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RIFM fragrance ingredient safety assessment, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate, CAS registry number 116044-44-1

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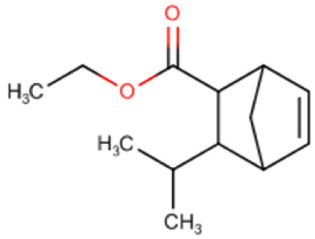
Name: (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate

CAS Registry Number: 116,044-44-1

Additional CAS*: 116,126-82-0

Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 3-(1-methylethyl)-, ethyl ester, (2-exo,3-endo)-*Included because the materials are isomers



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., 2015, 2017; Safford et al., 2015a, 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 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

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, 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,

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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.

(2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that (2-endo,3-exo)-ethyl 3-(1-methylethyl) bicyclo[2.2.1]hept-5-ene-2-carboxylate is not genotoxic. Data on (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate provide a calculated margin of exposure (MOE) > 100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the threshold of toxicological concern (TTC) for a Cramer Class II material, and the exposure to (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is below the TTC (0.009 mg/kg/day and 0.47 mg/day, respectively). Data from read-across analog methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo [2.2.2]oct-5-ene-2-carboxylate (CAS # 68,966-86-9) provided (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate a No Expected Sensitization Induction Level (NESIL) of 2200 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/violet (UV/Vis) spectra; (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo [2.2.1]hept-5-ene-2-carboxylate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate 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 genotoxic. (RIFM, 2007; RIFM, 2020b; RIFM, 1995c)

Repeated Dose Toxicity: NOAEL = 50 mg/kg/day. RIFM (1996a)

Reproductive Toxicity: No NOAEL available. Exposure is below TTC.

Skin Sensitization: NESIL = 2200 μg/cm^2. RIFM (2013b)

Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV/Vis Spectra; RIFM Database)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Critical Measured Value: 11% after 5 days (OECD 302C) RIFM (2008a)

Bioaccumulation:

Screening-level: 248.4 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:

Screening-level: 96-h Algae EC_{50}: 0.792 mg/L (ECOSAR; US EPA, 2012b)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito, 2002)

Critical Ecotoxicity Endpoint: 96-h Algae EC50: 0.792 mg/L (ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.0792 μg/L

- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

Chemical Name: (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate	Chemical Name: Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 3-(1-methylethyl)-, ethyl ester, (2-exo,3-endo)-
CAS Registry Number: 116,044-44-1	**CAS Registry Number:** 116,126-82-0
Synonyms: Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 3-(1-methylethyl)-, ethyl ester, (2-endo,3-exo)-; (2-endo,3-	**Synonyms:** 3-(1-Methyl ethyl) bicyclo (2.2.1) hept-5-ene-2-carboxylic acid ethyl ester; Herbanate; Herbanate

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exo)-Ethyl 3-isopropylbicyclo[2.2.1]hept-5-ene-2-carboxylate; Herbanate; (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate	38,330; Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 3-(1-methylethyl)-, ethyl ester, (2-exo,3-endo)-
Molecular Formula: $C_{13}H_{20}O_2$	**Molecular Formula:** $C_{13}H_{20}O_2$
Molecular Weight: 208.3	**Molecular Weight:** 208.3
RIFM Number: 6330	**RIFM Number:** 6923
Stereochemistry: 2-endo,3-exo isomer specified	**Stereochemistry:** 2-exo,3-endo isomer specified

2. Physical data

CAS # 116,044-44-1	CAS # 116,126-82-0
Boiling Point: 470 +/- 2K at 100.9–101.2 kPa (RIFM, 1995e), 254.37 °C (EPI Suite)	**Boiling Point:** 470 +/- 2K at 100.9–101.2 kPa (RIFM, 1995e), 254.37 °C (EPI Suite)
Flash Point: 102 ± 2 °C (RIFM, 1995f), 102 °C (Globally Harmonized System)	**Flash Point:** 102 ± 2 °C (RIFM, 1995f)
Log K_{OW}: Log10 Pow = 4.75 to 5.14 (RIFM, 1995e), log Pow = 3.9 and 4.2 (RIFM, 2009), 4.13 (EPI Suite)	**Log K_{OW}:** Log10 Pow = 4.75 to 5.14 (RIFM, 1995e), log Pow = 3.9 and 4.2 (RIFM, 2009), 4.13 (EPI Suite)
Melting Point: 24.97 °C (EPI Suite)	**Melting Point:** 24.97 °C (EPI Suite)
Water Solubility: 11.67 mg/L (EPI Suite)	**Water Solubility:** 1.64 × $10^{(-2)}$ g/L at 20.0 +/- 0.5 °C (RIFM, 1995e), 11.67 mg/L (EPI Suite)
Specific Gravity: Not Available	**Specific Gravity:** Not Available
Vapor Pressure: 2.4 Pa at 20 °C (RIFM, 2013a), 0.0215 mm Hg at 25 °C (EPI Suite), 0.0135 mm Hg at 20 °C (EPI Suite v4.0)	**Vapor Pressure:** 2.4 Pa at 20 °C (RIFM, 2013a), 0.0215 mm Hg at 25 °C (EPI Suite), 0.0135 mm Hg at 20 °C (EPI Suite v4.0)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
Appearance/Organoleptic: Not Available	**Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. 10–100 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)***

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.37% (RIFM, 2017)
2. **Inhalation Exposure*:** 0.00088 mg/kg/day or 0.058 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.0033 mg/kg/day (RIFM, 2017)

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

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in hydroalcoholics, inhalation exposure, and total exposure.

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class II, Intermediate (Expert Judgment)

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

*See the Appendix below for further details.

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** Methyl 4(or 1)-isopropyl-1(or 4)-methyl-bicyclo[2.2.2]oct-5-ene-2-carboxylate (CAS # 68,966-86-9)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   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

(2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate and bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 3-(1-methylethyl)-, ethyl ester, (2-exo,3-endo)- are 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

Dossier available for mixture of ethyl (2R,3R)-3-isopropylbicyclo[2.2.1]hept-5-ene-2-carboxylate; accessed on 05/06/21; no dossier available for ethyl (2S,3S)-3-isopropylbicyclo[2.2.1]hept-5-ene-2-carboxylate.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate 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)	0.15
2	Products applied to the axillae	0.050
3	Products applied to the face/body using fingertips	0.45
4	Products related to fine fragrances	0.94

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.24
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.24
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.24
5D	Baby cream, oil, talc	0.080
6	Products with oral and lip exposure	0.15
7	Products applied to the hair with some hand contact	0.61
8	Products with significant ano-genital exposure (tampon)	0.080
9	Products with body and hand exposure, primarily rinse-off (bar soap)	1.8
10A	Household care products with mostly hand contact (hand dishwashing detergent)	3.0
10B	Aerosol air freshener	0.15
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.080
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

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 (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate, the basis was the reference dose of 0.50 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 2200 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 v3.1.1.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate 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 (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. Weak increases (2- to 3-fold greater than the background) in the mean number of revertant colonies were observed at concentrations ≥1000 μg/plate for strain TA100 in the presence and absence of S9; negative results were observed in all other strains in the presence and absence of S9 (RIFM, 2007). Under the conditions of the study, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate was mutagenic in the Ames test.

In order to verify the weak increases observed in the bacterial assay, a mammalian cell gene mutation assay (HPRT assay) was conducted according to OECD TG 476 and GLP guidelines. Chinese hamster ovary cells were treated with (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate in DMSO at concentrations up to 125 μg/mL (as determined in a preliminary toxicity assay), for 5 h. Effects were evaluated both with and without metabolic activation. No statistically significant increases in the frequency of mutant colonies were observed with any concentration of the test material, either with or without metabolic activation (RIFM, 2020b). Under the conditions of the study, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate was not mutagenic to mammalian cells *in vitro*.

The clastogenicity of (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes were treated with (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate in DMSO at concentrations up to 250 μg/mL in the presence and absence of metabolic activation. A statistically significant increase in the frequency of cells with structural chromosomal aberrations was observed without S9 metabolic activation, but the increase was inside the historical control range and not considered to be indicative of clastogenic activity (RIFM, 1995c). Under the conditions of the study, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/15/20.

11.1.2. Repeated dose toxicity

The MOE of (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate for the repeated dose toxicity endpoint is adequate at the current use level.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate. In a GLP-compliant, 28-day oral gavage study, groups of 5 Sprague Dawley rats/sex/group were administered (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate at doses of 0, 50, 150, and 1000 mg/kg/day. No treatment-related mortality was observed in any dose group. In addition, no changes were reported for body weight and food consumption, and only transient clinical signs were observed in the high-dose group. A treatment-related decrease in red blood cell counts was reported in animals of both sexes receiving the highest dose. In addition, hemoglobin concentration and hematocrit were decreased in females of the high-dose group. In males, a dose-dependent increase in intra-epithelial eosinophilic droplets in the kidney proximal tubule was reported. However, this effect was attributed to α-2u-globulin nephropathy (confirmed by immunohistochemistry), which is considered a male rat-specific effect and is not relevant to human health. The underlying cause of the biochemical changes observed in high-dose females could not be determined. Liver hypertrophy was observed in the high-dose group of both sexes and was characterized by increased liver weights (absolute and relative), enlarged hepatocytes (females), inflammatory cell infiltration of the portal triads (males), degeneration of the bile duct epithelium (males),

bile duct hypertrophy, and multinucleated giant cells containing pigment in the portal regions (males). Along with liver hypertrophy, the presence of porphyrin in the intrahepatic bile ducts was also reported in both sexes. Since the changes related to liver hypertrophy were observed only in the high-dose group and were less than 2-fold in magnitude, this effect was considered to be an adaptive response to high-dose treatment (Hall, 2012). Based on the hematological changes, bile duct degeneration, and porphyrin deposition in the liver (both sexes), combined with female electrolyte changes at 1000 mg/kg/day, the NOAEL for repeated dose toxicity endpoint was considered to be 150 mg/kg/day (RIFM, 1996a).

A default safety factor of 3 was used when deriving a NOAEL from the 28-day studies (ECHA, 2012b). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 150/3 or 50 mg/kg/day.

Therefore, the MOE for the repeated dose toxicity endpoint is equal to the (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate NOAEL in mg/kg/day divided by the total systemic exposure to (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate, 50/0.0033, or 15,152.

11.1.3. Derivation of reference dose (RfD)

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, 2020a) and a reference dose of 0.50 mg/kg/day.

The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The reference dose for (2-endo, 3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 50 mg/kg/day by the uncertainty factor, 100 = 0.50 mg/kg/day.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/19/20.

11.1.4. Reproductive toxicity

There are insufficient reproductive toxicity data on (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate or any read-across materials. The total systemic exposure to (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

11.1.4.1. Risk assessment. There are no reproductive toxicity data on (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure (3.3 μg/kg/day) to (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material (9 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/30/20.

11.1.5. Skin sensitization

Based on the existing data and read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate (CAS # 68,966-86-9), (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is considered a skin sensitizer with a defined NESIL of 2200 μg/cm^2.

11.1.5.1. Risk assessment. Limited skin sensitization studies are available for (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate. Based on the existing data and data from read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate (CAS # 68,966-86-9; see Section VI), (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is considered a skin sensitizer. The chemical structures of these materials indicate that they would not be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a guinea pig maximization test, (2-endo,3-exo)-ethyl 3-(1-methylethyl) bicyclo[2.2.1]hept-5-ene-2-carboxylate presented reactions indicative of sensitization at 100% (RIFM, 1987). In 2 guinea pig maximization tests, read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate led to skin sensitization reactions when 100% of the material was used for topical induction (RIFM, 1982a; RIFM, 1982b). In another guinea pig maximization test, read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate did not lead to skin sensitization reactions when 30% of the material was used for topical induction (ECHA, 2017a; RIFM, 1994). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 4% (2204 μg/cm^2) of read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate in 1:3 ethyl alcohol:diethyl phthalate, no reactions indicative of sensitization were observed in any of the 107 volunteers (RIFM, 2013b).

Based on weight of evidence (WoE) from structural analysis, animal studies, and data on read-across material methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is a sensitizer with a WoE NESIL of 2200 μg/cm^2 (see 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, 2020a) and a reference dose of 0.50 mg/kg/day.

Additional References: RIFM, 1991; RIFM, 1988a; RIFM, 1988b; ECHA, 2017b; ECHA, 2012b.

Literature Search and Risk Assessment Completed On: 03/11/20.

11.1.6. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate would not be expected to present a concern for phototoxicity or photoallergenicity.

Table 1
Data summary for methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate as read-across material for (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate.

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
NA	Weak	2204	NA	NA	2200

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.

11.1.6.1. Risk assessment. There are no phototoxicity studies available for (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate does not present a concern for phototoxicity or photoallergenicity.

11.1.6.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/20/20.

11.1.7. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate is below the Cramer Class III* TTC value for inhalation exposure local effects.

11.1.7.1. Risk assessment. There are no inhalation data available on (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate. Based on the Creme RIFM Model, the inhalation exposure is 0.058 mg/day. This exposure is 8.1 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

*As per Carthew et al. (2009), Cramer Class II materials default to Cramer Class III for the local respiratory toxicity endpoint.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/02/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate was performed following the RIFM Environmental Framework (Salvito, 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, (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate was identified as a fragrance material with the 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) identified (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate as possibly persistent but not 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012b). 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). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. RIFM, 2008b: The inherent biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 302C guidelines. Biodegradation of 4% was observed after 28 days and 11% after 50 days.

RIFM, 1996b: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guidelines. No biodegradation was observed after 28 days.

RIFM, 2008a: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guidelines. Biodegradation of 0% was observed after 28 days and 1% after 50 days.

11.2.2.2. Ecotoxicity. RIFM, 1998: The acute fish (Rainbow trout) toxicity test was conducted according to the OECD 203 guidelines under dynamic test conditions. The 96-h LC_{50} value based on nominal concentrations was reported to be 7.5 mg/L (95% CI: 5.6–10 mg/L).

RIFM, 1995a: The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under static renewal conditions. The 48-h EC_{50} value based on the mean measured concentration was reported to be 5.7 mg/L (95% CI: 5.1–7.1 mg/L).

RIFM, 1995b: The acute fish (zebrafish) toxicity test was conducted according to the OECD 203 guidelines under continuous flow conditions. The 96-h LC_{50} value based on mean measured concentrations was reported to be 4.49 mg/L (95% CI: 3.20–6.31 mg/L).

RIFM, 1995d: The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC_{50} value based on mean measured concentrations for biomass and growth rate was reported to be > 5.9 mg/L.

	LC_{50} (Fish) (mg/L)	EC_{50} (*Daphnia*) (mg/L)	EC_{50} (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	0.52			1000000	0.00052	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	1.572	2.610	0.792	10000	0.0792	Esters
ECOSAR Acute Endpoints **(Tier 2)** v1.11	2.071	1.432	2.410			Neutral Organic SAR (Baseline toxicity)

11.2.2.3. Other available data. (2-endo,3-exo)-Ethyl 3-(1-methylethyl) bicyclo[2.2.1]hept-5-ene-2-carboxylate has been registered for REACH with no additional information available at this time.

11.2.2.4. Risk assessment refinement. Since (2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

Risk 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, 2002)

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	5.14	5.14
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	1–10	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined regional volume for both CAS #s.

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

The RIFM PNEC is 0.0792 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 04/02/20.

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
- **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 05/06/21.

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.2021.112624.

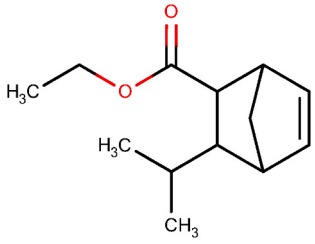
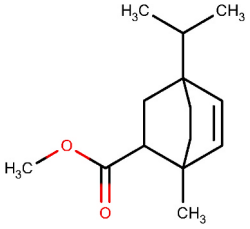
Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also 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, 2012a).

- 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 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
Principal Name	(2-endo,3-exo)-Ethyl 3-(1-methylethyl)bicyclo[2.2.1]hept-5-ene-2-carboxylate	Methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[2.2.2]oct-5-ene-2-carboxylate
CAS No.	116,044-44-1	68,966-86-9
Structure		
Similarity (Tanimoto Score)		0.87
Read-across Endpoint		• Skin sensitization
Molecular Formula	$C_{13}H_{20}O_2$	$C_{14}H_{22}O_2$
Molecular Weight	208.30000000	222.32000000
Melting Point (°C, EPI Suite)	24.97000	56.50000
Boiling Point (°C, EPI Suite)	254.37000	259.95000
Vapor Pressure (Pa @ 25 °C, EPI Suite)	2.86642	1.08524
Log K_{ow} (KOWWIN v1.68 in EPI Suite)	4.13000	4.62000
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	11.67000	3.76200
J_{max} (μg/cm^2/h, SAM)	1.13	0.43
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	51.67575	68.59703
Skin Sensitization		
Protein Binding (OASIS v1.1)	• No alert found	• No alert found
Protein binding (OECD)	• No alert found	• No alert found
Protein Binding Potency	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• No alert found	• No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No skin sensitization reactivity domains alerts identified.	• No skin sensitization reactivity domains alerts 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

Summary

There are insufficient toxicity data on (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate (CAS # 116,044-44-1). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[222]oct-5-ene-2-carboxylate (CAS # 68,966-86-9) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Methyl 4(or 1)-isopropyl-1(or 4)-methylbicyclo[222]oct-5-ene-2-carboxylate (CAS # 68,966-86-9) was used as a read-across analog for the target material (2-endo,3-exo)-ethyl 3-(1-methylethyl)bicyclo[221]hept-5-ene-2-carboxylate (CAS # 116,044-44-1) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of cyclic unsaturated aliphatic esters.
  - o The target material and the read-across analog share a cyclic bridged structure of the hydrocarbon skeleton.
  - o The key difference between the target material and the read-across analog is that the target material has the isopropyl group attached to the ring while the read-across analog has the isopropyl group at the bridge position. This structural difference is toxicologically insignificant.
  - 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 According to the OECD QSAR Toolbox v4.2, there are no *in silico* alerts for the target material or the read-across analog.
  - 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 endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

Explanation of Cramer Classification. Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

Q1. A normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and 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? (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? No
Q23. Aromatic? No
Q24. Monocarbocyclic with simple substituents? No
Q25. Cyclopropane (see explanation in Cramer et al., 1978)? No
Q26. Monocycloalkanone or a bicyclo compound? Yes Class Intermediate (class II)

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