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RIFM fragrance ingredient safety assessment, 4-*t*-amylcyclohexanone, CAS Registry Number 16587-71-6

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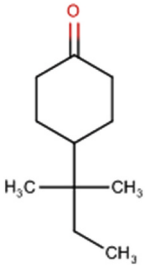
Name: 4-*t*-Amylcyclohexanone
CAS Registry Number: 16587-71-6



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., 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

(*continued on next column*)

(*continued*)

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.

4-*t*-Amylcyclohexanone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog 2-*tert*-butylcyclohexanone (CAS # 1728-46-7) show that 4-*t*-amylcyclohexanone is not expected to be genotoxic. Data on read-across analog 2-sec-butylcyclohexanone (CAS # 14765-30-1) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data provided 4-*t*-amylcyclohexanone a No Expected Sensitization Induction Level (NESIL) of 350 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 4-*t*-amylcyclohexanone is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to 4-*t*-amylcyclohexanone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 4-*t*-amylcyclohexanone 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.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic.	(RIFM, 2017a; RIFM, 2017b)
Repeated Dose Toxicity: NOAEL = 16 mg/kg/day.	RIFM (2018)
Reproductive Toxicity: Developmental toxicity: 226 mg/kg/day. Fertility: 226 mg/kg/day.	RIFM (2018)
Skin Sensitization: NESIL = 350 μg/cm^2.	RIFM (2012a)
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: 54% (OECD 302C)	RIFM (2010a)
Bioaccumulation:	
Screening-level: 80.9 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 48-h *Daphnia magna* LC50: 4.970 mg/L	(ECOSAR; US EPA, 2012b)
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: 48-h *Daphnia magna* LC50: 4.970 mg/L	(ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.4970 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1	

1. Identification

1. **Chemical Name:** 4-*t*-Amylcyclohexanone
2. **CAS Registry Number:** 16587-71-6
3. **Synonyms:** Cyclohexanone, 4-(1,1-dimethylpropyl)-; Orivone; 4-*tert*-Pentylcyclohexanone; 4-(1,1-Dimethylpropyl)cyclohexanone; 4-*tert*-Amylcyclohexanone; Isopentylcyclohexanone; アルキル(C = 1 ~ 5)シクロヘキサノン; p-*tert* Amyl cyclohexanone; 4-*t*-Amylcyclohexanone
4. **Molecular Formula:** $C_{11}H_{20}O$
5. **Molecular Weight:** 168.28
6. **RIFM Number:** 347
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 124 °C at 16 mm Hg (FMA), 229.87 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System), >200 °F; CC (FMA)
3. **Log K_{OW}:** 3.3 (RIFM, 2010c), 3.4 (EPI Suite)
4. **Melting Point**: 19.58 °C (EPI Suite)
5. **Water Solubility:** 78.72 mg/L (EPI Suite)
6. **Specific Gravity:** 0.918 (FMA)
7. **Vapor Pressure:** 0.0805 mm Hg at 20 °C (EPI Suite v4.0), 0.02 mm Hg at 20 °C (FMA), 0.121 mm Hg at 25 °C (EPI Suite)
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:** A colorless liquid with a powerful earthy odor (Arctander, 1969)

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 v1.0)

1. **95**th **Percentile Concentration in Hydroalcoholics***:** 0.085% (RIFM, 2016)
2. **Inhalation Exposure*:** 0.00021 mg/kg/day or 0.015 mg/day (RIFM, 2016)
3. **Total Systemic Exposure**:** 0.0027 mg/kg/day (RIFM, 2016)

*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).

* **See IFRA Category 4 in Section X for maximum acceptable concentrations in finished products.

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:** 2-*tert*-Butylcyclohexanone (CAS # 1728-46-7)
   b. **Repeated Dose Toxicity:** 2-sec-Butylcyclohexanone (CAS # 14765-30-1)
   c. **Reproductive Toxicity:** 2-sec-Butylcyclohexanone (CAS # 14765-30-1)
   d. **Skin Sensitization:** None
   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 (discrete chemical) or composition (NCS)

4-*t*-Amylcyclohexanone 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 05/12/21 (ECHA, 2018).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 4-*t*-amylcyclohexanone 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)	0.027
2	Products applied to the axillae	0.0080
3	Products applied to the face/body using fingertips	0.16
4	Products related to fine fragrances	0.15
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.038
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.038
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.038
5D	Baby cream, oil, talc	0.013
6	Products with oral and lip exposure	0.061
7	Products applied to the hair with some hand contact	0.24
8	Products with significant ano-genital exposure (tampon)	0.013
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.29
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.061
10B	Aerosol air freshener	1.1
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.013
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	61

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 4-*t*-amylcyclohexanone, the basis was the reference dose of 0.16 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 350 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, 4-*t*-amylcyclohexanone does not present a concern for genotoxicity.

11.1.1.1. Risk Assessment. 4-*t*-Amylcyclohexanone was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no data assessing the mutagenic and clastogenic activity of 4-*t*-amylcyclohexanone; however, read-across can be made to 2-*tert*-butylcyclohexanone (CAS # 1728-46-7; see Section VI).

The mutagenic activity of 2-*tert*-butylcyclohexanone has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 2-*tert*-butylcyclohexanone in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2017a). Under the conditions of the study, 2-*tert*-butylcyclohexanone was not mutagenic in the Ames test, and this can be extended to 4-*t*-amylcyclohexanone.

The clastogenic activity of 2-*tert*-butylcyclohexanone was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 2-*tert*-butylcyclohexanone in DMSO at concentrations up to 1543 μg/mL in the DRF study. Micronuclei analysis was conducted at concentrations up to 249 μg/mL in the presence and absence of S9 for 3 h and in the absence of metabolic activation for 24 h 2-*tert*-Butylcyclohexanone did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2017b). Under the conditions of the study, 2-*tert*-butylcyclohexanone was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 4-*t*-amylcyclohexanone.

Based on the available data, p-*tert*-butylcyclohexanone does not present a concern for genotoxic potential, and this can be extended to 4-*t*-amylcyclohexanone.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/28/21.

11.1.2. Repeated dose toxicity

The MOE for 4-*t*-amylcyclohexanone is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk Assessment. There are no repeated dose toxicity data for the target material. Read-across material 2-sec-butylcyclohexanone (CAS # 14765-30-1; see Section VI) has sufficient data for the repeated dose toxicity endpoint. In an OECD 422 and GLP-compliant study, 10 Wistar Hans rats/sex/dose were fed 2-sec-butylcyclohexanone (purity: >97%) orally with diet at doses of 0, 650, 2000, and 6000 ppm. Mean daily intakes of the test material at 650 ppm were 48 mg/kg/day for males and 88 mg/kg/day for females; at 2000 ppm they were 151 mg/kg/day for males and 226 mg/kg/day for females; and at 6000 ppm, they were 377 mg/kg/day for males and 508 mg/kg/day for females. Dosing in male and female rats started 2 weeks prior to mating and lasted up to euthanasia in males (at least 28 days); in females, it lasted up to 13 days post-partum (about 51–56 days for females with offspring and 42 days for females without offspring). No treatment-related mortalities were observed at any dose level. Treatment-related clinical signs like piloerection were observed in all females at 2000 ppm and in both sexes at 6000 ppm along with hunched posture in 1 male and most females at 6000 ppm from study week 3 onwards. Following week 4, there was a significant decrease in bodyweight gain in males and females at 2000 and 6000 ppm, as well as a decrease in food consumption at these doses. Treatment-related effects were observed in hematology, biochemistry, organ weights, and histopathology parameters. These were considered to be secondary to weight loss and presented with low severity. Based on the observed clinical signs at doses ≥2000 ppm, the NOAEL was considered to be 650 ppm (48 mg/kg/day) for both sexes (RIFM, 2018).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 422 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 48/3 or 16 mg/kg/day.

Therefore, the 4-*t*-amylcyclohexanone MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2-sec-butylcyclohexanone NOAEL (mg/kg/day) by the total systemic exposure (mg/kg/day) for 4-*t*-amylcyclohexanone, 16/0.0027, or 5926.

In addition, the total systemic exposure to 4-*t*-amylcyclohexanone (2.7 μg/kg/day) is below the TTC threshold (9 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose (RfD) of 0.16 mg/kg/day.

11.1.3. 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 4-*t*-amylcyclohexanone was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 16 mg/kg/day by the uncertainty factor, 100 = 0.16 mg/kg/day.

*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: ECHA, 2018.

Literature Search and Risk Assessment Completed On: 04/29/21.

11.1.4. Reproductive toxicity

The MOE for 4-*t*-amylcyclohexanone is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.4.1. Risk Assessment. There are no reproductive toxicity data for the target material. Read-across material 2-sec-butylcyclohexanone (CAS # 14765-30-1, see Section VI) has sufficient data for the reproductive toxicity endpoint. In an OECD 422/GLP combined repeated dose toxicity study with a reproduction/developmental toxicity screening test, groups of 10 Wistar Hans rats/sex/dose were fed diets containing 2-sec-butylcyclohexanone at doses of 0 (basal diet only), 650, 2000, or 6000 ppm (mean daily intake of 0, 48, 151, and 377 mg/kg/day for males and 0, 88, 226, and 508 mg/kg/day for females, respectively). The animals were dosed for 2 weeks prior to mating, during mating, and

continued until euthanization for males (at least 28 days) and up to 13 days after delivery for females (51–56 days for females with offspring and 42 days for females without offspring). In addition to systemic toxicity parameters, reproductive toxicity parameters were also assessed. High-dose dams exhibited statistically significant decreases in body weight and bodyweight gain, which was associated with decreased food consumption throughout the pre-mating, post-coitum, and lactation periods. Four females were not pregnant despite evidence of mating (1 control, 2 low-dose, and 1 high-dose); no abnormalities were observed in the reproductive organs. At 6000 ppm, significant changes in the length (1/10 dams) and acyclic estrous cycle (6/10 dams) were reported. However, most of these dams had normal litters, and no abnormalities were observed in the reproductive organs that could account for the effect on estrous cyclicity. This effect was considered to be possibly a secondary effect of the bodyweight loss (and stress related to the severely reduced food consumption) in the first treatment week (when vaginal lavage samples for estrous cycle examination were collected). One mid-dose dam (with a normal litter) also exhibited an acyclic estrous cycle, which can sporadically occur as background finding (1/316 control females, period 2015–2017). Given the low incidence of this finding (1/10 females) and in the absence of other potentially treatment-related reproductive or developmental effects at this dose, this incidence was not considered to be adverse. Statistically significant decreases in pup body weight and bodyweight gain were reported at 6000 ppm from birth (10%) and on PND 13 (30%). No other treatment-related adverse effects were reported for fertility or on the development of pups. Thus, the NOAEL for effects on fertility was considered to be 2000 ppm or 226 mg/kg/day, based on alterations in length and acyclic estrous cycle observed among the high-dose group dams. The NOAEL for developmental toxicity was considered to be 2000 ppm or 226 mg/kg/day, based on decreased pup body weight among high-dose group pups (RIFM, 2018; also available at ECHA, 2018). **Therefore, the 2-sec-butylcyclohexanone MOE for the reproductive toxicity endpoint can be calculated by dividing the 2-sec-butylcyclohexanone NOAEL in mg/kg/day by the total systemic exposure to 2-sec-butylcyclohexanone, 226/0.0027, or 83704.**

In addition, the total systemic exposure to 4-*t*-amylcyclohexanone (2.7 μg/kg/day) is below the TTC threshold (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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/29/21.

11.1.5. Skin sensitization

Based on the existing data, 4-*t*-amylcyclohexanone is considered a skin sensitizer with a defined NESIL of 350 μg/cm^2.

11.1.5.1. Risk Assessment. Based on the existing data, 4-*t*-amylcyclohexanone is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a modified murine local lymph node assay (LLNA), 4-*t*-amylcyclohexanone did not induce contact sensitization up to 50% (ECHA, 2018; 001 Key study). In a human maximization test with 4-*t*-amylcyclohexanone at 8% (5520 μg/cm^2) in petrolatum, no skin sensitization reactions were observed (RIFM, 1973). However, in 2 Confirmation of No Induction in Humans tests (CNIHs) with 1.25% or 689 μg/cm^2 4-*t*-amylcyclohexanone in 1:3 ethanol:diethyl phthalate (EtOH:DEP) and 2.5% (1938 μg/cm^2) of 4-*t*-amylcyclohexanone in 95% EtOH, reactions indicative of sensitization were observed in 1/100 and 1/42 volunteers, respectively (RIFM, 2012a; RIFM, 1964a). However, in 2 other CNIHs with 0.65% (358 μg/cm^2) 4-*t*-amylcyclohexanone in 1:3 EtOH:DEP or 1.25% (969 μg/cm^2) 4-*t*-amylcyclohexanone in 95% EtOH, no reactions indicative of sensitization were observed in any of the 108 and 41 volunteers, respectively (RIFM, 2012b; RIFM, 1964b).

Based on weight of evidence (WoE) from structural analysis and animal and human studies, 4-*t*-amylcyclohexanone is a sensitizer with a WoE NESIL of 350 μg/cm^2 (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 0.16 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/12/21.

11.1.6. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4-*t*-amylcyclohexanone would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.6.1. Risk Assessment. There are no phototoxicity studies available for 4-*t*-amylcyclohexanone 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, 4-*t*-amylcyclohexanone does not present a concern for phototoxicity or photoallergenicity.

11.1.7. 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: 04/29/21.

11.1.8. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 4-*t*-amylcyclohexanone is below the Cramer Class III TTC value for inhalation exposure local effects.

Table 1
Data summary for 4-*t*-amylcyclohexanone.

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
NA	NA	358	5520	689	350

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.

11.1.8.1. Risk Assessment. There are no inhalation data available on 4-*t*-amylcyclohexanone. Based on the Creme RIFM Model, the inhalation exposure is 0.015 mg/day. This exposure is 31.3 times 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: 05/04/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 4-*t*-amylcyclohexanone 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, 4-*t*-amylcyclohexanone 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 4-*t*-amylcyclohexanone 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, 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.

11.2.1.1. Risk Assessment. Based on the current Volume of Use (2015), 4-*t*-amylcyclohexanone presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. **RIFM, 2010a:** The biodegradability of the test material was evaluated according to the OECD 302C method. Based on the conditions of the study, biodegradation of 54% was observed after 61 days.

RIFM, 2010b: The ready biodegradability of the test material was evaluated in the manometric respirometry test according to the OECD 301F method. No biodegradation was observed after 42 days.

RIFM, 2017c: The ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D guideline. Biodegradation of 3% was observed after 28 days.

11.2.2.2. Ecotoxicity. No data available.

11.2.2.3. Other available data. 4-*t*-Amylcyclohexanone has been registered for REACH with no additional data available at this time.

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

Endpoints used to calculate PNEC are underlined.

	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>16.79</u>			1000000	0.01679	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	7.694	<u>4.970</u>	6.311	10000	0.4970	Neutral Organics

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.3	3.3
Biodegradation Factor Used	0.1	0.1
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 material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.4970 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 VoU.

Literature Search and Risk Assessment Completed On: 05/04/21.

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 05/12/21.

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 E. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fct.2021.112560.

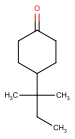
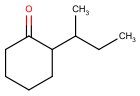
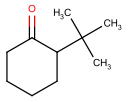
Appendix

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	4-*t*-Amylcyclohexanone	2-sec-Butylcyclohexanone	2-*tert*-Butylcyclohexanone
CAS No.	16587-71-6	14765-30-1	1728-46-7
Structure			
Similarity (Tanimoto Score)		0.87	0.87
Read-across Endpoint		• Repeated Dose Toxicity • Reproductive Toxicity	• Genotoxicity
Molecular Formula	$C_{11}H_{20}O$	$C_{10}H_{18}O$	$C_{10}H_{18}O$
Molecular Weight	168.28	154.25	154.25
Melting Point (°C, EPI Suite)	19.58	1.92	8.41
Boiling Point (°C, EPI Suite)	229.87	218.54	210.92
Vapor Pressure (Pa @ 25°C, EPI Suite)	16.13	28.26	40.93
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.40	2.94	2.91
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	78.72	222.70	239.80
J_{max} (μg/cm^2/h, SAM)	18.697	48.316	34.621
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	2.14E+001	1.61E+001	1.61E+001
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)	• No alert found		• 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
Repeated Dose Toxicity			
Repeated Dose (HESS)	• Not categorized	• Not categorized	
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH or NH2 group	• Non-binder, without OH or NH2 group	
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (good reliability)	• Toxicant (good reliability)	
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 4-*t*-amylcyclohexanone (CAS # 16587-71-6). 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, 2-sec-butylcyclohexanone (CAS # 14765-30-1) and 2-*tert*-butylcyclohexanone (CAS # 1728-46-7) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 2-sec-Butylcyclohexanone (CAS # 14765-30-1) was used as a read-across analog for the target material 4-*t*-amylcyclohexanone (CAS # 16587-71-6) for the reproductive toxicity and repeated dose toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to a class of alkyl-substituted cyclohexanones.
  - o The target material and the read-across analog share a cyclohexanone moiety.
  - o The key difference between the target material and the read-across analog is that the target material has a *tert*-pentyl substitution in position 4, whereas the read-across analog has a *sec*-butyl substitution in position 2. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across 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 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 Both the target material and the read-across analog are labeled as a toxicant for the developmental toxicity (CAESAR) classification scheme. The data described in the reproductive toxicity section show that the MOE is adequate at the current level of use. Therefore, the predictions 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.
- 2-*tert*-Butylcyclohexanone (CAS # 1728-46-7) was used as a read-across analog for the target material 4-*t*-amylcyclohexanone (CAS # 16587-71-6) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of alkyl-substituted cyclohexanones.
  - o The target material and the read-across analog share a cyclohexanone moiety.

- The key difference between the target material and the read-across analog is that the target material has a *tert*-pentyl substitution in position 4, whereas the read-across analog has a tert-butyl substitution in position 2. This structural difference is toxicologically insignificant.
- The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- 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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