Food and Chemical Toxicology 159 (2022) 112732

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

# RIFM fragrance ingredient safety assessment, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde, CAS Registry Number 68259-31-4

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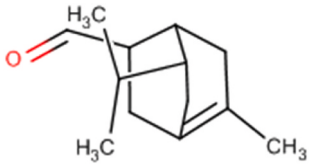
ARTICLE INFO

Handling editor: Dr. Jose Luis Domingo

Version: 100,121. 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: fragrancematerialsafetyresource.elsevier.com.



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**Name**: 5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde
**CAS Registry Number**: 68,259-31-4

**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

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https://doi.org/10.1016/j.fct.2021.112732
Received 7 October 2021; Accepted 27 November 2021
Available online 1 December 2021

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**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; Safford et al., 2017) compared to a deterministic aggregate approach
**DEREK** - Derek Nexus is an *in silico* tool used to identify structural alerts
**DST** - Dermal Sensitization Threshold
**ECHA** - European Chemicals Agency
**EU** - Europe/European Union
**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
**QRA** - Quantitative Risk Assessment
**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.**

5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Existing data and data from read-across analog 8-isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 67,845-30-1) show that 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material; exposure to 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is below the TTC (0.009 mg/kg/day, 0.009 mg/kg/day, and 0.47 mg/day, respectively). Existing data and read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8) provided 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde a defined No Expected Sensitization Induction Level (NESIL) of 4700 μg/cm$^2$ for skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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.

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**Human Health Safety Assessment**

**Genotoxicity:** Not expected to be genotoxic. (RIFM, 1988; RIFM, 2000a)

**Repeated Dose Toxicity:** No NOAEL available. Exposure is below the TTC.

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

**Skin Sensitization:** NESIL = 4700 μg/cm$^2$. RIFM (2018)

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

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

**Environmental Safety Assessment**

**Hazard Assessment:**

**Persistence:**
Critical Measured Value: −7.4% (OECD 301B) (ECHA REACH Dossier: 5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde; ECHA, 2018)

**Bioaccumulation:**
Screening-level: 306.3 L/kg (EPI Suite v4.11; US EPA, 2012a)

**Ecotoxicity:**
Screening-level: 48-h *Daphnia magna* LC50: 0.369 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 et al., 2002)

**Critical Ecotoxicity Endpoint:** 48-h *Daphnia magna* LC50: 0.369 mg/L (ECOSAR; US EPA, 2012b)

**RIFM PNEC is:** 0.0369 μg/L

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

## 1. Identification

1. **Chemical Name:** 5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde
2. **CAS Registry Number:** 68,259-31-4
3. **Synonyms:** Bicyclo[2.2.2]oct-5-ene-2-carboxaldehyde, 5(or 6)-methyl-7(or 8)-(1-methylethyl)-; Lierral; Maceal; 5(Or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde
4. **Molecular Formula:** $C_{13}H_{20}O$
5. **Molecular Weight:** 192.3
6. **RIFM Number:** 5902
7. **Stereochemistry:** Isomer not specified. Four chiral centers present and 16 total stereoisomers possible.

## 2. Physical data

1. **Boiling Point:** 262.71 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System)
3. **Log $K_{OW}$:** 4.27 (EPI Suite)
4. **Melting Point**: 40.12 °C (EPI Suite)
5. **Water Solubility:** 10.74 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0101 mm Hg at 25 °C (EPI Suite), 0.00569 mm Hg at 20 °C (EPI Suite v4.0)

8. **UV Spectra:** No absorbance between 290 and 500 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1}$ • $cm^{-1}$)
9. **Appearance/Organoleptic:** Not Available

## 3. Volume of use (worldwide band)

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

## 4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v3.0.4)

1. **95**th **Percentile Concentration in Fine Fragrance**: 0.012% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.000074 mg/kg/day or 0.0054 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.00022 mg/kg/day (RIFM, 2019)

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

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

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

*See Appendix for an explanation.

2. **Analogs Selected**:
   a. **Genotoxicity:** 8-Isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 67,845-30-1)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** α,α,6,6-Tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8)
   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

5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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

Available; accessed 10/01/21.

## 10. Conclusion

The maximum acceptable concentrations[a] in finished products for 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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) | 0.36 |
| 2 | Products applied to the axillae | 0.11 |
| 3 | Products applied to the face/body using fingertips | 2.2 |
| 4 | Products related to fine fragrances | 2.0 |
| 5A | Body lotion products applied to the face and body using the hands (palms), primarily leave-on | 0.51 |
| 5B | Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on | 0.51 |
| 5C | Hand cream products applied to the face and body using the hands (palms), primarily leave-on | 0.51 |
| 5D | Baby cream, oil, talc | 0.51 |
| 6 | Products with oral and lip exposure | 1.2 |
| 7 | Products applied to the hair with some hand contact | 4.1 |
| 8 | Products with significant ano-genital exposure (tampon) | 0.21 |
| 9 | Products with body and hand exposure, primarily rinse-off (bar soap) | 3.9 |
| 10A | Household care products with mostly hand contact (hand dishwashing detergent) | 14 |
| 10B | Aerosol air freshener | 14 |
| 11 | Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad) | 7.8 |
| 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 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde, the basis was the skin sensitization NESIL of 4700 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 v3.0.5.

## 11. Summary

### *11.1. Human health endpoint summaries*

#### *11.1.1. Genotoxicity*

Based on the current existing data, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde does not present a concern for genotoxicity.

*11.1.1.1. Risk assessment.* 5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation (RIFM, 2013). 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.

The mutagenic activity of 5(or 6)-methyl-7(or 8)-(1-methylethyl) bicyclo[2.2.2]oct-5-ene-2-carbaldehyde has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and guidelines equivalent to OECD TG 471 using the plate incorporation and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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 concentration in the presence or absence of S9 (RIFM, 2000a). Under the conditions of the study, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde; however, read-across can be made to 8-isopropyl-6-methylbicyclo [2.2.2]oct-5-ene-2-carbaldehyde (CAS # 67,845-30-1; see Section VI). The clastogenicity of 8-isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster ovary cells were treated with 8-isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde in DMSO for 6 h in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (RIFM, 1988). In addition, weight of evidence was made to 6,6-dimethylbicyclo [3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-51-7). The clastogenic activity of 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Micronuclei analysis was conducted at concentrations ranging up to 120 μg/mL in the presence and absence of S9 for 4 h and in the absence of metabolic activation for 24 h 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2--propionaldehyde did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2015a). Under the conditions of the study, 8-isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde and 6, 6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde were considered to be non-clastogenic to mammalian cells, and this can be extended to 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2] oct-5-ene-2-carbaldehyde.

Based on the data available, 5(or 6)-methyl-7(or 8)-(1-methylethyl) bicyclo[2.2.2]oct-5-ene-2-carbaldehyde does not present a concern for genotoxic potential.

**Additional References**: None.

**Literature Search and Risk Assessment Completed On**: 06/01/21.

**Table 1**
Data summary for α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde as read-across material for 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo [2.2.2]oct-5-ene-2-carbaldehyde.

| 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$^2$ |
| 4800 [1] | Weak | 4724 | NA | NA | 4700 |

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.

*11.1.2. Repeated dose toxicity*

There are insufficient repeated dose toxicity data on 5(or 6)-methyl-7 (or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde or any read-across materials. The total systemic exposure to 5(or 6)-methyl-7 (or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

*11.1.2.1. Risk assessment.* There are no repeated dose toxicity data on 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde (0.22 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

**Additional References**: None.

**Literature Search and Risk Assessment Completed On**: 05/20/21.

*11.1.3. Reproductive toxicity*

There are no reproductive toxicity data on 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde or on any read-across materials. The total systemic exposure to 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

*11.1.3.1. Risk assessment.* There are no reproductive toxicity data on 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde (0.22 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

**Additional References**: None.

**Literature Search and Risk Assessment Completed On**: 05/31/21.

*11.1.4. Skin sensitization*

Based on the existing data and read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8), 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is considered a skin sensitizer with a defined NESIL of 4700 μg/cm$^2$.

*11.1.4.1. Risk assessment.* Limited skin sensitization studies are available for 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde. Based on the existing data and read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8; see Section VI), 5(or 6)-methyl-7(or 8)-(1-methylethyl) bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is considered a skin sensitizer. The chemical structure of these materials indicate that they would be

expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2--propionaldehyde was found to be sensitizing with an EC3 value of 19.2% (4800 μg/cm$^2$) (RIFM, 2010). In a guinea pig maximization test, the target material, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde, presented reactions indicative of sensitization at 100% (RIFM, 2000b). In a guinea pig open epicutaneous test (OET), the target material 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde presented reactions indicative of sensitization (RIFM, 2001). In a Confirmation of No Induction in Humans test (CNIH) with 1% (500 μg/cm$^2$) of 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde in dimethyl phthalate, no reactions indicative of sensitization was observed in any of the 52 volunteers (RIFM, 2002). In 2 CNIHs, read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde did not present reactions indicative of sensitization when tested at 2.5% (1938 μg/cm$^2$) in alcohol SDA 39C or at 4% (4724 μg/cm$^2$) in 1:3 ethanol: diethyl phthalate (EtOH:DEP) in any of the 41 and 104 volunteers, respectively (RIFM, 1971; RIFM, 2018).

Based on the available data on read-across material α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde, summarized in Table 1, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is considered to be a weak skin sensitizer with a defined NESIL of 4700 μg/cm$^2$. 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, 2020).

**Additional References**: None.

**Literature Search and Risk Assessment Completed On**: 05/27/21.

*11.1.5. Phototoxicity/photoallergenicity*

Based on the available UV spectra, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde would not be expected to present a concern for phototoxicity or photoallergenicity.

*11.1.5.1. Risk assessment.* There are no phototoxicity studies available for 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde in experimental models. UV absorption spectra indicate no absorption between 290 and 500 nm. As such, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde does not present a concern for phototoxicity or photoallergenicity.

*11.1.5.2. UV spectra analysis.* The available spectra indicate no absorbance in the range of 290–500 nm. As the material does not absorb in the range of interest, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009).

**Additional References**: None.

**Literature Search and Risk Assessment Completed On**: 05/19/21.

*11.1.6. Local respiratory toxicity*

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde is below the Cramer Class III* TTC value for inhalation exposure local effects.

*11.1.6.1. Risk assessment.* There are no inhalation data available on 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde. Based on the Creme RIFM Model, the inhalation exposure is 0.0054 mg/day. This exposure is 87 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

*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**: 05/28/21.

*11.2. Environmental endpoint summary*

*11.2.1. Screening-level assessment*

A screening-level risk assessment of 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1,

| | LC50 (Fish) (mg/L) | EC50 (*Daphnia*) (mg/L) | EC50 (Algae) (mg/L) | AF | PNEC (μg/L) | Chemical Class |
|---|---|---|---|---|---|---|
| RIFM Framework Screening-level **(Tier 1)** | 0.203 | | | 1000000 | 0.000203 | |
| ECOSAR Acute Endpoints **(Tier 2)** v1.11 | 0.710 | 0.369 | 0.941 | 10000 | 0.0369 | Aldehydes (mono) |
| ECOSAR Acute Endpoints **(Tier 2)** v1.11 | 1.437 | 1.006 | 1.785 | | | Neutral Organics |

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, 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde 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 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).

Based on the current Volume of Use (2015), 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde presents a risk to the aquatic compartment in the screening-level assessment.

*11.2.2. Key studies*

*11.2.2.1. Biodegradation.* No data available.

*11.2.2.2. Ecotoxicity.* **RIFM, 2015b:** An algae inhibition test was conducted according to the OECD 201. The 72-h EC50 based on geometric mean measured concentration was reported to be 0.912 mg/L, 0.877 mg/L, and greater than 1.499 mg/L for yield, biomass, and growth rate, respectively.

*11.2.3. Other available data*

5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde has been registered for REACH with the following additional data available at this time (ECHA, 2017a):

The ready biodegradability of the test material was evaluated using the $CO_2$ evolution test according to the OECD 301B guideline. Biodegradation of −7.4% was observed after 28 days.

The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions. The 48-h EC50 value based on the mean measured concentration was reported to be 2.7 mg/L.

*11.2.4. Risk assessment refinement*

Since 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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 | 5.57 | 5.57 |
| 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 0.0369 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**: 05/24/21.

## 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
- **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 10/01/21.

## 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.2021.112732.

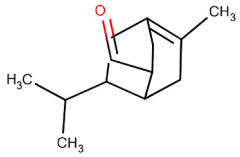
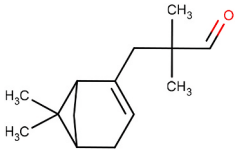
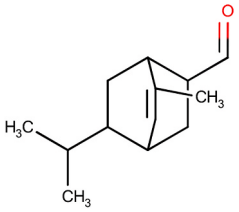
## Appendix

*Read-across Justification*

*Methods*

The read-across analogs were 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, 2017b).

- 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, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization 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).

| | Target Material | Read-across Material | Read-across Material |
|---|---|---|---|
| **Principal Name** | 5(or 6)-Methyl-7(or 8)-(1-methylethyl)bicyclo [2.2.2]oct-5-ene-2-carbaldehyde | α,α,6,6-Tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde | 8-Isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde |
| **CAS No.** | 68,259-31-4 | 33,885-52-8 | 67,845-30-1 |
| **Structure** |  |  |  |
| **Similarity (Tanimoto Score)** | | 0.84 | 0.93 |
| **Read-across Endpoint** | | • Skin Sensitization | • Genotoxicity |
| **Molecular Formula** | $C_{13}H_{20}O$ | $C_{14}H_{22}O$ | $C_{13}H_{20}O$ |
| **Molecular Weight** | 192.30 | 206.32 | 192.30 |
| **Melting Point (°C, EPI Suite)** | 40.12 | 54.98 | 30.74 |
| **Boiling Point (°C, EPI Suite)** | 262.71 | 263.89 | 259.26 |
| **Vapor Pressure (Pa @ 25°C, EPI Suite)** | 1.34 | 0.915 | 1.96 |
| **Log $K_{OW}$(KOWWIN v1.68 in EPI Suite)** | 4.27 | 4.63 | 4.14 |
| **Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)** | 10.74 | 4.492 | 13.86 |
| **$J_{max}$ (μg/cm$^2$/h, SAM)** | 52.42 | 9.48 | 39.63 |
| **Henry's Law (Pa·m$^3$/mol, Bond Method, EPI Suite)** | 3.70E+001 | 4.16E+001 | 3.13E+001 |
| ***Genotoxicity*** | | | |

(*continued on next page*)

(*continued*)

| | Target Material | Read-across Material | Read-across Material |
|---|---|---|---|
| **DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)** | • No alert found | | • No alert found |
| **DNA Binding (OECD QSAR Toolbox v4.2)** | • Schiff base formers\|Schiff base formers ≫ Direct Acting Schiff Base Formers\|Schiff base formers ≫ Direct Acting Schiff Base Formers ≫ Mono aldehydes | | • Schiff base formers\|Schiff base formers ≫ Direct Acting Schiff Base Formers\|Schiff base formers ≫ Direct Acting Schiff Base Formers ≫ Mono aldehydes |
| **Carcinogenicity (ISS)** | • Carcinogen (low reliability) | | • Carcinogen (low reliability) |
| **DNA Binding (Ames, MN, CA, OASIS v1.1)** | • No alert found | | • No alert found |
| ***In Vitro* Mutagenicity (Ames, ISS)** | • Simple aldehyde | | • Simple aldehyde |
| ***In Vivo* Mutagenicity (Micronucleus, ISS)** | • Simple aldehyde | | • Simple aldehyde |
| **Oncologic Classification** | • Aldehyde-type Compounds | | • Aldehyde-type Compounds |
| ***Skin Sensitization*** | | | |
| **Protein Binding (OASIS v1.1)** | • Schiff base formation\|Schiff base formation ≫ Schiff base formation with carbonyl compounds\|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes | • Schiff base formation\|Schiff base formation ≫ Schiff base formation with carbonyl compounds\|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes | |
| **Protein Binding (OECD)** | • Schiff Base Formers\|Schiff Base Formers ≫ Direct Acting Schiff Base Formers\|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls | • Schiff Base Formers\|Schiff Base Formers ≫ Direct Acting Schiff Base Formers\|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls | |
| **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)** | • Schiff base formation\|Schiff base formation ≫ Schiff base formation with carbonyl compounds\|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes | • Schiff base formation\|Schiff base formation ≫ Schiff base formation with carbonyl compounds\|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes | |
| **Skin Sensitization Reactivity Domains (Toxtree v2.6.13)** | • Alert for Schiff base formation | • Alert for Schiff base formation | |
| ***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 |

*Summary*

There are insufficient toxicity data on 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 68,259-31-4). 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, α,α,6,6-tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8) and 8-isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 67,845-30-1) were identified as read-across analogs with sufficient data for toxicological evaluation.

*Conclusions*

- α,α,6,6-Tetramethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33,885-52-8) was used as a read-across analog for the target material 5 (or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 68,259-31-4) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of alkyl aldehydes bearing an unsaturated bridged macrocyclic substituent.
  - o The target material and the read-across analog share an alkyl aldehyde group with a bridged unsaturated bicyclic substituent.
  - o The key difference between the target material and the read-across analog is that whereas the target material has a carbaldehyde group attached to the bridge of the bridged bicyclic structure, the read-across analog has a 2,2-dimethyl propanal group attached to a bridged bicyclic structure. The target material has 1 isopropyl and 1 methyl substitution, whereas the read-across analog has a dimethyl substitution. These structural differences are toxicologically insignificant.
  - o 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 comparison of their toxicological properties.
  - o Differences are predicted for $J_{max}$, which estimates skin absorption. $J_{max}$ for the target material corresponds to skin absorption $\leq$80% and $J_{max}$ for the read-across analog corresponds to skin absorption $\leq$40%. While percentage skin absorption estimated from $J_{max}$ indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.

- o Both the target material and the read-across analog display several alerts related to the aldehyde group. Data are consistent with *in silico* alerts.
- 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.

- 8-Isopropyl-6-methylbicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 67,845-30-1) was used as a read-across analog for the target material 5(or 6)-methyl-7(or 8)-(1-methylethyl)bicyclo[2.2.2]oct-5-ene-2-carbaldehyde (CAS # 68,259-31-4) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of alkyl aldehydes bearing an unsaturated bridged macrocyclic substituent.
  - o The target material and the read-across analog share an alkyl aldehyde group with a bridged unsaturated bicyclic substituent.
  - o The key difference between the target material and the read-across analog is the position of the vinylene in the bicyclic structure. This structural difference is toxicologically insignificant.
  - o 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 comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o Both the target material and the read-across analog display several alerts related to the aldehyde group. Data are consistent with *in silico* alerts.
  - 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 between 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.

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, 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 II (Class moderate)

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