity endpoint are consistent between the metabolites of the read-across analog and the target material.
- The structural differences between the target material and the read-across analog do not affect consideration of the reproductive toxicity endpoint.

- β-Ionone (CAS # 14901-07-6) was used as a read-across analog for the target material 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (CAS # 67801-38-1) for the repeated dose toxicity and local respiratory toxicity endpoints.
  - The target material and the read-across analog are structurally similar and belong to the structural class of cyclic aliphatic ketones.
  - The target material and the read-across analog share a cyclohexene ring with methyl substitutions and aliphatic ketone fragment.
  - The key difference between the target material and the read-across analog is that the read-across analog and the target differ in methyl substitutions on cyclohexene ring. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target material.
  - The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the QSAR OECD Toolbox (v4.2), structural alerts for the respiratory endpoint are consistent between the target material and the read-across analog.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the respiratory endpoint are consistent between the metabolites of the read-across analog and the target material.
  - The structural differences between the target material and the read-across analog do not affect consideration of the respiratory endpoint.
- B-Irone (CAS # 79-70-9) was used as a read-across analog for the target material 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (CAS # 67801-38-1) for the skin sensitization endpoint.
  - The target material and the read-across analog are structurally similar and belong to the structural class of cyclic aliphatic ketones.
  - The target material and the read-across analog share a cyclohexene ring with methyl substitutions and aliphatic ketone fragment.
  - The key difference between the target material and the read-across analog is that the read-across analog and the target differ in methyl substitutions on cyclohexene ring. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target material.
  - The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.
  - The read-across analog and the target material have several protein-binding alerts by *in silico* models, including Michael addition. It is predicted to have protein binding potency. In addition, the target material and the read-across analog are predicted to be skin sensitizers according to the CAESAR model for skin sensitization. The data described in the skin sensitization endpoint section shows that the read-across substance does not pose a concern for the skin sensitization endpoint. Hence, the alerts will be superseded by the data.
  - Since the target material is a major direct metabolite of the read-across analog, the structural differences between the target material and the read-across analog do not affect consideration of the toxicity endpoints.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the skin sensitization endpoint are consistent between the metabolites of the read-across analog and the target material.
  - The structural differences between the target material and the read-across analog do not affect consideration of the skin sensitization endpoint.

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