RIFM fragrance ingredient safety assessment, \textit{p-}\textit{tert}-butylcyclohexanone, CAS Registry Number 98-53-3

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The Panel Expert 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 (Appl 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 NSIL).

*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. p-tert-Butylcyclohexane was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data and read-across to 2-tert-butylcyclohexane (CAS # 1728-46-7) show that p-tert-butylcyclohexane is not expected to be genotoxic. Data on read-across material 2-sec-butylcyclohexanone (CAS # 14765-30-1) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints. Data from read-across analog 4-t-amylocyclohexane (CAS # 16587-71-6) provided a No Expected Sensitization Induction Level (NESIL) of 350 μg/cm² for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; p-tert-butylcyclohexane is not expected to be phototoxic/photoallergic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material, and the exposure to p-tert-butylcyclohexane is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; p-tert-butylcyclohexane 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 (Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (ECHA REACH Dossier: 4-tert-Butylcyclohexane; ECHA, 2011; RIFM, 2017)

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/cm². (RIFM 2012b)

Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergic. (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: 32% (OECD 301D) (ECHA REACH Dossier: 4-tert-Butylcyclohexane; ECHA, 2011)

Bioaccumulation: Screening-level: 38.3 L/kg (EPISuite v4.11; US EPA, 2012a)

Ecotoxicity: Screening-level: Fish LC50: 33.61 mg/L. (RIFM Framework; Salvo et al., 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) < 1 (RIFM Framework; Salvo et al., 2002)

Critical Ecotoxicity Endpoint: Fish LC50: 33.61 mg/L. (RIFM Framework; Salvo et al., 2002)

RIFM PNEC in: 0.03361 μg/L (RIFM Framework; Salvo et al., 2002)

• Revised PEC/PNECs (2015 IFRA VoU): North America and Europe: not applicable; cleared at screening-level

1. Identification

1. Chemical Name: p-tert-Butylcyclohexane

2. CAS Registry Number: 98-53-3

3. Synonyms: 4-tert-Butylcyclohexane; Cyclohexanone, 4-(1,1-dimethylethyl)-; 74444-1-5; C₁₅H₂₄O; p-tert-Butylcyclohexanone

4. Molecular Formula: C₁₅H₂₄O

5. Molecular Weight: 214.25

6. RIFM Number: 530
7. **Stereochemistry:** Isomer not specified. One chiral center and 2 enantiomers possible.

2. **Physical data**
   1. **Boiling Point:** 113 °C at 20 mm Hg (Fragrance Materials Association (FMA)), 210.92 °C (EPI Suite)
   2. **Flash Point:** 95 °C (Globally Harmonized System), 190 °F; CC (FMA)
   3. **Melting Point:** 2015; Safford et al., 2017; and Comiskey et al., 2017.
   4. **Vapor Pressure:** 0.105 mm Hg at 20 °C (EPI Suite v4.0), 0.06 mm Hg at 20 °C (FMA), 0.173 mm Hg at 25 °C (EPI Suite)
   5. **Appearance/Organoleptic:** Colorless or white crystals with a powerful, dry-camphoraceous, slightly minty odor with woody cedary-patchouli-like undertones
   6. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol⁻¹ cm⁻¹)
   7. **Water Solubility:** 239.8 mg/L (EPI Suite)
   8. **Melting Point:** [FMA], 210.92 °C (Globally Harmonized System), 0.173 mm Hg at 25 °C (EPI Suite)
   9. **Log Kow:** 2.91 (EPI Suite)

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. **95th Percentile Concentration in Hydroalcohols:** 0.0060% (RIFM, 2016)
   2. **Inhalation Exposure**: 0.000044 mg/kg/day or 0.0033 mg/day (RIFM, 2016)
   3. **Total Systemic Exposure**: 0.00034 mg/kg/day (RIFM, 2016)

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

   **95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 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 et al., 2015; Safford et al., 2017; and 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**
   1. **Cramer Classification:** Class II, Intermediate

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2. **Analogs Selected:**
   a. **Genotoxicity:** 2-tert-Butylcyclohexanone (CAS # 1728-46-7)
   b. **Repeated Dose Toxicity:** 2-sec-butylocyclohexanone (CAS # 14765-30-1)
   c. **Reproductive Toxicity:** 2-sec-Butylcyclohexanone (CAS # 14765-30-1)
   d. **Skin Sensitization:** 4-t-Amylcylohexanone (CAS # 16587-71-6)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None

7. **Metabolism**
   No relevant data available for inclusion in this safety assessment.

7.1. **Additional references**

8. **Natural occurrence**

   *p*-tert-Butylcyclohexanone is not reported to occur in foods by the VCP.


9. **REACH dossier**

   Available; accessed 03/26/20 (ECHA, 2011).

10. **Conclusion**

    The maximum acceptable concentrations in finished products for *p*-tert-butylcyclohexanone are detailed below.

    | IFRA Category | Description of Product Type | Maximum Acceptable Concentrations in Finished Products (%) |
    |--------------|------------------------------|----------------------------------------------------------|
    | 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 | 0.088 |
    | 7            | Products applied to the hair with some hand contact | 0.26 |
    | 8            | Products with significant anogenital 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) | 0.13 |
    | 10B          | Aerosol air freshener | 0.52 |
    | 11           | Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad) | 0.013 |

(continued on next page)
11. Summary

11.1. Genotoxicity

Based on the current existing data and use levels, p-tert-butylcyclohexanone does not present a concern for genetic toxicity.

11.1.1. Risk assessment

p-tert-Butylcyclohexanone 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.

The mutagenic activity of p-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 and preincubation methods. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA102 were treated with p-tert-butylcyclohexanone in an unspecified solvent 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 (ECHA, 2011a). Under the conditions of the study, p-tert-butylcyclohexanone was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of p-tert-butylcyclohexanone; however, read-across can be made to 2-tert-butylcyclohexanone (CAS # 1728-46-7; see Section VI). 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 dimethyl sulfoxide (DMSO) at concentrations up to 1543 μg/mL in a dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 400 μg/mL in the presence and absence of metabolic activation. 2-tert-Butylcyclohexanone did not induce binucleated cells with micronuclei when tested up to the cytotoxic level concentration in either the presence or absence of an S9 activation system (RIFM, 2017). 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 p-tert-butylcyclohexanone.

Based on the data available, 2-tert-butylcyclohexanone does not present a concern for genotoxic potential, and this can be extended to p-tert-butylcyclohexanone.

11.1.2. Additional references

None.

11.1.3. Literature search and risk assessment completed on 05/22/20.

11.2. Repeated dose toxicity

The MOE for p-tert-butylcyclohexanone is adequate for the repeated dose toxicity endpoint at the current level of use.

11.2.1. Risk assessment

There are no repeated dose toxicity data on p-tert-butylcyclohexanone. 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 (≥97% purity) 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 were 377 mg/kg/day and 508 mg/kg/day. Dosing in male and female rats started 2 weeks prior to mating and lasted up to euthanasia in males (at least 28 days) and in females 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 such as 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 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 p-tert-butylcyclohexanone 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 for p-tert-butylcyclohexanone, 16/0.0034, or 47059.

In addition, the total systemic exposure to p-tert-butylcyclohexanone (0.34 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

11.2.1.1. Derivation of reference dose (RFD). 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, 2020b) and a reference dose of 0.16 mg/kg/day.

The RIFM Criteria Document (Api et al., 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 p-tert-butylcyclohexanone 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 lowest NOAEL was derived from read-across material 2-sec-butylcyclohexane (CAS # 14765-30-1).

*The Expert Panel for Fragrance Safety is composed of scientific and
11.3. Reproductive toxicity

The MOE for p-tert-butylcyclohexanone is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

11.3.1. Risk assessment

There are no reproductive toxicity data on p-tert-butylcyclohexanone. Read-across material 2-sec-butylocyclohexanone (CAS # 14765-30-1; see Section VI) has sufficient reproductive toxicity data.

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-butylocyclohexanone 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 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 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 1/10 dams had a significant change of the estrous cycle, and 6/10 were reported to have an acyclic estrous cycle. 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 most likely 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 estrus cycle examination were collected). One mid-dose dam (with a normal litter) also exhibited an acyclic estrous cycle, which can sporadically occur as a 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, 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 1/10 dams had a significant change of the estrous cycle, and 6/10 were reported to have an acyclic estrous cycle. 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 most likely 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 estrus cycle examination were collected). One mid-dose dam (with a normal litter) also exhibited an acyclic estrous cycle, which can sporadically occur as a 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. 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 in ECHA, 2018a).

Therefore, the p-tert-butylcyclohexanone MOE for the reproductive toxicity endpoint can be calculated by dividing the 2-sec-butylocyclohexanone NOAEL in mg/kg/day by the total systemic exposure to p-tert-butyl-cyclohexanone. 226/0.00034, or 664705.

In addition, the total systemic exposure to 2-cyclohexylcyclohexanone (0.34 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

11.3.2. Additional references

ECHA, 2018a

11.3.3. Literature search and risk assessment completed on 09/03/20.

11.4. Skin sensitization

Based on the existing data and read-across material 4-t-amylocyclohexanone (CAS # 16587-71-6), p-tert-butylcyclohexanone is considered a skin sensitizer with a defined NESIL of 350 μg/cm².

11.4.1. Risk assessment

Limited skin sensitization studies are available for p-tert-butylcyclohexanone. Based on the existing data and read-across material 4-t-amylocyclohexanone (CAS # 16587-71-6; see Section VI), p-tert-butylcyclohexanone is considered a skin sensitizer. The chemical structure of these materials indicates that they would be expected to react with skin proteins directly, as well as through metabolites and autoxidation products (Roberts et al., 2007; OECD Toolbox v4.2; TIMES-SS v2.28.1). In separate modified murine local lymph node assays (LLNAs), p-tert-butylcyclohexanone and read-across material 4-t-amylocyclohexanone did not induce contact sensitization up to 50% (ECHA, 2011a; ECHA, 2018a). In human maximization tests with 6% (4140 μg/cm²) p-tert-butylcyclohexanone and 8% (5520 μg/cm²) read-across material 4-t-amylocyclohexanone in petrolatum, no skin sensitization reactions were observed (RIFM, 1974a; RIFM, 1973). In contrast, in 2 Confirmation of No Induction in Humans (CNIH) tests with 1.25% or 689 μg/cm² read-across material 4-t-amylocyclohexanone in 1:3 ethanol:diethyl phthalate (EtOH:DEP) and 2.5% (1938 μg/cm²) of read-across material 4-t-amylocyclohexanone in 95% EtOH, reactions indicative of sensitization were observed in 1/100 and 1/42 volunteers, respectively (RIFM, 2012; RIFM, 1964a). However, in 2 other CNIH tests with 0.65% (358 μg/cm²) read-across material 4-t-amylocyclohexanone in 1:3 ethanol: diethyl phthalate or 1.25% (969 μg/cm²) read-across material 4-t-amylocyclohexanone 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, animal and human studies, and data on the read-across material 4-t-amylocyclohexanone, p-tert-butylcyclohexanone is a sensitizer with a WoE NESIL of 350 μg/cm² (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 0.16 mg/kg/day.

11.4.2. Additional references

Klecak (1979); Klecak (1985).

11.4.3. Literature search and risk assessment completed on 05/08/20.

11.5. Phototoxicity/photoallergenicity

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

11.5.1. Risk assessment

There are no phototoxicity studies available for p-tert-butylcyclohexanone 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 et al., 2009). Based on the lack of absorbance, p-tert-butylcyclohexanone does not present a...
The 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 phototoxicity, 1000 L mol⁻¹ • cm⁻¹ (Henry et al., 2009).

### 11.5.3. Additional references
None.

### 11.5.4. Literature search and risk assessment completed on 05/04/20.

### 11.6. Local respiratory toxicity

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

#### 11.6.1. Risk assessment

There are no inhalation data available on p-tert-butylocyclohexanone. Based on the Creme RIFM Model, the inhalation exposure is 0.0033 mg/day. This exposure is 142.4 times lower than the Cramer Class III* TTC value of 0.47 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.

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

#### 11.6.2. Additional references
None.

#### 11.6.3. Literature search and risk assessment completed on 05/04/20.

2. Environmental Endpoint Summary:

11.7. Screening-level assessment

A screening-level risk assessment of p-tert-butylocyclohexanone 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 Kow, 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, p-tert-butylocyclohexanone was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified p-tert-butylocyclohexanone as possibly persistent but not bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent and bioaccumulative and toxic, or very persistent and very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFCBF looks for 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 BCFCBF found in EPI Suite v4.11).

#### 11.8. Risk assessment

Based on the current Volume of Use (2015), p-tert-butylocyclohexanone does not present a risk to the aquatic compartment in the screening-level assessment.

#### 11.9. Key studies

11.9.1. Biodegradation

No data available.

11.9.2. Ecotoxicity

No data available.

11.10. Other available data

p-tert-Butylocyclohexanone has been registered under REACH and the following data is available (ECHA, 2011a):

- The ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D guideline. After 28 days, biodegradation of 32% was observed.
- The algae growth inhibition test was conducted according to the OECD 201 guideline under static conditions. The 72-h EC50 values based on the mean measured concentration for growth rate and biomass...
were reported to be 60 mg/L and 45 mg/L, respectively.

11.10.1. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

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

<table>
<thead>
<tr>
<th>Exposure Information</th>
<th>Europe (EU)</th>
<th>North America (NA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log K_{ow} Used</td>
<td>2.91</td>
<td>2.91</td>
</tr>
<tr>
<td>Biodegradation Factor Used</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dilution Factor</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Regional Volume of Use Tonnage Band</td>
<td>1–10</td>
<td>1–10</td>
</tr>
<tr>
<td>Risk Characterization: PEC/PNEC</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

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

The RIFM PNEC is 0.03361 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

11.10.2. Literature search and risk assessment completed on 05/07/20.

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/
- **SciFinder**: https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **National Library of Medicine’s Toxicology Information Services**: https://toxnet.nlm.nih.gov/
- **IARC**: https://monographs.iarc.fr
- **EPA ACToR**: https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS**: https://ofmpub.epa.gov/opthpvp/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japan Existing Chemical Data Base (JECDB)**: http://dra4.nih.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 04/21/21.

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.2021.112709.

Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (RIFM, 2020a). 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, 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).

\( J_{\text{max}} \) values were calculated using the RIFM Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).

DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions 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.

The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

To keep continuity and compatibility with \textit{in silico} alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

| Principal Name | Target Material Read-across Material Read-across Material Read-across Material |
|----------------|---------------------------------|---------------------------------|---------------------------------|
| CAS No.        | p-tert-Butylcyclohexanone 98-53-3 | 2-tert-Butylcyclohexanone 1728-46-7 | 4-t-Amylcyclohexanone 16587-71-6 | 2-sec-Butylcyclohexanone 14765-30-1 |
| Molecular Weight | 154.253 | 154.253 | 168.28 | 154.253 |
| Melting Point (°C, EPI Suite) | 48.50 | 8.41 | 19.58 | 1.92 |
| Boiling Point (°C, EPI Suite) | 210.92 | 210.92 | 229.87 | 218.54 |
| Vapor Pressure (Pa @ 25°C, EPI Suite) | 2.31E+01 | 4.09E+01 | 1.61E+01 | 2.83E+01 |
| Log KOW | 2.91 | 2.91 | 3.4 | 2.94 |
| Henry's Law (Pa⋅m^3/mol, Bond Method, EPI Suite) | 17.06 | 17.06 | 7.32 | 16.40 |

**Summary**

There are insufficient toxicity data on \textit{p-tert}-butylcyclohexanone (CAS # 98-53-3). Hence, \textit{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-tert-
butylcyclohexanone (CAS # 1728-46-7), 4-amylocyclohexanone (CAS # 16587-71-6), and 2-sec-butylcyclohexanone (CAS # 14765-30-1) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 2-tert-Butylcyclohexanone (CAS # 1728-46-7) was used as a read-across analog for the target material p-tert-butylcyclohexanone (CAS # 98-53-3) for the genotoxicity endpoint.
- The target material and the read-across analog are structurally similar and belong to a class of substituted cyclohexanones.
- The target material and the read-across analog share a cyclohexanone substructure.
- The key difference between the target material and the read-across analog is that the target material has a p-tert-butyl group while the read-across has an o-tert-butyl group on the ring. This structural difference is toxicologically insignificant.
- 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.
- There are no alerts for the target material or the read-across analog. In silico alerts are consistent with data.
- 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.
- 4-tert-Amylocyclohexanone (CAS # 16587-71-6) was used as a read-across analog for the target material p-tert-butylocyclohexanone (CAS # 98-53-3) for the skin sensitization endpoint.
- The target material and the read-across analog are structurally similar and belong to a class of substituted cyclohexanones.
- The target material and the read-across analog share a cyclohexanone substructure.
- The key difference between the target material and the read-across analog is that the target material has a p-tert-butyl group, whereas the read-across material has a p-tert-amyl group on the ring. This structural difference is toxicologically insignificant.
- 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.
- There are no alerts for the target material or the read-across analog. In silico alerts are consistent with data.
- 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.

2-sec-Butylcyclohexanone (CAS # 14765-30-1) was used as a read-across analog for the target material p-tert-buyslocyclohexanone (CAS # 98-53-3) for the repeated dose toxicity and reproductive toxicity endpoints.

- The target material and the read-across analog are structurally similar and belong to a class of substituted cyclohexanones.
- The target material and the read-across analog share a cyclohexanone substructure.
- The key difference between the target material and the read-across analog is that the target material has a p-tert-butyl group, whereas the read-across has an o-sec-butyl group on the ring. This structural difference is toxicologically insignificant.
- 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.
- There are no alerts for the target material or the read-across analog. In silico alerts are consistent with data.
- 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.

References


