Food and Chemical Toxicology 134 (2019) 111009

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, 4,7,7-trimethyl-6-thiabicyclo [3.2.1]octane, CAS Registry Number 68398-18-5

A.M. Api[a], F. Belmonte[a], D. Belsito[b], S. Biserta[a], 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], S. Gadhia[a], L. Jones[a], K. Joshi[a], A. Lapczynski[a], M. Lavelle[a], D.C. Liebler[i], M. Na[a], D. O'Brien[a], A. Patel[a], T.M. Penning[j], G. Ritacco[a], F. Rodriguez-Ropero[a], J. Romine[a], N. Sadekar[a], D. Salvito[a], T.W. Schultz[k], F. Siddiqi[a], I.G. Sipes[l], G. Sullivan[a,*], Y. Thakkar[a], Y. Tokura[m], S. Tsang[a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE, 20502, Sweden*
[d] *Member RIFM Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member RIFM Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member RIFM Expert Panel, 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 RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member RIFM Expert Panel, 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 RIFM Expert Panel, 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 RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996-4500, USA*
[l] *Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member RIFM Expert Panel, 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*

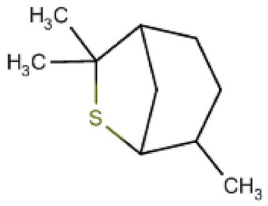
ARTICLE INFO

Keywords:
Genotoxicity
Repeated Dose, Developmental, and Reproductive Toxicity
Skin Sensitization
Phototoxicity/Photoallergenicity
Local Respiratory Toxicity
Environmental Safety

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

https://doi.org/10.1016/j.fct.2019.111009
Received 17 September 2019; Received in revised form 20 November 2019; Accepted 25 November 2019
Available online 29 November 2019

Version: 011619. This version replaces any previous versions.
Name: 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane CAS Registry Number: 68398-18-5
Additional CAS Numbers:
68921-26-6 Cyclohexene, 1-methyl-4-(1-methylethenyl)-, sulfurized
*Included because they are identical materials



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
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
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.

4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (CAS # 6784-08-3) show that 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class III material, and the exposure to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is below the TTC (0.0015 mg/kg/day, 0.0015 mg/kg/day, and 0.47 mg/day, respectively). The skin sensitization endpoint was completed using the DST for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (RIFM, 2016a; RIFM, 2016b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: No safety concerns at current, declared use levels; exposure is below the DST.
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:
Screening-level: 2.6 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:

Screening-level: 187 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: 48-h *Daphnia magna* LC50: 1.688 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: 1.688 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.1688 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: < 1

1. Identification

Chemical Name: 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane	Chemical Name: Cyclohexene, 1-methyl-4-(1-methylethenyl)-, sulfurized
CAS Registry Number: 68398-18-5	**CAS Registry Number:** 68921-26-6
Synonyms: 6-Thiabicyclo[3.2.1]octane, 4,7,7-trimethyl-; 6-Thiabicyclo[3.2.1]octane, 4,7,7-trimethyl-, (Z)-; Zestoril; 2,8 epithio-p-menthane; Thiocineol; 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane	**Synonyms:** Zestoril super; Dipentene, reaction product with sulfur; Corps 1490; Cyclohexene, 1-methyl-4-(1-methylethenyl)-, sulfurized
Molecular Formula: $C_{10}H_{18}S$	**Molecular Formula:** Not available
Molecular Weight: 170.31	**Molecular Weight:** Not available
RIFM Number: 5907	**RIFM Number:** 1214
Stereochemistry: Isomer not specified. Three chiral centers and 9 total diastereoisomers possible.	**Stereochemistry:** Isomer not specified. Three chiral centers and 9 total diastereoisomers possible.

2. Physical data

CAS # 68398-18-5	CAS # 68921-26-6
Boiling Point: 80 °C at 0.9 mm Hg (Private communication to FEMA), 209.1-°C (EPI Suite)	**Boiling Point:** Not available
Flash Point: 89 °C (Private communication to FEMA), 89 °C (GHS)	**Flash Point:** Not available
Log K_{OW}: 3.95 (EPI Suite)	**Log K_{OW}:** Not available
Melting Point: 29.72 °C (EPI Suite)	**Melting Point:** Not available
Water Solubility: 25.98 mg/L (EPI Suite)	**Water Solubility:** Not available
Specific Gravity: 0.999 (Private communication to FEMA)	**Specific Gravity:** Not available
Vapor Pressure: 0.191 mm Hg @ 25 °C (EPI Suite), 0.115 mm Hg @ 20 °C (-EPI Suite v4.0)	**Vapor Pressure:** Not available
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
Appearance/Organoleptic: Not Available	**Appearance/Organoleptic:** Not available

3. *Exposure to fragrance ingredient****

1. **Volume of Use (Worldwide Band):** 1–10 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.08% (RIFM, 2018)
3. **Inhalation Exposure*:** 0.000014 mg/kg/day or 0.0010 mg/day (RIFM, 2018)
4. **Total Systemic Exposure**:** 0.00011 mg/kg/day (RIFM, 2018)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015a; 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 4. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015a; Safford et al., 2017; and Comiskey et al., 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 or 97.5th percentile, inhalation exposure and total exposure.

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

1. **Cramer Classification:** Class III, High

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
III	III	III

2. Analogs Selected:
   a. **Genotoxicity:** 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (CAS # 6784-08-3)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

6. Metabolism

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

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

4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane is reported to occur in the following foods by the VCF*:
Citrus fruits.
*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.

8. IFRA standard

None.

IX. REACH Dossier.

Pre-registered for 2010; no dossier available as of 01/08/19.

9. Summary

9.1. Human health endpoint summaries

9.1.1. Genotoxicity

Based on the current existing data, 4,7,7-trimethyl-6-thiabicyclo [3.2.1]octane does not present a concern for genotoxicity.

9.1.1.1. Risk assessment

4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: < 80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a screening assay that assesses genotoxic stress through human-derived gene expression. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no data assessing the mutagenic/clastogenic activity of 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane; however, read-across can be made to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (CAS # 6784-08-3; see Section 5). The mutagenic activity of 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene 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 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene 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, 2016a). Under the conditions of the study, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene was not mutagenic in the Ames test, and this can be extended to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane.

The clastogenic activity of 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene in DMSO at concentrations up to 1317 μg/mL in the presence and absence of metabolic activation (S9) for 3 and 24 h 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene did not induce binucleated cells with micronuclei when tested up to the maximum dose in either non-activated or S9-activated test systems (RIFM, 2016b). Under the conditions of the study, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane.

Based on the current existing data, 4,7,7-trimethyl-6-thiabicyclo [3.2.1]oct-3-ene does not present a concern for genotoxic potential, and this can be extended to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane.

Additional References: None

Literature Search and Risk Assessment Completed On: 12/29/18.

9.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane nor any read-across materials. The total systemic exposure to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is below the TTC for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

9.1.2.1. Risk assessment

There are insufficient repeated dose toxicity data on 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane nor any read-across materials. The available oral subchronic 28-day study (RIFM, 2004) was conducted on 1 low-dose treatment level; hence, it is considered inadequate for establishing a true NOAEL for the study. Since the exposure level for the target material 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane (0.11 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use, human health hazard is not predicted for this material.

9.1.2.2. Weight of evidence

RIFM, 2004: In a subchronic GLP compliant study, 10 Crl:CD(SD) IGS BR rats/sex/dose were administered the test material 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane at doses of 0 and 10 mg/kg/day for a period of 28 days through oral gavage. The study followed the OECD 407 protocol except, only 2 dose groups were included in the study contrary to a minimum of 3 dose groups per test guidelines. No incidences of mortality among treated animals were reported. Although the mean bodyweight gain among treated females increased significantly during days 27–28, it was not considered to be adverse since the increases were within historical ranges. The food consumption among treated females increased significantly as compared to control during the first week of the study but was restored during the study duration. There were no alterations in clinical chemistry or hematology among treated animals. Urinalysis revealed coarsely granular casts among treated males; no such incidences were reported among treated females. The presence of granular casts correlated with the presence of renal tubular focal degeneration/regeneration observed histologically in male rat renal tubules and was considered to be a treatment-related effect. In males, absolute and relative kidney weights increased significantly, while in females, only the absolute kidney weight was increased. The kidney weight increases correlated with histopathological findings of focal tubular degeneration/regeneration in male rats. In fact, the affected tubules had a few cells with necrotic nuclei, an increase in eosinophilic cytoplasm, mitotic figures accompanied by the presence of hyaline droplets. These findings are consistent with early hyaline droplet nephropathy, a condition unique to male rats, and of no toxicological relevance to humans. The increases in female kidney weights are probably a reflection of increased mean daily food consumption (15.8 vs. 14.5 g/day) and a significant increase in mean body weights (223 vs. 212 g) for females in the test group in comparison to the control. The absence of any significant increase in relative kidney weight and gross or histopathologic evidence of damage to the kidneys in females suggests that these are adaptive changes. In contrast, in males there was an increase in relative liver weight only, whereas both absolute and relative liver weights increased in females in the treatment group. However, no associated gross, histopathological, and/or enzyme changes were reported. This suggests that the hepatotoxicity effects are not considered to be treatment-related adverse events. Similarly, the increases in absolute and relative heart weights were not supported by any gross or histopathological changes in females and were not considered to be adverse effects. Since the study included only one treatment dose, a true NOAEL could not be established.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/19/18.

9.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane or on any read-across materials. The total systemic exposure to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is

Table 1
Acceptable concentrations for 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Acceptable Concentrations in Finished Products Based on reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	NRU[b]
2	Products applied to the axillae	0.0015%	0.0012%
3	Products applied to the face using fingertips	0.029%	8.6×10^{-5}%
4	Fine fragrance products	0.027%	0.0013%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	0.0027%
6	Products with oral and lip exposure	0.016%	NRU[b]
7	Products applied to the hair with some hand contact	0.056%	1.0×10^{-4}%
8	Products with significant ano-genital exposure	0.0029%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054%	9.0×10^{-4}%
10	Household care products with mostly hand contact	0.19%	0.0045%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	0.063%

[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

below the TTC for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

9.1.3.1. Risk assessment

There are insufficient reproductive toxicity data on 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane (0.11 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: RIFM, 2004.

Literature Search and Risk Assessment Completed On: 12/10/18.

9.1.4. Skin sensitization

Based on existing data and the application of the DST, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane does not present a safety concern for skin sensitization under the current, declared levels of use.

9.1.4.1. Risk assessment

The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1; OECD Toolbox v4.2). No predictive skin sensitization studies are available for 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane. In 2 guinea pig maximization tests, the additional material cyclohexene, 1-methyl-4-(1-methylethenyl)-, sulfurized presented skin reactions indicative of sensitization at 5% (RIFM, 1982b; RIFM, 1982c). However, in a human maximization test, no skin sensitization reactions were observed with cyclohexene, 1-methyl-4-(1-methylethenyl)-, sulfurized in petrolatum at 0.1% or 69 μg/cm^2 (RIFM, 1982a). Acting conservatively due to the limited data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Roberts et al., 2015; Safford, 2008; Safford et al., 2011; Safford et al., 2015b). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 1 provides the acceptable concentrations for 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane that present no appreciable risk for skin sensitization based on the reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None

Literature Search and Risk Assessment Completed On: 11/29/18.

9.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane would not be expected to present a concern for phototoxicity or photoallergenicity.

9.1.5.1. Risk assessment

There are no phototoxicity studies available for 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane 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 et al., 2009). Based on the lack of absorbance, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane does not present a concern for phototoxicity or photoallergenicity.

9.1.5.2. 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 et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/20/18.

9.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane is below the Cramer Class III TTC value for inhalation exposure local effects.

9.1.6.1. Risk assessment

There are no inhalation data available on 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane. Based on the Creme RIFM Model, the inhalation exposure is 0.0010 mg/day. This exposure is 470 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/11/18.

9.2. Environmental endpoint summary

9.2.1. Screening-level assessment

A screening-level risk assessment of 4,7,7-trimethyl-6-thiabicyclo [3.2.1]octane 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 ECHA, 2012), 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, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane 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).

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	5.098			1000000	0.005098	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	2.484	1.688	2.647	10000	0.1688	Neutral Organics

A screening-level hazard assessment using EPI Suite v4.11 (US ECHA, 2012) did not identify 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane 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).

9.2.2. Risk assessment

Based on the current Volume of Use (2015), 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane presents a risk to the aquatic compartment in the screening-level assessment.

9.2.2.1. Key studies

9.2.2.1.1. Biodegradation

No data available.

9.2.2.1.2. Ecotoxicity

No data available.

9.2.2.1.3. Other available data

4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane has been pre-registered for REACH with no additional data.

9.2.3. 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	3.9	3.9
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	< 1	< 1
Risk Characterization: PEC/PNEC	< **1**	< **1**

*Combined Regional Volume of Use.

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

The RIFM PNEC is 0.1688 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: 01/03/19.

10. 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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** 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 05/31/19.

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.2019.111009.

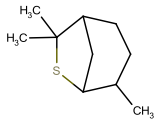
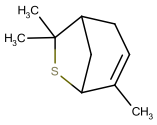
Appendix

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, 2016).

- 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 ECHA, 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).
- 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
Principal Name	4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane	4,7,7-Trimethyl-6-thiabicyclo[3.2.1]oct-3-ene
CAS No.	68398-18-5	6784-08-3
Structure		
Similarity (Tanimoto Score)		0.41
Read-across Endpoint		• Genotoxicity
Molecular Formula	$C_{10}H_{18}S$	$C_{10}H_{16}S$
Molecular Weight	170.31	168.30
Melting Point (°C, EPI Suite)	29.72	30.99
Boiling Point (°C, EPI Suite)	209.10	215.59
Vapor Pressure (Pa @ 25°C, EPI Suite)	25.5	17.9

Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.95	3.86
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	25.98	31.4
J_{max} (μg/cm^2/h, SAM)	52.18	182.96
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.49E+002	1.55E+002
Genotoxicity		
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• No alert found	• No alert found
DNA Binding (OECD QSAR Toolbox v4.2)	• No alert found	• No alert found
Carcinogenicity (ISS)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)
DNA Binding (Ames, MN, CA, OASIS v1.1)	• No alert found	• No alert found
***In Vitro* Mutagenicity (Ames, ISS)**	• No alert found	• No alert found
***In Vivo* Mutagenicity (Micronucleus, ISS)**	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified
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 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane (CAS # 68398-18-5). 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, 4,7,7-trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (CAS # 6784-08-3) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (CAS # 6784-08-3) was used as a read-across analog for the target material 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane (CAS # 68398-18-5) for the genotoxicity endpoint.
  - ○ The target material and the read-across analog are structurally similar and belong to a class of sulfur-containing bridged macrocycles.
  - ○ The target material and the read-across analog share the same number of carbons in the sulfur-containing bridged macrocycle and the same number of methyl groups at the same positions.
  - ○ The key difference between the target material and the read-across analog is that while the target material is fully saturated, the read-across analog has 1 unsaturation within the macrocycle. This structural difference is toxicologically insignificant.
  - ○ 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 comparison of their toxicological properties.
  - ○ According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - ○ Data are consistent with *in silico* alerts.
  - ○ 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 endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

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

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

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

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

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

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

IFRA (International Fragrance Association), 2015. Volume of Use Survey, February 2015.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.

OECD, 2015. Guidance Document On the Reporting Of Integrated Approaches To Testing And Assessment (IATA). ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.

OECD, 2018. The OECD QSAR Toolbox, v3.2–4.2. Retrieved from. http://www.qsartoolbox.org/.

RIFM (Research Institute for Fragrance Materials, Inc.), 1982. Report on Human Maximization Studies. Report to RIFM. RIFM Report Number 1643. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 1982. Biological Studies on P-Menthylene Sulfide 1. Unpublished Report from Parish, W.E. RIFM Report Number 2197. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 1982. Guinea Pig Skin Sensitisation Test with P-Menthylene Sulfide 1. Unpublished Report from Quest International. RIFM Report Number 46901. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2004. 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]octane (2,8 Epithio-P-Menthane): Repeated-Dose Oral Toxicity 28-day Gavage Study in Rats. Report to FEMA. Unpublished Report from Finlay, C. RIFM Report Number 52966. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2013. Report on the testing of 4,7,7-trimethyl-6-thiabicyclo[3.2.1]octane in the BlueScreen HC Assay (-/+ S9 metabolic activation). RIFM Report Number 65881. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016. 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]oct-3-ene: Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA1537, TA98 and TA100, and Escherichia coli WP2 uvrA/pKM101. RIFM Report Number 69828. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016. 4,7,7-Trimethyl-6-thiabicyclo[3.2.1]oct-3-ene (Pamplover 100): Genetic Toxicity Evaluation Using a Micronucleus Test in Human Lymphocyte Cells. RIFM Report Number 70468. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2018. Exposure Survey 19, January 2018.

Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of high potency category chemicals for which the dermal sensitization threshold (DST) approach should not be applied. Regul. Toxicol. Pharmacol. 72 (3), 683–693.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015b. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.

Safford, R.J., 2008. The dermal sensitisation threshold–A TTC approach for allergic contact dermatitis. Regul. Toxicol. Pharmacol. 51 (2), 195–200.

Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015a. Extension of the dermal sensitization threshold (DST) approach to incorporate chemicals classified as reactive. Regul. Toxicol. Pharmacol. 72 (3), 694–701.

Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regul. Toxicol. Pharmacol. 60 (2), 218–224.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.

Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74, 164–176.

US EPA, 2012. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.