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RIFM fragrance ingredient safety assessment, 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene, CAS Registry Number 564-94-3

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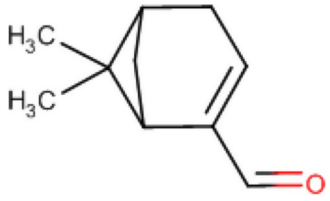
Version: 032318. This version replaces any previous versions.



Name: 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene CAS Registry Number: 564-94-3

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., 2015, 2017) compared to a deterministic aggregate approach

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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 under the limits 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 use of this material under current conditions is supported by existing information.

2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2-formyl-6,6-dimethylbicyclo(3.1.1) hept-2-ene is not genotoxic. Data show that there are no safety concerns for 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene for skin sensitization under the current declared levels of use. The repeated dose, repoductive, and local respiratory toxicity endpoints were completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class I material (0.030 mg/kg/day, 0.030 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra. The environmental endpoints were evaluated; 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene 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, 2017a; RIFM, 2017b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC	
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC	
Skin Sensitization: Not a concern for skin sensitization.	(RIFM, 2012)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic.	(UV Spectra, RIFM DB)
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: 42.9 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 42.47 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: 42.47 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.04247 µg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at the screening level

1. Identification

1. **Chemical Name:** 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene
2. **CAS Registry Number:** 564-94-3
3. **Synonyms:** Benihinal; Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-; 6,6-Dimethyl-2-norpinene-2-carboxaldehyde; Myrtenal; Pin-2-ene-1-carbaldehyde; ミルテナール; 2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene-1-carbaldehyde; Myrtenal PFG; 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene
4. **Molecular Formula:** $C_{10}H_{14}O$
5. **Molecular Weight:** 150.22
6. **IFM Number:** 1174
7. **Stereochemistry:** Isomer not specified. Two stereocenters and 4 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 199 °C (FMA), 209.52 °C (US EPA, 2012a)
2. **Flash Point:** 183°F; CC (FMA)
3. **Log K_{OW}:** 2.78 (EPI Suite)
4. **Melting Point**: 22.64 °C (US EPA, 2012a)
5. **Water Solubility:** 215.9 mg/L (US EPA, 2012a)
6. **Specific Gravity:** 0.984 (FMA)
7. **Vapor Pressure:** 0.0842 mm Hg @ 20 °C (US EPA, 2012a), 0.08 mm Hg 20 °C (FMA), 0.128 mm Hg @ 25 °C (US EPA, 2012a)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** Colorless liquid with a minty, green, and cooling odor with spicy, woody notes.*

*http://www.thegoodscentscompany.com/data/rw1008861.html#tooccur, 09/14/17.

3. Exposure

1. **Volume of Use (worldwide band):** < 0.1 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.00026% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.0000007 mg/kg/day or 0.000051 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.00055 mg/kg/day (RIFM, 2016)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM aggregate exposure model (Comiskey et al., 2015; Safford et al., 2015; and 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 IV. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

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 I, Low* (Expert Judgment)

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

*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** below for further detail.

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

6. Metabolism

No relevant data available for inclusion in this safety assessment.

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

2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene is reported to occur in the following foods by the VCF* and is found in some natural complex substances (NCS):

*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

Calabash nutmeg (*Monodora myristica* Dunal)	*Mangifera* species
Calamus (sweet flag) (*Acorus calamus* L.)	Mastic (*Pistacia lentiscus*)
Camomile	Melon
Cherimoya (*Annona cherimolia* Mill.)	Mentha oils
Citrus fruits	Myrtle (*Myrtus communis* L.)
Cumin seed (*Cuminum cyminum* L.)	Nutmeg (*Myristica fragrans* Houttuyn)
Custard apple, atemoya (*Annona atemoya*)	Parsley (*Petroselium* species)
Eucalyptus oil (*Eucalyptus globulus* Labill)	Pepper (*Piper nigrum* L.)
Ginger (*Zingiber* species)	Pistachio oil (*Pistacia vera*)
Juniperus communis	*Pistacia atlantica*
Lamb's lettuce (*Valerianella locusta*)	Raspberry, blackberry and boysenberry
Laurel (*Laurus nobilis* L.)	Thyme (*Thymus* species)
Lemon balm (*Melissa officinalis* L.)	Turpentine oil (*Pistacia terebinthus*)
Licorice (*Glycyrrhiza* species)	Walnut (*Juglans* species)
Mace (*Myristica fragrans* Houttuyn)	*Xylopia* species

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.

9. REACH dossier

Pre-Registered for 2010; No dossier available as of 03/15/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2014). The mutagenic activity of 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-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 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-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 dose in the presence or absence of S9 (RIFM, 2017a). Under the conditions of the study, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was not mutagenic in the Ames test.

The clastogenic activity of 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-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 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene in DMSO at concentrations up to 1500 μg/mL in the presence and absence of metabolic activation (S9) for 4 and 24 h 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2017b). Under the conditions of the study, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 2-formyl-6,6-dimethylbicyclo(3.1.1) hept-2-ene does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/27/2017.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene or any read-across materials. The total systemic exposure to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene or any read across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene (0.55 μg/kg bw/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/25/17.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene or any read across materials. The total systemic exposure to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene or any read across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene (0.55 μg/kg bw/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/25/17.

10.1.4. Skin sensitization

Based on the existing data, 2-formyl-6,6-dimethylbicyclo(3.1.1) hept-2-ene does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the available data 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene does not present a concern for skin

sensitization. The chemical structure of this material indicates that it would be expected to react directly with skin proteins (Roberts et al., 2007; Roberts et al., 2007; OECD toolbox v3.4). In a local lymph node assay (LLNA), 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was negative up to a maximum tested concentration of 50% or 12500 μg/cm2 (RIFM, 2012). Additionally, no reactions were observed when 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was tested in a human maximization test at 1% or 690μg/cm2 in petrolatum. Based on weight of evidence from structural analysis as well as animal and human studies, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/25/17.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of significant absorbance in the critical range, 2-formyl-6,6-dimethylbicyclo(3.1.1) hept-2-ene does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene were obtained. The spectra indicate minor 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: 08/02/17.

10.1.6. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exosure level for 2-Formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene is below the Cramer Class I TTC value for inhafation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene. Based on the Creme RIFM Model, the inhalation exposure is 0.000051 mg/day. This exposure is 27451 times lower than the Cramer Class I TTC value of 1.4 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: 09/06/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2-formyl-6,6-dimethylbicyclo (3.1.1)hept-2-ene 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, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene 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 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene 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 $\geq$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.

10.2.2. Risk assessment

Based on the current Volume of Use (2015), 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene does not present a risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

10.2.3. Other available data

2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene has been re-registered for REACH with no additional data at this time.

10.2.4. 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 Environmental 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)**	42.47			1,000,000	0.04247	

Exposure	Europe	North America
Log K_{ow} used	2.7	2.7
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.04247 μg/L. The revised PEC/PNECs for EU and NA: Not applicable; cleared at the screening level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 8/15/17.

11. Literature search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- OECD Toolbox
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** http://monographs.iarc.fr
- **OECD SIDS:** http://webnet.oecd.org/hpv/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:** http://www.safe.nite.go.jp/english/db.html
- **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.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix

Explanation of cramer classification:

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

Q1 Normal constituent of the body? No
Q2 Contains functional groups associated with enhanced toxicity? No
Q3 Contains elements other than C, H, O, N, and divalent S? No
Q5 Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6 Benzene derivative with certain substituents? No
Q7 Heterocyclic? No
Q16 Common terpene? (see Cramer et al., 1978 for detailed explanation)? Yes, Class I (Low Class)

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.

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 Chem. 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/.

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.

RIFM (Research Institute for Fragrance Materials, Inc), 2012. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene: Local Lymph Node Assay. RIFM Report Number 68397. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2014. Report on the Testing of 2-

formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM Report Number 67328. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2016. Exposure Survey 13 November 2016.
RIFM (Research Institute for Fragrance Materials, Inc), 2017a. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene: Bacterial Reverse Mutation Assay. RIFM Report Number 72517. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2017b. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene: in Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). RIFM Report Number 72921. RIFM, Woodcliff Lake, NJ, USA.
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., 2015. 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.
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.
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.