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Short Review

RIFM fragrance ingredient safety assessment, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene, CAS registry number 67674-46-8

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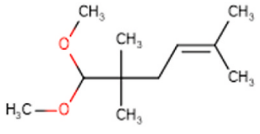
Name: 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene



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CAS Registry Number: 67674-46-8

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
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)

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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
DRF - Dose Range Finding
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 Observed 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
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
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.

6,6-Dimethoxy-2,5,5-trimethylhex-2-ene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is not genotoxic. Data on 6,6-dimethoxy-2,5,5-trimethylhex-2-ene provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog (*E*)-1-(1-methoxypropoxy)hex-3-ene (CAS # 97358-54-8) show that there are no safety concerns for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is not expected to be photoirritating/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 2002d; RIFM, 2003a; RIFM, 2017; RIFM, 2015)
Repeated Dose Toxicity: NOAEL = 205nullmg/kg/day.	RIFM (2018)
Reproductive Toxicity: Developmental toxicity NOAEL = 386nullmg/kg/day. Fertility NOAEL = 1102nullmg/kg/day.	RIFM (2018)
Skin Sensitization: No safety concerns under the current, declared levels of use.	RIFM (2001)
Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic.	(UV/Vis Spectra; RIFM Database)

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

Environmental Safety Assessment

Hazard Assessment:	
Persistence: Critical Measured Value: 14% (OECD 302C)	RIFM (2003b)
Bioaccumulation: Screening-level: 80.1nullL/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: 48-h *Daphnia magna* LC50: 5.571nullmg/L	(EPI Suite v4.11; US EPA, 2012a)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 5.571nullmg/L	(EPI Suite v4.11; US EPA, 2012a)
RIFM PNEC is: 0.5571nullμg/L	
• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe <1	

1. Identification

1. **Chemical Name:** 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene
2. **CAS Registry Number:** 67674-46-8
3. **Synonyms:** 2-Hexene, 6,6-dimethoxy-2,5,5-trimethyl-; Amarocit; Methyl pamplemousse; アルケナール（C = 6 ~ 12）ジアルキル（C = 1 ~ 2）アセタール; 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene
4. **Molecular Formula:** $C_{11}H_{22}O_2$
5. **Molecular Weight:** 186.29nullg/mol
6. **RIFM Number:** 5836
7. **Stereochemistry:** One geometric center and 2 isomers are possible.

2. Physical data

1. **Boiling Point:** 195.99 °C (EPI Suite), 201 °C (474 K) at 1025 ± 1 hPa (RIFM, 2016)
2. **Flash Point:** 80 °C (Globally Harmonized System), 73 °C (RIFM, 2016), half-life at 20, 25, 30 and 50 °C = 7.9, 4.1, 2.1 and 0.22 hours for pH 4, respectively; at 25, 50 and 70 °C = 137 days, 6.3 days and 18 hours for pH 7, respectively and >1 year at 25 °C for pH 9 (RIFM, 2016)
3. **Log K_{OW}:** 3.8 (RIFM, 2014), 4.3 (RIFM, 2004), 3.39 (EPI Suite)

4. **Melting Point**: −22.91 °C (EPI Suite), less than −80 °C (<193 K) (RIFM, 2016)
5. **Water Solubility:** 65.23nullmg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.399 mm Hgnullat 20 °C (EPI Suite v4.0), 0.626 mm Hgnullat 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 Lnullmol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. 100–1000 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0.3)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.35% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00070nullmg/kg/day or 0.051 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0064nullmg/kg/day (RIFM, 2019)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer Classification

Class I, Low (Expert Judgment)

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

*See the Appendix below for details.

6.2. Analogs selected

a. **Genotoxicity:** Weight of Evidence (WoE) material - Citral dimethyl acetate (CAS# 7549-37-3)
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** (*E*)-1-(1-Methoxypropoxy)hex-3-ene (CAS # 97358-54-8)
e. **Photoirritation/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. Environmental Toxicity:

6.3. Read-across justification

See Appendix below.

7. Metabolism

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

8. Natural occurrence

6,6-Dimethoxy-2,5,5-trimethylhex-2-ene is not reported to occur in foods by the VCF*.

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

Available; accessed on 06/14/22.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation at 5000 μM (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures (Thakkar et al., 2022). Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 6,6-dimethoxy-2,5,5-trimethylhex-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, TA97a, and TA102 were treated with 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in dimethyl sulfoxide (DMSO) at concentrations up to 5 mg/plate (5000 μg/plate). No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2002d). Under the conditions of the study, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was not mutagenic in the Ames test.

The mutagenic activity of 6,6-dimethoxy-2,5,5-trimethylhex-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 and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in DMSO at concentrations up to 5000 μg/plate. Statistically significant increases in the mean number of revertant colonies were observed in strain TA100 in the presence (2.7-fold) or absence (2.1-fold) of S9 only when using the plate incorporation method (RIFM, 2003a). A verification study assessed concentrations up to 3330 μg/plate in strain TA100 in the presence and absence of S9 in triplicate plates using the plate incorporation method. Statistically significant, dose-related increases in

the frequency of revertant mutations were observed in strain TA100 in the presence (2.3-fold) or absence (1.5-fold) of an S9 activation system using the plate incorporation method. Although there were no increases observed when using the preincubation method, the increases observed when using the plate incorporation method were considered biologically relevant. Under the conditions of the study, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was mutagenic in the Ames test.

To address the positive results observed in the second Ames test, a more biologically relevant mammalian cell gene mutation assay (mouse lymphoma assay) was conducted according to OECD TG 476/GLP guidelines. Mouse lymphoma cells were treated with 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in DMSO at concentrations up to 145nullμg/mL (as determined in a preliminary toxicity assay), for 3 and 24 hours. Effects were evaluated both with and without metabolic activation. A statistically significant increase in the frequency of mutant colonies was observed in the first mutagenicity test at 90nullμg/mL in the absence of S9, with a relative total growth (RTG) of 27% (RIFM, 2017). However, since this increase was not reproducible in the second mutagenicity test, was only observed at a single toxic dose with an RTG of 27%, and had a low cloning efficiency of 67% when compared to lower and higher test concentrations, the increase was considered to be not biologically relevant. Under the conditions of the study, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was not mutagenic to mammalian cells *in vitro*.

The increases in the mutation frequency were observed only at higher doses where there was a reduction in background lawn in plate incorporation assays. The results were negative in the pre-incubation studies. Moreover, another Ames test on the same material that had negative results both with and without S9 testing conditions (RIFM, 2002d). Hence, to verify the biological relevance of the study outcome, an additional mammalian cell line gene mutation assay was conducted. Some data show that chemicals that are positive in the Ames test and negative in different *in vitro* mammalian cell line tests evaluating two different endpoints may lack in vivo genotoxic or carcinogenic activity (Kirkland et al., 2014). As additional WoE, the structurally related material citral dimethyl acetate (CAS# 7549-37-3) was also negative in the mutagenicity study (RIFM, 1986).

The clastogenic activity of 6,6-dimethoxy-2,5,5-trimethylhex-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 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in DMSO at concentrations up to 512nullμg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 175nullμg/mL in the presence and absence of metabolic activation. 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (RIFM, 2015). Under the conditions of the study, 6,6-dimethoxy-2,5,5-trimethylhex-2--ene was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene does not present a concern for genotoxic potential.

Additional References: RIFM, 1986.

Literature Search and Risk Assessment Completed On: 01/21/22.

11.1.2. Repeated dose toxicity

The MOE of 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene has sufficient data to support the repeated dose toxicity endpoint. In an OECD 422, and GLP-compliant study, 12 SPF-bred Wistar Han rats/sex/dose were administered 6,6-dimethoxy-2,5,5-trimethylhex-2-ene by dietary administration at doses of 0, 1500, 5000, and 15000 ppm (equivalent to 0, 113, 386, and 1102nullmg/kg/day in males and 0, 204, 615, and 1548nullmg/kg/day in females respectively). Males were treated for 29 days (2 weeks prior to mating, during mating, and up to termination). Females were treated for 50–55 days or 63 days (one female) (during 2 weeks prior to mating, during mating, during post-coitum, and during 13–15 days of lactation). No treatment-related mortality or clinical signs of toxicity were reported throughout the study. A significant reduction in food intake (40%) was observed in females in the highest dose group. This did not result in a significant reduction in their body weight. However, the magnitude of change in food intake of females in the high-dose group and given that this is considered to have a significant effect on pup body weights at 15000 ppm, this can be considered an adverse change at this dose level. Microscopic examination revealed adverse treatment-related increased incidence and severity of hyaline droplet accumulation in the kidneys of male rats at 5000 and 15000 ppm. This type of renal toxicity is specific to male rats and is not considered to be relevant for human risk assessment. Low grades of centrilobular hepatocellular hypertrophy were observed in the livers of a few males and females at 15000 ppm. However, In the absence of any degenerative or inflammatory changes, these treatment-related hepatic changes were not considered to be adverse. No adverse changes were noted in hematology and clinical

Table 1
Summary of existing data on (E)-1-(1-methoxypropoxy)hex-3-ene as a read-across for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene.

WoE Skin Sensitization Potency Category[a]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2	LLNA[d] Weighted Mean EC3 Value μg/cm^2	GPMT[e]	Buehler[e]
No evidence of sensitization[g]	NA	NA	NA	NA		NA	NA
	***In vitro* Data[f]**				***In silico* protein binding alerts (OECD Toolbox v4.2)**		
	KE 1	**KE 2**	**KE 3**	**Target Material**	**Autoxidation simulator**	**Metabolism simulator**	
	NA	NA	NA	No alert found	No alert found	Schiff base formation	

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; KE = Key Event; NA = Not Available.

[a] WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

[d] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[e] Studies conducted according to the OECD TG 406 are included in the table.

[f] Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

[g] Determined based on Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients (Api et al., 2015).

biochemistry parameters at any dose groups. Thus, the NOAEL under the conditions of this study was 5000 ppm (615nullmg/kg/day for females), based on significantly lower food intake of females at 15000 ppm (RIFM, 2018).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 422 studies (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

The derived NOAEL for the repeated dose toxicity data is 615/3 or 205nullmg/kg/day.

Therefore, the MOE for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was calculated by dividing the 6,6-dimethoxy-2,5,5-trimethylhex-2-ene NOAEL (mg/kg/day) by the total systemic exposure to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in mg/kg/day, 205/0.0064 or 32031.

In addition, the total systemic to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene (6.4nullμg/kg/day) is below the TTC (30nullμg/kg/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: 01/05/22.

11.1.3. Reproductive toxicity

The MOE of 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene has sufficient data to support the reproductive toxicity endpoint. In an OECD 422, and GLP-compliant study, 12 SPF-bred Wistar Han rats/sex/dose were administered 6,6-dimethoxy-2,5,5-trimethylhex-2-ene by dietary administration at doses of 0, 1500, 5000, and 15000 ppm (equivalent to 0, 113, 386, and 1102nullmg/kg/day in males and 0, 204, 615, and 1548nullmg/kg/day in females respectively). Males were treated for 29 days (2 weeks prior to mating, during mating, and up to termination). Females were treated for 50–55 days or 63 days (one female) (during 2 weeks prior to mating, during mating, during post-coitum, and during 13–15 days of lactation). No treatment-related mortality or clinical signs of toxicity were reported throughout the study. No treatment-related effects were seen in any of the reproductive parameters up to the highest dose levels. All females of all dose groups showed clear evidence of mating. However, a lower number of pregnant females (fertility index) was recorded at 5000 ppm and 15000 ppm (7/10 females with living pups for both doses). The incidence of the non-pregnancies showed no clear dose-related response (8/10, 9/10, 7/10, and 7/10 females with living pups for 0, 1500, 5000, and 15000 ppm respectively). There were no treatment-related morphological findings in the reproductive organs of either sex, and stage-aware evaluation of the testes did not reveal any indication of abnormal spermatogenesis. Furthermore, no abnormalities were seen in the reproductive organs of the infertile couples, which could account for their non-pregnancy. Thus, the lower fertility index at 5000 and 15000 ppm is not considered to be related to exposure to the test material but rather is a chance finding. No treatment-related changes were noted in the mating index, precoital time, and the number of implantation sites up to 15000 ppm. Pups in the high-dose group showed reduced bodyweight gain (30%). The magnitude of this change was considered to represent an adverse effect on pup development and was considered to be related to the presence of significantly reduced food consumption in the dams. No treatment-related changes were noted in any of the other developmental parameters. Thus, the NOAEL for fertility was considered to be 15000 ppm (1102nullmg/kg/day for males and 1548nullmg/kg/day for females), the highest dose tested. The NOAEL for developmental toxicity was considered to be 5000 ppm (386nullmg/kg/day for males and 615nullmg/kg/day for females), based on the significant reduction in pup body weight at 15000 ppm. Taking a conservative approach, the NOAEL for developmental toxicity was considered to be 386nullmg/kg/day, and the NOAEL for fertility was considered to be 1102nullmg/kg/day (RIFM, 2018).

Therefore, the MOE for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene for the developmental toxicity endpoint can be calculated by dividing the 6,6-dimethoxy-2,5,5-trimethylhex-2-ene NOAEL in mg/kg/day by the total systemic exposure to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene, 386/0.0064 or 60313. The MOE for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene for the fertility endpoint can be calculated by dividing the 6,6-dimethoxy-2,5,5-trimethylhex-2-ene NOAEL in mg/kg/day by the total systemic exposure to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene, 1102/0.0064, or 172188.

In addition, the total systemic exposure to 6,6-dimethoxy-2,5,5-trimethylhex-2-ene (6.4nullμg/kg/day) is below the TTC (30nullμg/kg/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: 01/05/22.

11.1.4. Skin sensitization

Based on the existing data on the target material and read-across analog (*E*)-1-(1-methoxypropoxy)hex-3-ene (CAS # 97358-54-8), 6,6-dimethoxy-2,5,5-trimethylhex-2-ene does not present a safety concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene. Therefore, read-across material (*E*)-1-(1-methoxypropoxy)hex-3-ene (CAS # 97358-54-8; see Section VI) was used for the risk assessment of 6,6-dimethoxy-2,5,5-trimethylhex-2-ene. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). The read-across material was tested in a murine local lymph node assay (LLNA) as an isomeric mixture of 71.1% *trans* and 26.7% *cis* 1-(1-methoxypropoxy)hex-3-ene and was not found to be sensitizing up to a maximum tested concentration of 30% (7500nullμg/cm^2) (RIFM, 2001). However, in a guinea pig maximization test with 6,6-dimethoxy-2,5,5-trimethylhex-2-ene, 2 out of 10 animals showed signs of positive reactions, which were not sufficient to reach the threshold for classification as a sensitizer according to ECETOC 87 criteria (RIFM, 2002c). In a Confirmation of No Induction in Humans test (CNIH), 1000nullμg/cm^2 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in dimethyl phthalate did not induce sensitization in any of the 53 subjects (RIFM, 1996).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies on the read-across material as well as the target material, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene does not present a concern for skin sensitization.

Additional References: RIFM, 1979.

Literature Search and Risk Assessment Completed On: 01/20/22.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene would not be expected to present a concern for photoirritation or photoallergenicity.

11.1.5.1. Risk assessment. There are no photoirritation studies available for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for photoirritation and photoallergenicity (Henry

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	15.57			1000000	0.01557	
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	8.630	5.571	7.057 L	10000	0.557	Neutral Organics

et al., 2009). Based on the lack of absorbance, 6,6-dimethoxy-2,5,5-trimethylhex-2-ene does not present a concern for photoirritation or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for photoirritating effects, 1000 Lnullmol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/10/22.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 6,6-dimethoxy-2,5,5-trimethylhex-2-ene is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 6,6-dimethoxy-2,5,5-trimethylhex-2-ene. Based on the Creme RIFM Model, the inhalation exposure is 0.051 mg/day. This exposure is 27.5 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: 01/17/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 6,6-dimethoxy-2,5,5-trimethylhex-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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 6,6-Dimethoxy-2,5,5-trimethylhex-2-ene was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified 6,6-dimethoxy-2,5,5-trimethylhex-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, 2017a). 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 VoU (2019), 6,6-dimethoxy-2,5,5-trimethylhex-2-ene presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. ***Biodegradation:***

RIFM, 2002a: The ready biodegradability of the test material was evaluated in a closed bottle test according to the OECD 301D method. No biodegradation was observed after 28 days.

RIFM, 2003b: Inherent biodegradability of 6,6-dimethoxy-2,5,5-trimethylhex-2-ene was evaluated according to the OECD 302C method. Biodegradation of 14% was observed over a 32-day period.

RIFM, 2003c: The ready biodegradability of the test material was evaluated in a Manometric Respirometry test according to the OECD 301F method. No biodegradation was observed after 32 days.

Ecotoxicity:

RIFM, 2002b: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC50 was reported to be 50.7nullmg/L.

Other available data:

6,6-Dimethoxy-2,5,5-trimethylhex-2-ene has been pre-registered for REACH with no additional data at this time.

11.2.3. Risk assessment refinement

Since 6,6-dimethoxy-2,5,5-trimethylhex-2-ene has passed the screening criteria, measured data are included for completeness only and have not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.3	4.3
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	10–100	10–100
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 0.5571nullμg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 05/24/22.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA ChemView:** https://chemview.epa.gov/chemview/
- **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 06/14/22.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.113758.

Appendix

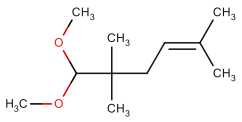
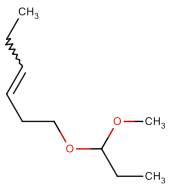
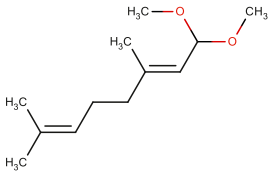
Read-across Justification

Methods

The read-across analog was identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017b).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material	WoE Material
Principal Name	6,6-Dimethoxy-2,5,5-trimethylhex-2-ene	(E)-1-(1-Methoxypropoxy)hex-3-ene	Citral dimethyl acetal
CAS No.	67674-46-8	97358-54-8	7549-37-3
Structure			
Similarity (Tanimoto Score)		0.34	0.38
SMILES	COC(OC)C(C)(C)CC=C(C)C	CCC=CCCOC(CC)OC	COC(OC)C=C(C)CCC=C(C)C
Endpoint		Skin sensitization	Genotoxicity
Molecular Formula	$C_{11}H_{22}O_2$	$C_{10}H_{20}O_2$	$C_{12}H_{22}O_2$
Molecular Weight	186.295	172.268	198.306
Melting Point (°C, EPI Suite)	−22.91	−21.19	−16.62
Boiling Point (°C, EPI Suite)	195.99	201.42	234.99
Vapor Pressure (Pa @ 25°C, EPI Suite)	8.35E+01	6.45E+01	1.25E+01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	6.52E+01	1.80E+02	2.37E+01
Log KOW	3.39	2.95	3.83
J_{max} (μg/cm^2/h, SAM)	4.40	9.39	2.05
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	5.14E+01	3.27E+01	7.07E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found		No alert found
DNA Binding (OECD QSAR Toolbox v4.2)	No alert found		No alert found
Carcinogenicity (ISS)	Structural alert for nongenotoxic carcinogenicity| Substituted n-alkylcarboxylic acids (Nongenotox)		No alert found
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
Skin Sensitization			
Protein Binding (OASIS v1.1)	No alert found	No alert found	
Protein Binding (OECD)	No alert found	No alert found	
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found	
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domains alerts identified.	No skin sensitization reactivity domains alerts identified.	
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on 6,6-dimethoxy-2,5,5-trimethylhex-2-ene (CAS # 67674-46-8). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, (E)-1-(1-methoxypropoxy)hex-3-ene (CAS # 97358-54-8) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- (E)-1-(1-Methoxypropoxy)hex-3-ene (CAS # 97358-54-8) was used as a read-across analog for the target material, 6,6-dimethoxy-2,5,5-trimethyl-hex-2-ene (CAS # 67674-46-8), for the skin sensitization endpoint.
  - o The target material and the read-across analog belong to the class of acetals.
  - o The key difference between the target and the read-across analog is that the target material has a vinylene on the main chain, whereas the read-across analog has a vinylene on the alkoxy fragment. Moreover, there are 3 methyl substituents in the target material which are lacking in the read-across analog. These structural differences are toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score.
  - o Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for both the target material and the read-across analog corresponds to skin absorption $\leq$40%. While the percentage of skin absorption estimated from J_{max} indicates exposure to the material, it does not represent

hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v4.2, the structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.
- Citral dimethyl acetal (CAS # 7549-37-3) was used as a WoE analog for the target material 6,6-dimethoxy-2,5,5-trimethylhex-2-ene (CAS # 67674-46-8) for the genotoxicity endpoint.
  - o The target material and the WoE analog are structurally similar and belong to the class of acetals.
  - o The key difference between the target material and the WoE analog is that the WoE analog has two isolated vinylene groups in the main chain compared to the target material which has one vinylene in the main chain. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the WoE analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the WoE analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the WoE analog.
  - o The target material has an alert for non-genotoxic carcinogenicity due to substituted n-alkylcarboxylic acids while the WoE material has no structural alerts. However, data on the target material has shown no evidence for carcinogenicity. Therefore, the predictions are superseded by data.
  - o The target material and the WoE 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 WoE analog and the target material.

Explanation of Cramer Classification

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

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q43. Possibly harmful divalent sulfur (not detected via Q3) No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q42. Possibly harmful analog of benzene No
Q7. Heterocyclic? No
Q16. Common terpene? (see Cramer et al., 1978 for detailed explanation) No
Q17. Readily hydrolyzed to a common terpene? Yes
Q18. One of the list? (see Cramer et al., 1978 for a detailed explanation on list of categories) No, Low (Class I)

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