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Short Review

# RIFM fragrance ingredient safety assessment, 2,4,4,7-tetramethylnona-6,8-dien-3-one, CAS Registry Number 81782-89-0

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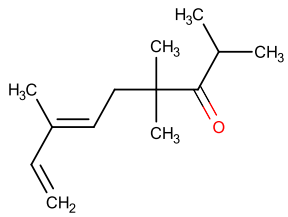


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**Abbreviation/Definition List:**

**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; Safford et al., 2015b, 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
**DEREK** - Derek Nexus is an *in silico* tool used to identify structural alerts
**DST** - Dermal Sensitization Threshold
**ECHA** - European Chemicals Agency
**EU** - Europe/European Union
**GLP** - Good Laboratory Practice
**IFRA** - The International Fragrance Association
**LOEL** - Lowest Observable Effect Level
**MOE** - Margin of Exposure
**MPPD** - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
**NA** - North America
**NESIL** - No Expected Sensitization Induction Level
**NOAEC** - No Observed Adverse Effect Concentration
**NOAEL** - No Observed Adverse Effect Level
**NOEC** - No Observed Effect Concentration
**NOEL** - No Observed Effect Level
**OECD** - Organisation for Economic Co-operation and Development
**OECD TG** - Organisation for Economic Co-operation and Development Testing Guidelines
**PBT** - Persistent, Bioaccumulative, and Toxic
**PEC/PNEC** - Predicted Environmental Concentration/Predicted No Effect Concentration
**QRA** - Quantitative Risk Assessment
**REACH** - Registration, Evaluation, Authorisation, and Restriction of Chemicals
**RfD** - Reference Dose
**RIFM** - Research Institute for Fragrance Materials
**RQ** - Risk Quotient
**Statistically Significant** - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
**TTC** - Threshold of Toxicological Concern
**UV/Vis spectra** - Ultraviolet/Visible spectra
**VCF** - Volatile Compounds in Food
**VoU** - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
**WoE** - Weight of Evidence

**The Expert Panel for Fragrance Safety* concludes that this material is safe 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.**

2,4,4,7-Tetramethylnona-6,8-dien-3-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data on 2,4,4,7-tetramethylnona-6,8-dien-3-one and from read-across analog 3,5,6,6-tetramethyl-4-methyleneheptan-2-one (CAS# 81786-75-6) show that 2,4,4,7-tetramethylnona-6,8-dien-3-one is not expected to be genotoxic. The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm$^2$); exposure is below the DST. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to 2,4,4,7-tetramethylnona-6,8-dien-3-one is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra and data; 2,4,4,7-tetramethylnona-6,8-dien-3-one is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2,4,4,7-tetramethylnona-6,8-dien-3-one was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients (i.e., PEC/PNEC) could not be calculated as there was no volume of use reported in Europe or North America.

**Human Health Safety Assessment**

**Genotoxicity**: Not expected to be genotoxic. (RIFM, 1985; RIFM, 2009)

**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 phototoxic/photoallergenic. (UV Spectra, RIFM DB; RIFM, 1980a; RIFM, 1980b)

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

**Environmental Safety Assessment**

**Hazard Assessment:**

**Persistence:** Critical Measured Value: 4% (OECD 301F) (RIFM, 2003b)
**Bioaccumulation:** Screening-level: 272 L/kg (EPI Suite v4.11; US EPA, 2012a)
**Ecotoxicity: Not applicable**
**Conclusion:** Not PBT or vPvB as per IFRA Environmental Standards

**Risk Assessment:**
**Screening-level:** PEC/PNEC (North America and Europe): Not applicable; no Volume of Use reported in 2015 for Europe or North America

## 1. Identification

1. **Chemical Name:** 2,4,4,7-Tetramethylnona-6,8-dien-3-one
2. **CAS Registry Number:** 81782-89-0
3. **Synonyms:** 6,8-Nonadien-3-one, 2,4,4,7-tetramethyl-; Labienone; 2,4,4,7-Tetramethylnona-6,8-dien-3-one
4. **Molecular Formula:** $C_{13}H_{22}O$
5. **Molecular Weight:** 194.18
6. **RIFM Number:** 5997
7. **Stereochemistry:** Isomer not specified. Two geometric isomers possible.

## 2. Physical data

1. **Boiling Point:** 230.71 °C (EPI Suite)
2. **Flash Point:** 202.00 °F. TCC (94.20 °C)*
3. **Log $K_{ow}$:** Log $P_{ow}$ = 4.5 (RIFM, 2004), 4.2 (EPI Suite)
4. **Melting Point**: 0.35 °C (EPI Suite)
5. **Water Solubility:** 12.2 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.116 mm Hg @ 25 °C (EPI Suite), 0.0772 mm Hg @ 20 °C (EPI Suite v4.0)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L · $mol^{-1}$ · $cm^{-1}$)
9. **Appearance/Organoleptic:** Not Available

*http://www.thegoodscentscompany.com/data/rw1382261.html#toorgano, retrieved 10/12/2017.

## 3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** < 0.1 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.0026% (RIFM, 2017)
3. **Inhalation Exposure*:** 0.000066 mg/kg/day or 0.0049 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.00015 mg/kg/day (RIFM, 2017)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM aggregate exposure model (Comiskey et al., 2015, 2017; Safford et al., 2015b, 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, 2017; Safford et al., 2015b, 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* | 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 detail.

2. Analogs Selected:
   a. **Genotoxicity:** 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one (CAS # 81786-75-6)
   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.

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

2,4,4,7-Tetramethylnona-6,8-dien-3-one is not reported to occur in food by the VCF*.

*VCF Volatile Compounds in Food: database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database that contains 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 2013, no dossier available as of 10/12/2017.

## 10. Summary

### *10.1. Human health endpoint summaries*

#### *10.1.1. Genotoxicity*

Based on the current existing data, 2,4,4,7-tetramethylnona-6,8-dien-3-one does not present a concern for genotoxicity.

*10.1.1.1. Risk assessment.* The mutagenic activity of 2,4,4,7-tetramethylnona-6,8-dien-3-one 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,

**Table 1**
Acceptable concentration limits for 2,4,4,7-tetramethylnona-6,8-dien-3-one based on non-reactive DST.

| IFRA Category[a] | Description of Product Type | Acceptable Concentrations in Finished Products | Reported 95th Percentile Use Concentrations in Finished Products |
|---|---|---|---|
| 1 | Products applied to the lips | 0.07% | 0.00% |
| 2 | Products applied to the axillae | 0.02% | 0.00%[b] |
| 3 | Products applied to the face using fingertips | 0.41% | 0.00%[b] |
| 4 | Fine fragrance products | 0.39% | 0.00%[b] |
| 5 | Products applied to the face and body using the hands (palms), primarily leave-on | 0.10% | 0.00%[b] |
| 6 | Products with oral and lip exposure | 0.23% | 0.00% |
| 7 | Products applied to the hair with some hand contact | 0.79% | 0.00%[b] |
| 8 | Products with significant ano-genital exposure | 0.04% | No Data |
| 9 | Products with body and hand exposure, primarily rinse-off | 0.75% | 0.00%[b] |
| 10 | Household care products with mostly hand contact | 2.70% | 0.01% |
| 11 | Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate | 1.50% | No Data |
| 12 | Products not intended for direct skin contact, minimal or insignificant transfer to skin | Not Restricted | 0.12% |

Note: [a]For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b]Negligible exposure (< 0.01%).

TA1535, TA1537, and TA1538 were treated with 2,4,4,7-tetramethylnona-6,8-dien-3-one in dimethyl sulfoxide (DMSO) at concentrations up to 150 µl/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 1985). Under the conditions of the study, 2,4,4,7-tetramethylnona-6,8-dien-3-one was not mutagenic in the Ames test.

There are no data assessing the clastogenic activity of 2,4,4,7-tetramethylnona-6,8-dien-3-one; however, read-across can be made to the commercial mixture of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one and its related isomers (CAS # 81786-75-6; see Section V). The clastogenicity of the commercial mixture of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one and its related isomers was evaluated in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster ovary cells were treated with 3,5,6,6-tetramethyl-4-methyleneheptan-2-one in DMSO at concentrations up to 5000 µg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test item, either with or without S9 metabolic activation (RIFM, 2009). Under the conditions of the study, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was considered to be non-clastogenic to mammalian cells in the *in vitro* chromosome aberration assay.

Based on the data available, 2,4,4,7-tetramethylnona-6,8-dien-3-one does not present a concern for genotoxic potential.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 10/02/2017.

*10.1.2. Repeated dose toxicity*

There are insufficient repeated dose toxicity data on 2,4,4,7-tetramethylnona-6,8-dien-3-one or any read-across materials. The total systemic exposure to 2,4,4,7-tetramethylnona-6,8-dien-3-one 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 2,4,4,7-tetramethylnona-6,8-dien-3-one or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,4,4,7-tetramethylnona-6,8-dien-3-one (0.15 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:** 10/12/2017.

*10.1.3. Reproductive toxicity*

There are insufficient reproductive toxicity data on 2,4,4,7-tetramethylnona-6,8-dien-3-one or any read-across materials. The total systemic exposure to 2,4,4,7-tetramethylnona-6,8-dien-3-one 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 2,4,4,7-tetramethylnona-6,8-dien-3-one or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2,4,4,7-tetramethylnona-6,8-dien-3-one (0.15 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:** 10/12/2017.

*10.1.4. Skin sensitization*

Based on existing data and the application of DST, 2,4,4,7-tetramethylnona-6,8-dien-3-one does not present a safety concern for skin sensitization under the current, declared levels of use.

*10.1.4.1. Risk assessment.* The chemical structure of this material indicates that it would not be expected to react with skin proteins (Toxtree 2.6.13; OECD toolbox v3.4). In an open epicutaneous test (OET), no sensitization reactions were observed (RIFM, 1981). Additionally, in a human repeat insult patch test (HRIPT) with 4000 µg/cm$^2$ of 2,4,4,7-tetramethylnona-6,8-dien-3-one, no reactions indicative of sensitization were observed in any of the 51 subjects (RIFM, 1982).

Acting conservatively, due to the limited data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 µg/cm$^2$ (Safford et al., 2015a). The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the acceptable concentration for 2,4,4,7-tetramethylnona-6,8-dien-3-one, which presents no appreciable risk for skin sensitization based on the non-reactive DST.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 09/29/2017.

*10.1.5. Phototoxicity/photoallergenicity*

Based on UV/Vis absorption spectra, and the available *in vivo* study data, 2,4,4,7-tetramethylnona-6,8-dien-3-one would not be expected to present a concern for phototoxicity or photoallergenicity.

*10.1.5.1. Risk assessment.* 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). In guinea pig phototoxicity and photoallergenicity studies, 10% 2,4,4,7-tetramethylnona-6,8-dien-3-one in rectified alcohol did not result in phototoxicity or photoallergenicity (RIFM, 1980a; RIFM, 1980b). Based on lack of absorbance, and the available *in vivo* study data, 2,4,4,7-tetramethylnona-6,8-dien-3-one does not present a concern for phototoxicity or photoallergenicity.

*10.1.5.2. 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, $1000\,L \cdot mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009).

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 10/02/17.

*10.1.6. Local Respiratory Toxicity*

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 2,4,4,7-tetramethylnona-6,8-dien-3-one is below the Cramer Class I TTC value for inhalation exposure local effects.

*10.1.6.1. Risk assessment.* There are no inhalation data available on 2,4,4,7-tetramethylnona-6,8-dien-3-one. Based on the Creme RIFM Model, the inhalation exposure is 0.0049 mg/day. This exposure is 286 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:** 10/05/17.

*10.2. Environmental endpoint summary*

*10.2.1. Screening-level assessment*

A screening-level risk assessment of 2,4,4,7-tetramethylnona-6,8-dien-3-one 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, 2,4,4,7-tetramethylnona-6,8-dien-3-one was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 2,4,4,7-tetramethylnona-6,8-dien-3-one as possibly persistent nor 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.

*10.2.2. Risk assessment*

Not applicable.

*10.2.2.1. Biodegradation.* RIFM, 2003a: The inherent biodegradability of the test material was evaluated by the Manometric respirometry test according to the OECD 302C method. Under the conditions of this study, no biodegradation was observed after 28 days.

RIFM, 2003b: The ready biodegradability of the test article was evaluated by the Manometric respirometry test according to the OECD 301F method. Under the conditions of this study, biodegradation of 4% was observed at day 28.

*10.2.2.2. Ecotoxicity.* No data available.

*10.2.2.3. Other available data.* No data available.

*10.2.3. Risk Assessment Refinement*

Not applicable.

**Literature Search and Risk Assessment Completed On:** 10/4/17.

## 11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** http://tools.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 A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fct.2018.08.073.

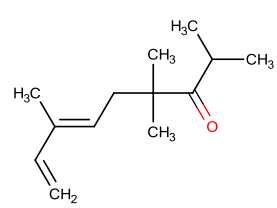
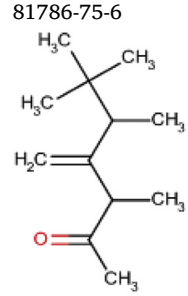
## Appendix

*Read-across Justification*

*Methods*

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity 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 was 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 substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- $J_{max}$ values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v3.4 (OECD, 2012).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2012).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 (OECD, 2012).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2012).

| | Target Material | Read-across Material |
|---|---|---|
| Principal Name | 2,4,4,7-Tetramethylnona-6,8-dien-3-one | 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one |
| CAS No. | 81782-89-0 | 81786-75-6 |
| Structure |  |  |
| Similarity (Tanimoto Score) | | 0.87 |
| Read-across Endpoint | | • Genotoxicity |
| Molecular Formula | $C_{13}H_{22}O$ | $C_{12}H_{22}O$ |
| Molecular Weight | 194.32 | 182.31 |
| Melting Point (°C, EPI Suite) | 0.35 | −21.02 |
| Boiling Point (°C, EPI Suite) | 230.71 | 193.77 |
| Vapor Pressure (Pa @ 25°C, EPI Suite) | 15.5 | 0.695 |
| Log Kow (KOWWIN v1.68 in EPI Suite) | 4.20 | 3.85 |
| Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite) | 12.2 | 27.84 |
| $J_{max}$ (mg/cm$^2$/h, SAM) | 8.891 | 6.959 |
| Henry's Law (Pa·m$^3$/mol, Bond Method, EPI Suite) | 5.38E+001 | 5.66E+001 |
| *Genotoxicity* | | |
| DNA Binding (OASIS v1.4, QSAR Toolbox v3.4) | • No alert found | • No alert found |
| DNA Binding (OECD QSAR Toolbox v3.4) | • 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 v3.4) | See Supplemental Data 1 | See Supplemental Data 2 |

*Summary*

There are insufficient toxicity data on 2,4,4,7-tetramethylnona-6,8-dien-3-one (CAS # 81782-89-0). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism, physical–chemical properties, and expert judgment, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one (CAS # 81786-75-6) was identified as a read-across material with sufficient data for toxicological evaluation.

*Conclusions*

- 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one (CAS # 81786-75-6) was used as a read-across analog for the target material 2,4,4,7-tetramethylnona-6,8-dien-3-one (CAS # 81782-89-0) for the genotoxicity endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to the class of aliphatic ketones.
  - o The target substance and the read-across analog share a ketone moiety with an unsaturated, branched aliphatic structure.
  - o The key difference between the target substance and the read-across analog is that the target substance is a C13 molecule and the read-across analog is a C12 molecule. This structural difference is toxicologically insignificant.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the ketone moiety. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

*Explanation of Cramer Classification:*

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? No
Q16. Common terpene (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? Yes
Q20. Aliphatic with some functional groups (see Cramer et al., 1978 for detailed explanation)? Yes
Q21. 3 or more different functional groups? No
Q18. One of the list (see Cramer et al., 1978 for detailed explanation on list of categories)? No, Class I (Low Class)

Research Institute for Fragrance Materials, Inc.

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