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RIFM fragrance ingredient safety assessment, cyclohexadecanone, CAS Registry Number 2550-52-9

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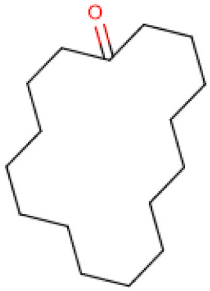

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Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
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., 2020)
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.,

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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 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
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, 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.

Cyclohexadecanone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that cyclohexadecanone is not genotoxic. Data on cyclohexadecanone provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- (CAS # 259854-71-2 and 259854-70-1, respectively) provided cyclohexadecanone a No Expected Sensitization Induction Level (NESIL) of 10000 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; cyclohexadecanone is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class II material, and the exposure to cyclohexadecanone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; cyclohexadecanone 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 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, 2001c; RIFM, 2001d)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day. RIFM (2001b)
Reproductive Toxicity: Developmental: 100 mg/kg/day. Fertility: 100 mg/kg/day. RIFM (2017a)
Skin Sensitization: NESIL = 10000 μg/cm^2. RIFM (2006)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/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: 79% (OECD 302C) RIFM (2017b)
Bioaccumulation: Screening-level: 4468 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Critical Ecotoxicity Endpoint: 96-h Fish LC50 (OECD 203) > 0.10 mg/L (RIFM, 2001e)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards
Risk Assessment:
Screening-level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: 96-h Fish LC50 (OECD 203): >0.10 mg/L (RIFM, 2001e)
RIFM PNEC is: 0.10 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1

1. Identification

1. **Chemical Name:** Cyclohexadecanone
2. **CAS Registry Number:** 2550-52-9
3. **Synonyms:** Isomuscone; Homoexaltone; Cyclohexadecanone
4. **Molecular Formula:** $C_{16}H_{30}O$
5. **Molecular Weight:** 238.41
6. **RIFM Number:** 6663
7. **Stereochemistry:** One possible stereoisomer

2. Physical data

1. **Boiling Point:** 339.75 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System)
3. **Log K_{OW}:** 6.04 (EPI Suite), Log Pow = > 5.7 (RIFM, 2001i), log Pow = 7.77 (RIFM, 2016a)
4. **Melting Point**: 52.35 °C (EPI Suite)
5. **Water Solubility:** 0.1915 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
   Vapor Pressure: 0.000138 mm Hg at 20 °C (EPI Suite v4.0), 0.000262 mm Hg at 25 °C (EPI Suite)
7. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
8. **Appearance/Organoleptic:** Solid white or colorless crystals which have a tenacious animal, mostly musk-like odor

3. Volume of use (worldwide band)

1. 1–10 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.48% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00036 mg/kg/day or 0.025 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0070 mg/kg/day (RIFM, 2019)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class II, Intermediate

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
II	II	II

2. Analogs Selected:

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** 5-Cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- (CAS # 259854-71-2, 259854-70-1, respectively)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

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

Cyclohexadecanone 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 03/16/20 (ECHA, 2019).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for cyclohexadecanone are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.25
2	Products applied to the axillae	0.23
3	Products applied to the face/body using fingertips	4.4
4	Products related to fine fragrances	4.3
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	1.1
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	1.1
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	1.1
5D	Baby cream, oil, talc	0.37
6	Products with oral and lip exposure	0.25
7	Products applied to the hair with some hand contact	7.4
8	Products with significant ano-genital exposure (tampon)	0.37
9	Products with body and hand exposure, primarily rinse-off (bar soap)	8.4
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.98
10B	Aerosol air freshener	13
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.37
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For cyclohexadecanone, the basis was the reference dose of 1 mg/kg/day, a predicted skin absorption value of 10%, and a skin sensitization NESIL of 10000 μg/cm^2.
[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.1.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data and use levels, cyclohexadecanone does not present a concern for genetic toxicity.

11.1.1.1. Risk assessment. The mutagenic activity of cyclohexadecanone 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 *Escherichia coli* strain WP2uvrA were treated with cyclohexadecanone in dimethyl sulfoxide (DMSO) at concentrations up to 5

mg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2001c). Under the conditions of the study, cyclohexadecanone was not mutagenic in the Ames test.

The clastogenicity of cyclohexadecanone was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations. Chinese hamster lung cells were treated with cyclohexadecanone in DMSO at concentrations up to 500 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 material, either with or without S9 metabolic activation (RIFM, 2001d). Under the conditions of the study, cyclohexadecanone was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, cyclohexadecanone does not present a concern for genotoxic potential.

Additional References: RIFM, 2016e.

Literature Search and Risk Assessment Completed On: 04/15/20.

11.1.2. Repeated dose toxicity

The MOE for cyclohexadecanone is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient data on cyclohexadecanone for the repeated dose toxicity endpoint. In a subchronic toxicity study, groups of 5 Wistar Crl:WI BR rats/sex/dose were administered cyclohexadecanone via gavage at doses of 0 (deionized water), 0 (vehicle control: corn oil), 300, 600, or 1000 mg/kg for 28 days. An additional 5 Wistar rats/sex/dose at 0 mg/kg/day and 1000 mg/kg/day were maintained for a 14-day recovery period after the 28-day treatment period. Observations included clinical signs, mortality, sensory reactivity (auditory, visual, and proprioceptive stimuli), grip strength, motor activity, bodyweight changes, food consumption, hematology, serum biochemistry, histology, organ weights, and macroscopic changes. Histopathology was only performed on tissues from the control and 1000 mg/kg/day groups. No mortality was observed during the study period. No treatment-related effects were seen on clinical observations, body weight, bodyweight gain, or food consumption. The serum cholesterol level was increased in both sexes at all doses ≥300 mg/kg/day and was irreversible in females. The total serum protein and albumin level was increased in females at 600 and 1000 mg/kg/day but was reversed during the recovery period. The absolute and relative liver weights were increased (within historical control ranges) in all treated animals and were irreversible in females. Absolute liver weight changes were statistically significant in males at the low and high doses, while relative liver weight changes were statistically significant in males at all doses and females at the mid and high doses. Intracellular vacuoles, indicating fat deposits, were observed in the hepatocytes in all animals of the vehicle control group and 1000 mg/kg/day group, suggesting that the vehicle contributed to this effect. The minor liver and clinical chemistry changes observed were determined to be non-adverse. Based on no treatment-related adverse effects seen up to the highest dose, the NOAEL was determined to be 1000 mg/kg/day (RIFM, 2001b).

A default safety factor of 3 was used when deriving a NOAEL from a 28-day study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 1000/3, or 333 mg/kg/day.

Therefore, the cyclohexadecanone MOE for the repeated dose toxicity endpoint can be calculated by dividing the cyclohexadecanone NOAEL in mg/kg/day by the total systemic exposure to cyclohexadecanone, 333/0.007, or 47571.

In addition, the total systemic exposure to cyclohexadecanone (7 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/24/20.

11.1.3. Reproductive toxicity

The MOE for cyclohexadecanone is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on cyclohexadecanone that can be used to support the reproductive toxicity endpoint. An OECD 421/GLP reproduction/developmental toxicity screening test was conducted in Sprague Dawley (Crl:CD[SD]) rats. Groups of 10 rats/sex/dose were administered test material cyclohexadecanone via the oral gavage in corn oil at doses of 0, 100, 300, or 1000 mg/kg/day. Males were dosed for 42 days (2 weeks prior to mating, 2 weeks through the mating period, and 2 weeks post-mating) while females were dosed for 2 weeks prior to mating, throughout the gestation period, and for 13 days after delivery. No treatment-related adverse effects were observed with respect to the estrous cycle, mating period, mating index, gestation period, mean litter size, body weights of pups, external examination of pups, and sex ratio of pups in the treatment groups. No mortality was observed in males; 1 female died in each of the 100, 300, and 1000 mg/kg/day groups during the parturition or postpartum period. Black foci on the mucosa of the glandular stomach (erosion), small thymus (atrophy), and small spleen (white pulp atrophy) were observed in the dead animals at 1000 mg/kg/day. Black foci on the mucosa of the glandular stomach (erosion), small thymus (atrophy), and small spleen (white pulp atrophy) were observed in the dead animals at 1000 mg/kg/day. The death in the 100 mg/kg/day group was not considered to have toxicological relevance as there were no treatment-related adverse effects in other parameters at 100 mg/kg/day. The number of dams whose pups died, including stillbirths, was increased at 300 and 1000 mg/kg/day. An increase in post-implantation loss rate, decreases of the live birth index and viability index (postnatal days [PNDs] 0 and 4) of pups, and abnormal deliveries were observed at 300 and 1000 mg/kg/day. Stillbirth or prolonged gestation (GD 24) and dystocia were observed in 2 females at 300 mg/kg/day, and vaginal bleeding, prolonged gestation (GD 25), delayed delivery, stillbirth, and dystocia in 1 female were observed at 1000 mg/kg/day on postpartum day 0. The NOAEL for developmental toxicity was considered to be 100 mg/kg/day, based on decreases of the live birth index, viability index, and abnormal deliveries observed at 300 and 1000 mg/kg/day. The NOAEL for fertility was also considered to be 100 mg/kg/day, based on the increase in stillbirth, dystocia, an increase in post-implantation loss, prolonged gestation, and vaginal bleeding (RIFM, 2017a).

Therefore, the cyclohexadecanone MOE for the reproductive toxicity endpoint can be calculated by dividing the cyclohexadecanone NOAEL in mg/kg/day by the total systemic exposure to cyclohexadecanone, 100/0.007, or 14286.

In addition, the total systemic exposure to cyclohexadecanone (7 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 1 mg/kg/day.

Derivation of reference dose (RfD)

The RIFM Criteria Document (Api, 2015) calls for a default MOE of

100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The reference dose for cyclohexadecanone was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 100 mg/kg/day by the uncertainty factor, 100 = 1 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/14/20.

11.1.4. Skin sensitization

Based on the existing data and read-across 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- (CAS # 259854-71-2, 259854-70-1, respectively), cyclohexadecanone is considered a skin sensitizer with a defined NESIL of 10000 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for cyclohexadecanone. Based on the existing data and read-across materials 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- (CAS # 259854-71-2, 25984-70-1; see Section VI), cyclohexadecanone is considered a skin sensitizer. The chemical structures of these materials indicate that they would be expected to react with skin proteins directly (Roberts, 2007; OECD Toolbox v4.2). The read-across material, 5-cyclotetradecen-1-one, 3-methyl-, (5E)-, was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens test but positive in the human cell line activation test (h-CLAT) (RIFM, 2016c; RIFM, 2016d; RIFM, 2016f). However, in a guinea pig maximization test, cyclohexadecanone did not present reactions indicative of sensitization up to 25% (RIFM, 2001a). In a murine local lymph node assay (LLNA), read-across material 5-cyclotetradecen-1-one, 3-methyl-, (5Z)- was found to be sensitizing with an EC3 value of 16.4% (4100 μg/cm^2) (RIFM, 2004b). In a guinea pig open epicutaneous test, read-across material 5-cyclotetradecen-1-one, 3-methyl-, (5Z)- did not present reactions indicative of sensitization (RIFM, 2005a). Additionally, in 3 Confirmation of No Induction in Humans tests (CNIH) with 20% (10000 μg/cm^2), 10% (5000 μg/cm^2), and 6% (3000 μg/cm^2) of read-across material 5-cyclotetradecen-1-one, 3-methyl-, (5Z)- in 3:1 diethyl phthalate:ethanol and dimethyl phthalate, no reaction indicative of sensitization was observed in any of the 97, 103, and 54 volunteers, respectively (RIFM, 2006; RIFM, 2005b; RIFM, 2004a).

Based on the weight of evidence (WoE) from structural analysis, animal studies, and data on the read-across material 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)-, cyclohexadecanone is a sensitizer with a WoE NESIL of 10000 μg/cm^2 (see Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 1 mg/kg/day.

Additional References: RIFM, 2005a; ECHA, 2019: Skin sensitization 001 key study.

Literature Search and Risk Assessment Completed On: 04/14/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, cyclohexadecanone would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for cyclohexadecanone in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, cyclohexadecanone does not present a concern for phototoxicity or photoallergenicity.

Table 1
Data summary for 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- as read-across material for cyclohexadecanone.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[a]	Human 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
4100 [1]	Weak	10000	NA	NA	10000

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

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

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/20/20.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for cyclohexadecanone is below the Cramer Class III* TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on cyclohexadecanone. Based on the Creme RIFM Model, the inhalation exposure is 0.025 mg/day. This exposure is 18.8 lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

*As per Carthew et al. (2009), Cramer Class II materials default to Cramer Class III for the local respiratory toxicity endpoint.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/14/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of cyclohexadecanone was performed following the RIFM Environmental Framework (Salvito, 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, cyclohexadecanone 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 cyclohexadecanone as not possibly persistent but 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on current VoU (2015), cyclohexadecanone presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. RIFM, 2001h: The ready biodegradability of the test material was evaluated using the modified strum test according to the OECD 301B guideline. Biodegradation of 43% was observed after 28 days.

RIFM, 2016b: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guideline. Biodegradation of 47% was observed after 28 days.

RIFM, 2017b: The inherent biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 302C guideline. Biodegradation of 79% was observed after 28 days.

11.2.2.1.2. Ecotoxicity. RIFM, 2001g: An algae inhibition test (limit test) was performed according to the OECD 201 guideline under static conditions. The 72-h EC50 values for growth rate and biomass-based on measured concentrations were both reported to be > 0.18 mg/L.

RIFM, 2001f: A *Daphnia magna* acute immobilization test (limit test) was performed according to the OECD 202 guideline under static conditions. The 48-h EC50 value based on the mean measured concentration was reported to be > 0.19 mg/L.

RIFM, 2001e: The acute fish (zebrafish) toxicity test (limit test) was performed according to the OECD 203 guideline under semi-static conditions. The 96-h LC50 value based on measured concentration was reported to be > 0.10 mg/L.

11.2.2.1.3. Other available data. Cyclohexadecanone has been registered for REACH with no additional data available at this time.

11.2.3. Risk assessment refinement

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>0.0031</u>			1000000	0.0000031	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.046	<u>0.038</u>	0.133	10000	0.0038	Neutral Organics

Tier 3: Measured Data Including REACH

	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	<u>>0.10</u>			1000	0.10	
Daphnia		>0.19				
Algae		>0.18				

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	7.77	7.77
Biodegradation Factor Used	1	1

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(*continued*)

Exposure	Europe (EU)	North America (NA)
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.10 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 04/14/20.

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
- **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 HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 04/19/21.

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.2021.112487.

Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US 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).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	Cyclohexadecanone	5-Cyclotetradecen-1-one, 3-methyl-,(5E)-	5-Cyclotetradecen-1-one, 3-methyl-, (5Z)-
CAS No.	2550-52-9	259854-70-1	259854-71-2
Structure			

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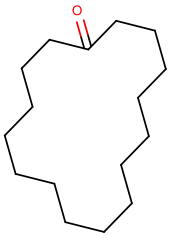
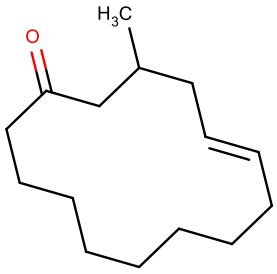
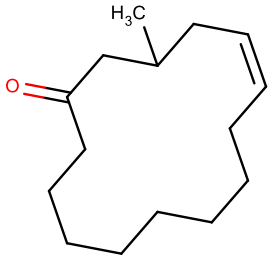
	Target Material	Read-across Material	Read-across Material
			
Similarity (Tanimoto Score)		0.42	0.42
Endpoint		• Skin sensitization	• Skin sensitization
Molecular Formula	$C_{16}H_{30}O$	$C_{15}H_{26}O$	$C_{15}H_{26}O$
Molecular Weight	238.41	222.37	222.37
Melting Point (°C, EPI Suite)	52.35	44.10	44.10
Boiling Point (°C, EPI Suite)	339.75	322.85	322.85
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.03	0.10	0.10
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	0.19	1.08	1.08
Log K_{OW}	6.04	5.26	5.26
J_{max} (μg/cm^2/h, SAM)	0.03	0.16	0.16
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	88.05	58.36	58.36
Skin Sensitization			
Protein Binding (OASIS v1.1)	No alert found	No alert found	No alert found
Protein Binding (OECD)	No alert found	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)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	Nucleophilic addition|Nucleophilic addition ≫ Addition to carbon-hetero double bonds|Nucleophilic addition ≫ Addition to carbon-hetero double bonds ≫ Ketones	Nucleophilic addition|Nucleophilic addition ≫ Addition to carbon-hetero double bonds|Nucleophilic addition ≫ Addition to carbon-hetero double bonds ≫ Ketones	Nucleophilic addition|Nucleophilic addition ≫ Addition to carbon-hetero double bonds|Nucleophilic addition ≫ Addition to carbon-hetero double bonds ≫ Ketones
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domains alerts identified.	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 cyclohexadecanone (CAS # 2550-52-9). 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, 5-cyclotetradecen-1-one, 3-methyl-,(5E)- (CAS # 259854-70-1) and 5-cyclotetradecen-1-one, 3-methyl-, (5Z)- (CAS # 259854-71-2) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 5-Cyclotetradecen-1-one, 3-methyl-,(5E)- (CAS # 259854-70-1) and 5-cyclotetradecen-1-one, 3-methyl-, (5Z)- (CAS # 259854-71-2) were used as read-across analogs for the target material 3-methyl-1-cyclopentadecanone (CAS # 541-91-3) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of ketones.
  - o The key difference between the target material and the read-across analog is that the read-across has an additional double bond at the fifth position. Moreover, the target material has a slightly larger cyclic ring than the read-across. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score presented in the table above. The differences in the structures that are responsible for a Tanimoto score <1 are not relevant from a toxicological perspective.

- o The physical–chemical properties of the target material and the read-across 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 read-across analog.
- o The target material and the read-across analogs have an alert for undergoing nucleophilic addition to carbon-hetero double bonds in carbonyl compounds by the protein Binding (OASIS v1.1 QSAR Toolbox v4.2) *in silico* model for skin sensitization. A chemical with this structural alert could interact with proteins via nucleophilic addition to ketones. Simple ketones are usually too weakly reactive to sensitize unless log P is very high. This is taken into account in the TIMES SS model by defining a threshold of log K_{ow} >4 for weak skin sensitizers. Both the target material and read-across analogs are simpler ketones with log K_{ow} >4. Based on the existing data and read-across to 5-cyclotetradecen-1-one, 3-methyl-, (5E)- and (5Z)- (CAS # 259854-71-2, 25984-70-1), 3-methyl-1-cyclopentadecanone is considered a skin sensitizer with a defined NESIL of 10000 μg/cm^2. Therefore, based on the structural similarity between the target material and the read-across analogs as well as the data for the read-across analogs, the *in silico* alerts on these materials are superseded by the data.
- 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.

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