Food and Chemical Toxicology 134 (2019) 110724

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, CAS Registry Number 1125-12-8

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], 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

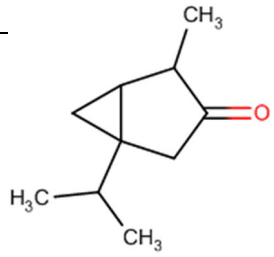
Keywords:
Genotoxicity
Repeated dose, developmental, and reproductive toxicity
Skin sensitization
Phototoxicity/photoallergenicity
Local respiratory toxicity
Environmental safety

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

https://doi.org/10.1016/j.fct.2019.110724
Received 4 April 2019; Received in revised form 24 July 2019; Accepted 26 July 2019
Available online 14 August 2019

Version: 031419. This version replaces any previous versions.

Name Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-
CAS Registry Number: 1125-12-8
Additional CAS Numbers*:
546-80-5 α-Thujone
471-15-8 β-Thujone (no reported use)
76231-76-0 α,β-Thujone (no reported use)
*These materials are included in this assessment because they are isomers



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
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
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications. Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

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

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

Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is not genotoxic. Data on bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- provide a calculated MOE > 100 for the repeated dose and reproductive toxicity endpoints. The developmental and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class II material, and the exposure to bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is below the TTC (0.009 mg/kg/day and 0.47 mg/day, respectively). Data and the application of the DST show that there are no safety concerns for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- for skin sensitization under the current declared levels of use; exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- 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 Genotoxic	NTP (2011)
Repeated Dose Toxicity: NOAEL = 11 mg/kg/day.	NTP (2011)
Reproductive Toxicity: Developmental toxicity: No NOAEL available. Exposure is below the TTC. Fertility: NOAEL = 25 mg/kg/day.	NTP (2011)
Skin Sensitization: Not a sensitization concern. 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: Critical Measured Value: 67% (OECD 301F)	RIFM (2010a)
Bioaccumulation: Screening-level: 25.88 L/kg	(EPI Suite; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 33.85 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 33.85 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.03385 μg/L	
•**Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not Applicable; cleared at screening-level	

1. Identification

Chemical Name: Bicyclo [3.1.0] hexan-3-one, 4-methyl-1-(1-methylethyl)-	**Chemical Name:** α-Thujone	**Chemical Name:** β-Thujone	**Chemical Name:** α,β-Thujone
CAS Registry Number: 1125-12-8	**CAS Registry Number:** 546-80-5	**CAS Registry Number:** 471-15-8	**CAS Registry Number:** 76231-76-0
Synonyms: 4-Methyl-1-(1-methylethyl) bicyclo [3.1.0] hexan-3-one; 3-Thujanone; 1-Isopropyl-4-methylbicyclo [3.1.0]hexan-3-one; Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	**Synonyms:** 3-Thujanone, (1s,4r,5r)-(−)-; 1-Isopropyl-4-methyl-bicyclo [3.1.0] hexan-3-one; Thujone; α-Thujone	**Synonyms:** Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, (1S,4S,5R)-	**Synonyms:** Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, (1S,5R)-, α,β-Thujone
Molecular Formula: $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$
Molecular Weight: Not available	**Molecular Weight:** 152.23	**Molecular Weight:** Not available	**Molecular Weight:** 152.23
RIFM Number: 698	**RIFM Number:** 698	**RIFM Number:** None	**RIFM Number:** 698
Stereochemistry: Isomer Not specified. Three stereocenters present and 8 total stereoisomers possible.	**Stereochemistry:** 1S,4R, 5R isomer specified. Three stereocenters present and 8 total stereoisomers possible.	**Stereochemistry:** 1S,4S, 5R isomer specified. Three stereocenters present and 8 total stereoisomers possible.	**Stereochemistry:** 1S, 5R isomer specified. Three stereocenters present and 8 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 201 °C (FMA Database), 200.58 °C (EPI Suite)
2. **Flash Point:** 148 °F; CC (FMA Database)
3. **Log K_{OW}:** 2.65 (EPI Suite), Log Pow = 2.9 (RIFM, 2010b)
4. **Melting Point**: 20.76 °C (EPI Suite)
5. **Water Solubility:** 407.7 mg/L (EPI Suite)
6. **Specific Gravity:** 0.92 (FMA Database)
7. **Vapor Pressure:** 0.308 mm Hg @ 20 °C (EPI Suite v4.0), 0.2 mm Hg 20 °C (FMA Database), 0.449 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance in the region 290–700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** No data available

*Physical data is identical for all materials included in this assessment.

3. Volume of use (Worldwide Band)

1. **Volume of Use (Worldwide Band):** 0.1–1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient*** (Creme RIFM aggregate exposure model v2.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.069% (RIFM, 2018)
2. **Inhalation Exposure*:** 0.000077 mg/kg/day or 0.0055 mg/day (RIFM, 2018)
3. **Total Systemic Exposure**:** 0.00089 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 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., 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, inhalation exposure, and total exposure.

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See Appendix below for further details.

2. Analogs Selected:
   a. **Genotoxicity:** None
   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: None

7. Metabolism

Hold et al., 2001: The metabolism of thujone was studied *in vitro* in mouse, rat, and human liver microsomes as well as *in vivo* in mice and rats. In all species, 7-hydroxy-α-thujone and 4-hydroxy-β-thujone were found to be the major metabolites from α- and β-thujone, respectively. Other metabolites observed were 2-hydroxy-α-thujone, 4-hydroxy-α-thujone, 4-hydroxy-β-thujone, and 7,8-dehydro-α-thujone from α-thujone and 2-hydroxy-β-thujone, 4-hydroxy-α-thujone, 7-hydroxy-β-thujone, and 7,8-dehydro-β-thujone from β-thujone. The pattern of metabolism among liver microsomes from rats, humans, and mice was similar. However, unlike mice, 2-hydroxythujones were not observed in microsomal incubations from rats and humans. A single dose of α- or β-thujone in mice resulted in the formation of glucuronide conjugates of 2-hydroxy-α-thujone and 7-hydroxy-β-thujone, respectively, as well as 4-hydroxy-α-thujone. The formation of 2-hydroxythujone was specific to mice and was detected only following administration of α-thujone. Based on these observations, the scheme presented in Fig. 1 is proposed for the metabolism of α- and β-thujones in rodents. However, the toxicological significance of these metabolites is unknown.

NTP, 2011: A single intravenous or oral gavage dose of α-thujone or α,β-thujone was administered to F344/N rats and B6C3F1 mice (of either sex) in independent studies to monitor the toxicokinetic parameters of α-thujone. The absorption of α-thujone was rapid and independent of dose, species, and sex following oral administration of either α-thujone or α,β-thujone. α-Thujone was detected in the brain within 2 min of administration with the brain:plasma ratios greater than 1.00 independent of gender and species. In another study, following oral (gavage) administration of α,β-thujone the *AUC*∞ (*predicted*) was compared to that of intravenous administration of α,β-thujone in order to estimate bioavailability of α-thujone. The oral bioavailability was 23.6% and 21.5% in male rats vs. 54.4% and 58.5% in female rats following administration of 25 and 50 mg/kg, respectively. The oral bioavailability was 9.56% and 52.9% in male mice vs. 9.77% and 26.8% in female mice following administration of 40 and 80 mg/kg, respectively. Male and female mice showed a greater than dose-dependent increase in *AUC*∞ suggesting possible saturation of elimination kinetics following administration of 80 mg/kg. Hence, the oral bioavailability of α-thujone in female rats was greater than in male rats following administration of α,β-thujone. However, the sex-specific difference in oral bioavailability of α-thujone was not observed in mice.

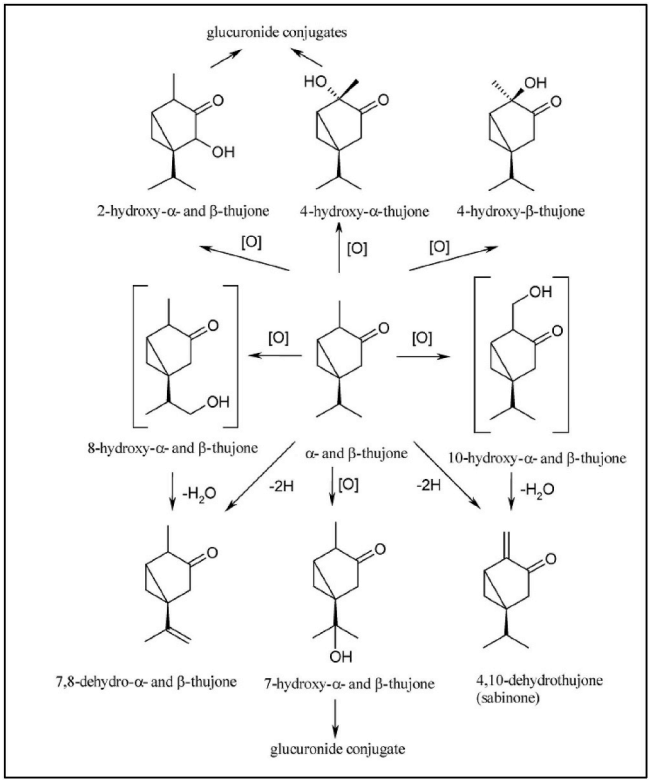


Fig. 1. (Adapted from Hold et al., 2001).

8. Natural occurrence (Discrete chemical) or composition (NCS)

Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is a component of the following naturals (essential oils):

COMPONENT	Artemesia absinthum L. oil (Wormwood)	Artemesia herba alba Asso oil	Artemesia arborescens L.	Artemesia vulgaris L. oil (Armoise)	Boswellia spp. absolute (Olibanum)
α-Thujone	2.8	39	nqd	50	
β-Thujone	42	10		7.5	0.2
Total Thujones	44.8	49	nqd	57.5	0.2
Current Use Level of α-Thujone mg/kg/day	0.0016	0.00255		0.0019	

COMPONENT	Boswellia carterii oil (Olibanum)	Boswellia spp.	Hyssopus officianalis L. oil	Mentha longifolia (L.) Huds.oil (Horsemint)	Peumus boldus Mol.oil (Boldo)
α-Thujone			0.14	7.1	14.3
β-Thujone	0.4	0.4	0.13	1	7.1
Total Thujones	0.4	0.4	0.27	8.1	21.4
Current Use Level of α-Thujone mg/kg/day			0.00068		

COMPONENT	Salvia officinalis L. oil (Sage dalmation)	Salvia officinalis L. oleoresin (Sage dalmation)	Salvia lavandifolia Vahl spanish oil	Sassafras albidum (Nutt.) Nees oil	Satureja hortensis L. oil (Savoury summer)
α-Thujone	24	7.5	3	nqd	1.5
β-Thujone	8.9	2.5	0.6		0.5
Total Thujones	32.9	10	3.6	nqd	2
Current Use Level of α-Thujone mg/kg/day	0.0016		0.00048		

COMPONENT	Picea mariana (Mill.) Britton oil (black Spruce)	Tanacetum vulgare L. oil (Tansy)	Thuja occidentalis L. oil (Cedar leaf)	Platycladus orientalis (L.) Franco oil (Cedar leaf China)
α-Thujone	0.17	0.5	43 (31-47)	43 (31-47)
β-Thujone		71	12 (9-14)	12 (9-14)
Total Thujones	0.17	71.5	55	55
Current Use Level of α-Thujone mg/kg/day	0.0033		0.0019	0.0021

Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is reported to occur in the following foods by the VCF*.

Capsicum species Caraway (*Carum carvi* L.)Citrus fruits ClamDate (*Phoenix dactylifera* L.)Dill (*Anethum species*)Elderberry (*Sambucus nigra* L.)Fennel (*Foeniculum vulg., ssp. capillaceum; var.*)Lemon balm (*Melissa officinalis* L.)Lemon grass oil Licorice (*Glycyrrhiza glabra* L.)Litchi (*Litchi chinensis* Sonn.) Mentha oils *Ocimum* species Origanum (Spanish) (*Coridothymus cap.* (L.) Rchb.)Pistacia atlantica Rosemary (*Rosmarinus officinalis* L.)*Salvia* species *Satureja* species Tarragon (*Artemisia dracunculus* L.)Thyme (*Thymus* species)Wormwood oil (*Artemisia absinthium* L.).

*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

Bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is pre-registered for 2013; no dossier available as of 03/14/19.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.0077
2	Products applied to the axillae	0.0081
3	Products applied to the face/body using fingertips	0.0011
4	Products related to fine fragrances	0.070
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.0076
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.0013
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0019
5D	Baby cream, oil, talc	No data
6	Products with oral and lip exposure	8.7×10^{-4}
7	Products applied to the hair with some hand contact	0.0013
8	Products with significant ano-genital exposure (tampon)	No data
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.0048
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.011
10B	Aerosol air freshener	0.011
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	No data
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	0.38

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 bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, the basis was the reference dose of 0.11 mg/kg/day and a predicted skin absorption value of 80%.

[b]For a description of the categories, refer to the IFRA RIFM Information Booklet. (www.rifm.org/doc).

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of additional material α-thujone 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 TA97, TA98, TA100, TA1535, and *Escherichia coli* strain WP2uvrA/pKM101 were treated with α-thujone at concentrations up to 10000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (NTP, 2011). Under the conditions of the study, α-thujone was not mutagenic in the Ames test.

The clastogenic activity of additional material α-thujone was evaluated in an *in vivo* micronucleus test. The test material was administered to groups

Table 1

Rats			
Sex	Thujone Dose (mg/kg/day)	Clonic convulsions	Mortality
Male	0	**1**/50	25/50
Female		**1**/50	15/50
Male	12.5	5/50	25/50
Female		3/50	17/50
Male	25	43/50	33/50
Female		47/50	31/50
Male	50	50/50	50/50
Female		50/50	50/50

Table 2

Mice			
Sex	Thujone Dose (mg/kg/day)	Clonic convulsions	Mortality
Male	0	0/50	10/50
Female		**1**/50	1350
Male	3	0/50	8/50
Female		**1**/50	17/50
Male	6	0/50	9/50
Female		0/50	11/50
Male	12	0/50	13/50
Female		0/50	9/50
Male	25	44/50	37/50
Female		50/50	50/50

of male and female B6C3F1 mice. Doses of 6.25, 12.5, 25, and 50 mg/kg body weight were administered. Mice from each dose level were euthanized, and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow in the peripheral blood of male mice. However, in female mice receiving a dose of 50 mg/kg, a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-months was reported. No significant changes in the percentage of reticulocytes among total erythrocytes were seen in either male or female mice at the end of the 3-month study, suggesting that α-thujone did not induce bone marrow toxicity (NTP, 2011). Under the conditions of the study, α-thujone was considered to be not clastogenic in the *in vivo* micronucleus test.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/24/18.

11.1.2. Repeated dose toxicity

The margin of exposure for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on additional material α-thujone. A 2-year carcinogenicity study was conducted on groups of 50 F344 rats/sex/group. The rats were administered α,β-thujone (70% and 11%, respectively) via gavage at doses of 0, 12.5, 25, or 50 mg/kg/day in 0.5% methylcellulose, 5 days per week, for up to 105 weeks. All animals in the highest dose group (50 mg/kg/day) died before the end of the study. The survival rates in the 25 mg/kg/day dose group were significantly less than the control. Seizures were reported in animals that received 12.5 mg/kg/day and above. The incidences of kidney mineralization were significantly increased in males of all dosed groups. In rats, there were increased incidences of seizures, increased incidences of non-neoplastic lesions in the brain, spleen, kidney (male rats), and the pituitary gland (female rats). Under the study conditions, the carcinogenic potential of the test material was only observed in male rats highlighted by increased incidences of preputial gland neoplasms and increased incidences of benign pheochromocytoma at 12 or 25 mg/kg/day. However, it was reported that the incidences of pheochromocytomas were not related to the occurrence of seizures or stress associated with seizures since this neoplasm was not observed in female rats experiencing seizures. Altogether, these findings suggest the neoplasms observed are not relevant to humans (NTP, 2011). Both lesions of the adrenal medulla (pheochromocytoma) and preputial gland are considered irrelevant to humans (Maronpot et al., 2004; Greim et al., 2009); therefore, the observed effects of preputial gland tumors, as well as pheochromocytoma, are not considered to be treatment-related adverse effects.

In a 2-year chronic study in mice, groups of 50 B6C3F1 mice/sex/dose were administered α,β-thujone (70% and 11%, respectively), via gavage at doses of 0, 3, 6, 12, or 25 mg/kg/day in 0.5% methylcellulose, 5 days per week, for up to 105 weeks. High incidences of mortality were reported among high-dose group mice. In female animals receiving the 25 mg/kg/day dose, mean body weights were 16% lower than those of the controls after week 29. Body weights of the other dose groups were within 10% of the control group. Seizures were observed in 44/50 male and 50/50 female mice in the 25 mg/kg/day dose group. This finding was not reported among treated mice of the lower dose groups. Although there were instances of small intestine carcinoma, the incidence did not exceed the historical controls. Moreover, due to the lack of dose-response, these neoplasms were not considered to be treatment-related. Under the conditions of the 2-year gavage study, there was no evidence of carcinogenic activity of the test material in male or female mice.

A benchmark dose (BMD v2.6.0.1) analysis was conducted on the occurrence of seizures (clonic convulsions) and mortality on rats and mice as shown in Tables 1–3. The most conservative $BMDL_{10}$ value of 11 mg/kg/day was considered for results obtained on the incidences of clonic convulsions among treated rats. Using an earlier version of the software, a study by Lachenmeier et al. (Lachenmeier and Uebelacker, 2010) reported a similar $BMDL_{10}$ value further substantiating the presented conclusions.

Therefore, the thujone MOE for repeated dose toxicity can be calculated by dividing the thujone BMD in mg/kg/day by the total systemic exposure to thujone, 11/0.00089, or 12360.

In addition, the total systemic exposure to α-thujone (0.89 μg/kg/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for

Table 3

Study, Animal model	Endpciirit	Sex	Model	P- Value	BMD1D (mg/kg/day)	BMDL10 (mg/kg/day)
NTP (2011), Rats	Clonic Convulsions	Male	Gamma Multi-Hit	0.98	13	11
		Female	LogProbit	0.99	13.S	12.2
	Mortality	Male	Log-Logistic	0.98	23	16.4
		Female	Gamma Multi-Hit	0.91	13.7	12.4
NTP (2011), **Mice**	Clonic Convulsions	Male	LogProbit	1	19.4	14.2
		Female	Weibull	0.58	19.2	12.9
	Mortality	Male	LogProbit	0.37	12.1	8,3
		Female	Weibull	0.29	19	12.2

the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account a reference dose of 0.11 mg/kg/day.

The RfD for α-thujone was calculated by dividing the NOAEL of 11 mg/kg/day by the uncertainty factor, 100 = 0.11 mg/kg/day.

The desired acceptable concentrations for 95th percentile aggregate exposure per unit body weight was calculated using the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015a and Safford et al., 2017) based on the reference dose of 0.11 mg/kg bw/day. Section X provides the maximum acceptable concentration in finished products based on this reference dose.

Additional References: Lachenmeier et al., 2006a; Lachenmeier et al., 2006b; Pelkonen et al., 2013; Bar and Griepentrog, 1967; Perry et al., 2001; Waidyanatha et al., 2013; Ishida et al., 1989; Hold et al., 2000; Hold et al., 2001; Longenecker et al., 1939; Le Bourhis and Soenen, 1973; Sampson and Fernandez, 1939; Meyer (1965); Smith and Margolis, 1954; Nikolayeva (1957); Pellacani (1883); Martin et al., 2004; Fox (1930); Ezeyza (1952); Stoner et al., 1973; Leuschner (1997); Tinwell et al., 2002.

Literature Search and Risk Assessment Completed On: 05/07/18.

11.1.3. Reproductive toxicity

There are insufficient developmental toxicity data on bicyclo [3.1.0] hexan-3-one, 4-methyl-1-(1-methylethyl)- or on any read-across materials. The total systemic exposure to bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is below the TTC for the developmental toxicity endpoint of a Cramer Class II material at the current level of use.

The margin of exposure for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is adequate for the fertility endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no developmental toxicity data on bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- or on any read-across materials that can be used to support the developmental toxicity endpoint. The total systemic exposure to α-thujone (0.89 μg/kg/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental toxicity endpoint of a Cramer Class II material at the current level of use.

There is sufficient fertility data on additional material α-thujone. A 3-month GLP gavage study was conducted by the US NTP on test material α,β-thujone (70% and 11%, respectively). Groups of 10 F344 rats/sex/dose were administered α,β-thujone at doses of 0, 12.5, 25, 50, 75, or 100 mg/kg/day in 0.5% methylcellulose, 5 days per week, for 14 weeks. The groups that received 75 mg/kg/day and 100 mg/kg/day doses demonstrated 50% and 85% mortality, respectively, before the end of the study. Detailed analysis on male and female reproductive systems among treated animals suggested the test material had no effects on the reproductive system; therefore, the NOAEL was considered to be 50 mg/kg/day since mortality was reported among the higher dose group animals. (NTP, 2011). In another study, test material α,β-thujone (70% and 11%, respectively) was administered via gavage to groups of 10 B6C3F1 mice/sex/dose at doses of 0, 6.25, 12.5, 25, 50, or 75 mg/kg/day in 0.5% methylcellulose, 5 days per week, for 14 weeks. Only 4 animals survived in the 50 mg/kg/day group, while 100% mortality was reported in the 75 mg/kg/day dose group. Detailed analysis of male and female reproductive systems revealed no significant differences in sperm parameters of male mice or the estrous cyclicity of female mice in comparison to the control group. The NOAEL for the reproductive toxicity endpoint was considered to be 25 mg/kg/day, based on mortality among the higher dose group animals (NTP, 2011). The most conservative NOAEL of 25 mg/kg/day obtained from the 14-week study on mice was considered for the reproductive toxicity endpoint. **Therefore, the α-thujone MOE for fertility can be calculated by dividing the α-thujone NOAEL in mg/kg/day by the total systemic exposure to α-thujone, 25/0.00089, or 28090.**

In addition, the total systemic exposure to α-thujone (0.89 μg/kg/day) is below the TTC (9 μg/kg bw/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: Lachenmeier et al., 2006a; Lachenmeier et al., 2006b; Pelkonen et al., 2013; Bar and Griepentrog, 1967; Perry et al., 2001; Waidyanatha et al., 2013; Ishida et al., 1989; Hold et al., 2000; Hold et al., 2001; Longenecker et al., 1939; Le Bourhis and Soenen, 1973; Sampson and Fernandez, 1939; Meyer (1965); Smith and Margolis, 1954; Nikolayeva (1957); Pellacani (1883); Martin et al., 2004; Fox (1930); Ezeyza (1952); Stoner et al., 1973; Leuschner (1997); Tinwell et al., 2002.

Literature Search and Risk Assessment Completed On: 05/07/18.

11.1.4. Skin sensitization

Based on the available data and application of the DST, bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- does not present a concern for skin sensitization.

11.1.4.1. 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 2.6.13; OECD toolbox v3.4). No predictive skin sensitization studies are available for bicyclo [3.1.0] hexan-3-one, 4-methyl-1-(1-methylethyl)-. In a human maximization test, no skin sensitization reactions were observed (RIFM, 1975). Acting conservatively, due to the limited data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 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 non-reactive materials when evaluated in all QRA categories. Table 4 provides the maximum acceptable concentrations for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- that present no appreciable risk for skin sensitization based on the non-reactive DST. These concentrations are not limits; they represent acceptable concentrations based on the DST approach.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/10/18.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- 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 α-thujone 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, α-thujone does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate 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 http://rifmdatabase.rifm.org/RifmDatabase/Studies/63035).

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/11/18.

Table 4
Maximum acceptable concentrations for α-thujone that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST (%)	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.069	0.0077
2	Products applied to the axillae	0.021	0.0081
3	Products applied to the face/body using fingertips	0.41	0.0011
4	Products related to fine fragrances	0.39	0.070
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.10	0.0076
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.10	0.0013
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.10	0.0019
5D	Baby cream, oil, talc	0.10	No data
6	Products with oral and lip exposure	0.23	8.7×10^{-4}
7	Products applied to the hair with some hand contact	0.79	0.0013
8	Products with significant ano-genital exposure (tampon)	0.041	No data[b]
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.75	0.0048
10A	Household care products with mostly hand contact (hand dishwashing detergent)	2.7	0.011
10B	Aerosol air freshener	2.7	0.011
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	1.5	No data[b]
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	0.38

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

11.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- is below the Cramer Class III* TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There is limited inhalation data available on α-thujone. Based on the Creme RIFM Model, the inhalation exposure is 0.0055 mg/day. This exposure is 85.5 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.

Additional References: Rice and Coats, 1994; Helmig et al., 1999a; Helmig et al., 1999b.

Literature Search and Risk Assessment Completed On: 12/15/16.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class–specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- 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). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), bicyclo [3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Biodegradation. **RIFM, 2010a**: Ready biodegradation of the

test material was evaluated according to the OECD 301F method. Under the test conditions, the test material undergoes 64% biodegradation after 28 days (67% after 31 days).

Ecotoxicity: No Data Available.

Other available data: α-Thujone has been pre-registered for REACH with no additional data.

11.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	33.85			1,000,000	0.03385	

Exposure	Europe (EU)	North America (NA)
Log Kow Used	2.9	2.9
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band (α-thujone only)	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.03385 μg/L μg/L. The revised PEC/PNECs for EU and North America are: not applicable. The material was cleared at screening-level and therefore does not present a risk to the aquatic environment at the currently reported volumes of use.

Literature Search and Risk Assessment Completed On: 02/28/19.

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**
- **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 01/22/19.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of interests

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

Explanation for Cramer Class

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. A normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? No
Q16. Common terpene? (see Cramer et al., 1978 for a detailed

explanation) No
Q17. Readily hydrolyzed to a common terpene? No
Q19. An 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 Intermediate)

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.

Bar, V.F., Griepentrog, F., 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel Fur Lebensmittel. (Where we stand concerning the evaluation of flavoring substances from the viewpoint of health). Medizin Ernahr 8, 244–251.

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

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

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.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food Cosmet. Toxicol. 16 (3), 255–276.

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

Ezeyza, S., 1952. Absence of carcinogenic properties of guaiacol, camphor, and rhubarb and intensive irritative action of the last substance; subcutaneous tests in rats. Sem. Med. 100 (21), 663–666.

Fox, N., 1930. The effect of camphor, eucalyptol and menthol on the nasal mucosa. Arch. Otolaryngol. 11, 48–54.

Greim, H., Hartwig, A., Reuter, U., Richter-Reichhelm, H.-B., Thielmann, H.-W., 2009. Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit. Rev. Toxicol. 39 (8), 695–718.

Helmig, D., Klinger, L.F., Guenther, A., Vierling, L., Geron, C., Zimmerman, P., 1999a. Biogenic volatile organic compound emissions (BVOCs). I. Identifications from three continental sites in the U.S. Chemosphere 38 (9), 2163–2187.

Helmig, D., Klinger, L.F., Guenther, A., Vierling, L., Geron, C., Zimmerman, P., 1999b. Biogenic volatile organic compound emissions (BVOCs). II. Landscape flux potentials from three continental sites in the U.S. Chemosphere 38 (9), 2189–2204.

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

Hold, K.M., Sirisoma, N.S., Casida, J.E., 2001. Detoxification of alpha- and beta-thujones (the active ingredients of absinthe): site specificity and species differences in cytochrome P450 oxidation in vitro and in vivo. Chem. Res. Toxicol. 14 (5), 589–595.

Hold, K.M., Sirisoma, N.S., Ikeda, T., Narahashi, T., Casida, J.E., 2000. Alpha-thujone (the active component of absinthe): gamma-Aminobutyric acid type A receptor modulation and metabolic detoxification. Proc. Natl. Acad. Sci. U.S.A. 97 (8), 3826–3831.

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

Ishida, T., Toyota, M., Asakawa, Y., 1989. Terpenoid biotransformation in mammals -V. metabolism of (+)-citronellal, (+-)-7-hydroxycitronellal, citral, (-)-perillaldehyde, (-)-myrtenal, cuminaldehyde, thujone, and (+-)-carvone in rabbits. Xenobiotica 19 (8), 843–855.

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.

Lachenmeier, D.W., Uebelacker, M., 2010. Risk assessment of thujone in foods and medicines containing sage and wormwood - evidence for a need of regulatory changes? Regul. Toxicol. Pharmacol. 58 (3), 437–443.

Lachenmeier, D.W., Emmert, J., Kuballa, T., Sartor, G., 2006b. Thujone-Cause of absinthism? Forensic Sci. Int. 156, 1–8.

Lachenmeier, D.W., Walch, S.G., Padosch, S.A., Kroner, L.U., 2006a. Absinthe-A review. Crit. Rev. Food Sci. Nutr. 46, 365–377.

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.

Le Bourhis, B., Soenen, A.M., 1973. Studies on the psychotropic action of some aromatic compounds used in food. Food Cosmet. Toxicol. 11 (1), 1–9.

Leuschner, J., 1997. Reproductive toxicity studies of d-camphor in rats and rabbits. Arzneimittel-Forschung (Drug Res.). Arzneimittel-Forschung (Drug Res.) 47 (2), 124–128.

Longenecker, H.E., Musulin, R.R., Tully III, R.H., King, C.G., 1939. An acceleration of vitamin C synthesis and excretion by feeding known organic compounds to rats. J. Biol. Chem. 129, 445–453.

Maronpot, R.R., Flake, G., Huff, J., 2004. Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol. Pathol. 32 (S1), 40–48.

Martin, D., Valdez, J., Boren, J., Mayersohn, M., 2004. Dermal absorption of camphor, menthol, and methyl salicylate in humans. J. Clin. Pharmacol. 44, 1151–1157.

Meyer, F. (1965). Penetrating agents. Patent. British, 1,001,949, M49750IVa/30h, 7/20/61.

National Toxicology Program, 2011. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Alpha, Beta-Thujone (CAS No. 76231-76-0) in F344 Rats and B6C3F1 Mice. (gavage studies) Unpublished.

Nikolayeva, N.I., 1957. Toxicological evaluation of camphor vapors. Gig. Sanit. 22 (11), 83–86.

Pelkonen, O., Abass, K., Wiesner, J., 2013. Thujone and thujone-containing herbal medicinal and botanical products: toxicological assessment. Regul. Toxicol. Pharmacol. 65 (1), 100–107.

Pellacani, P., 1883. Studies from the laboratory for experimental pharmacology in Strasbourg. 49. On the pharmacology of the camphor group. Archiv. Fuer. Exp. Pathologie. 17, 369–391.

Perry, N.S.L., Houghton, P.J., Sampson, J., Theobald, A.E., Hart, S., Lis-Balchin, M., Hoult, J.R.S., Evans, P., Jenner, P., Miligan, S., Perry, E.K., 2001. In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease. J. Pharm. Pharmacol. 53 (10), 1347–1356.

Rice, P.J., Coats, J.R., 1994. Insecticidal properties of several monoterpenoids to the house fly (Diptera: muscidae), red flour beetle (Coleoptera: tenebrionidae) and southern corn root worm (Coleoptera: chrysomelidae). J. Econ. Entomol. 87 (5), 1172–1179.

RIFM (Research Institute for Fragrance Materials, Inc), 1975. Report on Human Maximization Studies. Report to RIFM. RIFM Report Number 1799. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2010a. Ready Biodegradability of Alpha-Thujone. Unpublished report from Givaudan. RIFM report number 59634. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2010b. Partition Coefficient N-Octanol/water of Alpha-Thujone. Unpublished report from Givaudan. RIFM report number 59633. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2018. Expos. Surv. 22 November 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.

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.

Sampson, W.L., Fernandez, L., 1939. Experimental convulsions in the rat. J. Pharmacol. Exp. Ther. 65 (1), 275–280.

Smith, A.G., Margolis, G., 1954. Camphor poisoning. Anatomical and pharmacologic study; report of a fatal case; experimental investigation of protective action of barbiturate. Am. J. Pathol. 30 (5), 857–869.

Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K., Go, G.B., 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33 (12), 3069–3085.

Tinwell, H., Lefevre, P.A., Moffat, G.J., Burns, A., Odum, J., Spurway, T.D., Orphanides, G., Ashby, J., 2002. Confirmation of uterotrophic activity of 3-(4-methylbenzylidine) camphor in the immature rat. Environ. Health Perspect. 110 (5), 533–536.
US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.
Waidyanatha, S., Johnson, J.D., Hong, S.P., Robinson, V.G., Gibbs, S., Graves, S.W., Hooth, M.J., Smith, C.S., 2013. Toxicokinetics of alpha-thujone following intravenous and gavage administration of alpha-thujone or alpha and beta-thujone mixture in male and female F344/N rats B6C3F1 mice. Toxicol. Appl. Pharmacol. 271 (2), 216–228.