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Short Review

RIFM fragrance ingredient safety assessment, cyclohexanone, CAS Registry Number 108-94-1

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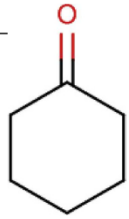
Name: Cyclohexanone
CAS Registry Number: 108-94-1



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 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
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications. Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database v2.0.5 (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) among reliable studies.

*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.

Cyclohexanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that cyclohexanone is not genotoxic. Data on cyclohexanone provide a calculated MOE > 100 for the repeated dose toxicity and developmental and reproductive toxicity endpoints. The skin sensitization endpoint was completed using the DST for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; cyclohexanone 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 cyclohexanone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; cyclohexanone was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (ECHA REACH Dossier: Cyclohexanone; ECHA, 2011)

Repeated Dose Toxicity: NOAEL = 3.04 mg/kg/day. (Lim et al., 2018a,b)

Developmental and Reproductive Toxicity:

Developmental NOAEL = 500 mg/kg/day; Reproductive NOAEL = 469 mg/kg/day. (ECHA REACH Dossier: Cyclohexanone; ECHA, 2011; US EPA, 2010)

Skin Sensitization: No safety concerns at current, declared use levels; exposure is below the DST.

Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM Database)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Screening-level: 90–100% (OECD 301 F)	(ECHA REACH Dossier: Cyclohexanone; ECHA, 2011)
Bioaccumulation:	
Screening-level: 3.16 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 756.39 mg/L	(RIFM Framework; Salvito 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; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 756.39 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.756 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** Cyclohexanone
2. **CAS Registry Number:** 108-94-1
3. **Synonyms:** Anon; Hexanon; Hytrol O; Ketohexamethylene; Nadone; Pimelic ketone; Sextone; Cyclohexyl ketone; Cyclohexanone
4. **Molecular Formula:** $C_6H_{10}O$
5. **Molecular Weight:** 98.14
6. **RIFM Number:** 6111
7. **Stereochemistry:** No stereocenter and no stereoisomers possible.

2. Physical data

1. **Boiling Point:** 154.98 °C (EPI Suite)
2. **Flash Point:** 44 °C (GHS)
3. **Log K_{OW}:** 0.81 (Patel et al., 2002); 1.13 (EPI Suite)
4. **Melting Point**: 29.56 °C (EPI Suite)
5. **Water Solubility:** 24080 mg/L (EPI Suite)
6. **Specific Gravity:** 0.94700 to 0.95000 @ 25.00 °C*
7. **Vapor Pressure:** 2.94 mm Hg @ 20 °C (EPI Suite v4.0), 4.04 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** A colorless oily liquid with a powerful, minty-camphoraceous, cool, and solvent-like odor

*http://www.thegoodscentscompany.com/data/rw1098591.html, retrieved 5/21/2015

3. Exposure

1. **Volume of Use (worldwide band):** < 0.1 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.00084% (RIFM, 2014)
3. **Inhalation Exposure*:** 0.0000001 mg/kg/day or 0.0000057 mg/day (RIFM, 2014)
4. **Total Systemic Exposure**:** 0.000012 mg/kg/day (RIFM, 2014)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported inSection IV. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford, 2015a, 2017; and Comiskey et al., 2017).

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
II	II	II

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Developmental and Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. **Read-across Justification:** None

6. Metabolism

In humans, cyclohexanone is metabolized to cyclohexanol, which is conjugated with glucuronic acid and excreted mainly in urine, where very little cyclohexanone or cyclohexanol is found. The metabolism and kinetics of cyclohexanone were studied in a group of volunteers (4 males and 4 females) during and after 8-h inhalation exposures to 101, 207, and 406 mg/m^3. Following exposure to the highest dose, the metabolic yields of urinary cyclohexanol, 1,2- and 1,4- cyclohexanediol and their glucuronide conjugates were 1%, 39%, and 18%, respectively. The elimination half-times (t½) of the 1,2- and 1,4-diols, were 16 h and 18 h, respectively. Consequently, after repeated exposure over 5 days, there was no accumulation of urinary cyclohexanol, whereas there was cumulative excretion of the diols. The permeation rate of cyclohexanone liquid through the skin was 37–69 mg/cm^2hour, indicating that occupational exposure by this route is of minor importance (IARC, 1989; Mraz et al., 1994) (see Fig. 1).

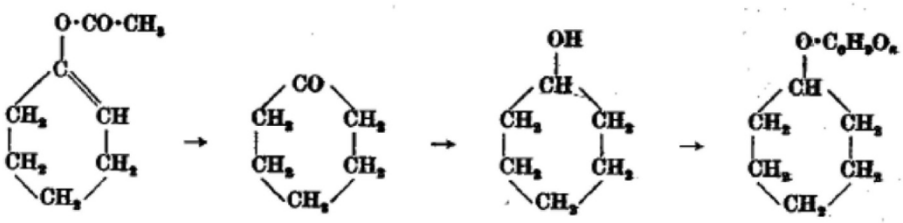


Fig. 1. Reduction and conjugation of cyclohexanone (adapted from Elliott et al., 1959).

Additional References: Deichmann and Thomas, 1943; US EPA, 1987a; US EPA, 1987b; ECHA, 2011 (accessed 10/31/18); Elliott et al., 1959; James and Waring, 1971; Longenecker et al., 1939.

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

Cyclohexanone is reported to occur in the following foods by the VCF*:

Acerola (*Malpighia*)
Avocado (*Persea americana* Mill.)
Beef.
Guinea hen.
Milk and milk products.
Mountain papaya (*C. candamarcensis, C. pubescens*)
Papaya (*Carica papaya* L.)
Tea.
Vanilla.
Wine.

*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. This is a partial list.

8. IFRA standard

None.

9. REACH dossier

Available; accessed 10/09/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. Based on the available data, there are controversial results in the literature regarding *in vitro* assays conducted on cyclohexanone. The majority of these assays are older studies and were not performed according to today's accepted guidelines and hence not accepted as the most reliable data for the assessment. In summary, several Ames tests were reported with negative results using a variety of protocols, strains, and methodologies (Kubo et al., 2002; Zeiger, 1997; US EPA ACToR; ECHA, 2011). In addition, a study with limited information was reported with Ames positive results (Massoud et al., 1980). An HPRT in Chinese hamster ovary cells was conducted with positive results without S9 but negative with S9 (Aaron et al., 1985). Several mouse lymphoma assays were conducted with paradoxical results reported (Mitchell et al., 1997; US EPA ACToR). Several chromosome aberration studies were conducted with mixed results (Aaron et al., 1985; Collin, 1971; Dyshlovoi et al., 1981; US EPA ACToR). Micronuclei were assessed in bovine peripheral lymphocytes with negative results (US EPA ACToR). Negative results were reported in a sister chromatid exchange assay in Chinese hamster ovary cells (OECD guideline deleted in 2014) (Aaron et al., 1985). *In vivo* assays (dominant lethal test in male rats via inhalation exposure, sperm abnormality tests in male mice via inhalation exposure, the cytogenetic test in male and female rat bone marrow cells after inhalation exposure, and the sex-linked recessive lethal in drosophila) were performed and were all considered negative (NIOSH, 1980). Based on these mixed data, only well-documented and well performed studies conducted in accordance with current-day scientific standards were used in this risk assessment.

The mutagenic activity of cyclohexanone 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 WP2*uvrA* were treated with cyclohexanone in 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 (ECHA, 2011). Under the conditions of the study, cyclohexanone was not mutagenic in the Ames test.

Additionally, lack of mutagenic activity was confirmed in a mammalian cell gene mutation assay. A forward gene mutation assay at the HPRT locus was conducted according to GLP regulations and OECD TG 476. Chinese hamster ovary cells were treated with cyclohexanone in DMSO at concentrations up to 980 μg/mL for 4 h in the presence and absence of metabolic activation and for 24 h in the absence of metabolic activation. No increases in the frequency of mutant colonies were observed with any concentration of the test item, either with or without metabolic activation (ECHA, 2011). Under the conditions of the study, cyclohexanone was not mutagenic to mammalian cells *in vitro*.

Therefore, based on the weight of evidence, cyclohexanone does not present a mutagenic concern.

For clastogenicity, in an *in vivo* mammalian bone marrow chromosomal aberration test conducted equivalent to the OECD 475 guideline, male and female CD-1 mice were exposed to vapors of cyclohexanone up to 400 ppm, and the test was negative (ECHA, 2011).

Cyclohexanone was assessed in the hen's egg test for analysis of micronucleus formation (HET-MN), which is more applicable to systemic exposure of a test material. Eggs were exposed to cyclohexanone for 3 days at concentrations up to 100 mg/egg. Although S9 metabolic activation is not explicitly added to the test system, evidence shows that the human metabolism is well reflected, and the HET-MN has a high intrinsic metabolic capacity which mirrors ADME. Harvested erythrocytes were assessed for micronuclei. Since this test was part of an inter-laboratory validation, 2 laboratories conducted the assay independently and in a blinded manner (material not identified). Both laboratories concluded that there was no induction of micronucleus formation, and cyclohexanone was negative in the HET-MN (Reisinger et al., 2017; Greywe et al., 2012).

These test results are supported by the absence of DNA damage and repair in an unscheduled DNA synthesis assay performed according to the OECD guideline 482 (deleted in 2014) in human fibroblasts

(NIOSH, 1980) and the absence of DNA repair synthesis in an assay assessing the uptake of tritiated thymidine uptake into mammalian cells (ECHA, 2011).

Therefore, based on the weight of evidence, cyclohexanone does not present a concern for clastogenicity.

As further evidence of lack of genotoxic potential, the carcinogenicity potency database (CPDB) reported negative experimental results in mice and rats for carcinogenicity effects of cyclohexanone (CPDB).

In conclusion, taking into account the full weight of evidence of available data from *in vitro* and *in vivo* tests that were conducted according to accepted scientific standards, cyclohexanone does not present a concern for genotoxic potential.

Additional References: Reus et al., 2012.

Literature Search and Risk Assessment Completed On: 11/10/18.

10.1.2. Repeated dose toxicity

The margin of exposure (MOE) for cyclohexanone is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on cyclohexanone a 90-day (OECD 408/GLP-compliant) study was conducted with Wistar rats (10 animals/sex/dose), where cyclohexanone (purity: 99.9%) was administered orally in drinking water at doses of 500, 2000, and 7000 ppm (equivalent to 40, 143, and 407 mg/kg/day). No treatment-related adverse effects were reported for mortality, clinical signs, body weight, food consumption, ophthalmoscopy, clinical chemistry, necropsy, organ weight, and histopathology up to the highest tested dose. Therefore, the NOAEL was considered to be 407 mg/kg/day (ECHA, 2011 [accessed 11/02/18]).

A 13-week inhalation toxicity study (GLP and non-guideline) was conducted using 10 F344 rats/sex/dose; animals were exposed to cyclohexanone at concentrations of 0, 100, 250, and 625 ppm (equivalent to 114.1, 285.2, and 713 mg/kg/day) for 6 h/day; 5 days/week. Liver weights in male rats were significantly increased at 250 (relative) and 625 (absolute and relative) ppm doses; female animals demonstrated a dose-dependent increase (no statistical significance). In addition, relative spleen weight was increased in males (625 ppm) and females (250 ppm) along with increased absolute kidney weight in males receiving the highest dose. Several hematological changes were reported in either sex at the highest dose along with changes in clinical chemistry at the 250 and 625 ppm doses. The hematological changes included increased MCH levels in males receiving the highest dose, while increased platelet counts and decreased prothrombin time were reported in females of the same group. At the 250 and 625 ppm doses, male AST and ALT levels increased, and in females ALT levels increased, but ALP levels decreased dose dependently. In animals of both sexes, BUN, albumin, and cholesterol levels increased dose dependently with changes being statistically significant only at the highest dose. Moreover, in males exposed to the highest dose, white focal lesions in the lungs were reported, while adhesions in the accessory lobe of the liver and right kidney were reported in females exposed to the 250 ppm dose; the findings were confirmed as indicative of alveolar macrophage aggregation and adhesion of hepatocytes to the renal capsule and atrophy of the glomeruli and tubules in the renal cortex, respectively. In male rats, bile duct hyperplasia in the liver was reported in 1, 2, and 4 animals in the 0, 250, and 625 ppm groups, respectively. Since treatment-related adverse effects were observed at all doses, a NOAEL could not be identified. Hence, the Expert Panel for Fragrance Safety elected to follow the American Conference of Governmental Industrial Hygienists guidelines to set a threshold limit of 25 ppm (equivalent to 28.9 mg/kg/day). In addition, the material was not considered to be genotoxic following extensive review of the available data (See Genotoxicity section above). The derived no effect level (DNEL) of 2 ppm was derived by applying an interspecies safety factor (2.5), intraspecies safety factor (5), and exposure duration factor (2) as default assessment factors (ECHA, 2012). **Therefore, a conservative NOAEL of 3.04 mg/kg/day was considered for repeated dose toxicity.**

Therefore, the cyclohexanone MOE for the repeated dose toxicity endpoint can be calculated by dividing the cyclohexanone NOAEL (mg/kg/day) by the total systemic exposure for cyclohexanone, 3.04/0.000012 or 253333.

In addition, the total systemic exposure to cyclohexanone (0.012 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.

Duration in detail	GLP/Guideline	No. of animals/dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/NOEL	Justification of NOAEL/LOAEL/NOEL	Reference
13 weeks	Not reported	B6C3F1 mice, 10 animals/sex/dose	Oral (drinking water)	0, 400, 2300, 6500, 13000, 25000, 34000, 47000 ppm (Equivalent to 0, 99, 568, 1600, 3210, 6170, 8390, and 11600 mg/kg/day for males and 0, 106, 608, 1720, 3430, 6610, 8980, and 12400 mg/kg/day for females) (purity: 96%)	Derived NOAEL - 25000 ppm in females and 13000 ppm in males (equivalent to 3210 mg/kg/day for males and 6610 mg/kg/day for females, respectively)	Based on decreased weight gain and mortality found at 2 highest doses (25000, selected for MTD in the report)	Lijinsky and Kovatch, 1986; US EPA, 2010
13 weeks, 6 h/day, 5 days per week	Not reported	B6C3F1 mice, 10 animals/sex/dose	Inhalation	0, 100, 250, and 625 ppm (equivalent to 170.3, 425.8 and 1064.4 mg/kg/day)	Reported NOAEL - 625 ppm (equivalent to 1064.4 mg/kg/day)	Based on no effects observed up to high dose tested	Lim et al., 2018a,b

10.1.2.2. Additional References. OECD SIDS on Cyclohexanone; US EPA, 2010; HSDB on Cyclohexanone (accessed 11/02/18)

10.1.2.3. Literature Search and Risk Assessment Completed On. 11/30/18.

10.1.3. Developmental and reproductive toxicity

The MOE for cyclohexanone is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.3.1. Risk assessment. There are sufficient developmental and reproductive toxicity data on cyclohexanone.

In a multi-generation GLP-compliant reproduction toxicity study (similar to OECD 416), Sprague Dawley rats (30 animals/sex/group) were exposed to cyclohexanone for 2 consecutive generations through whole-body inhalation at concentrations of 0, 250, 500, or 1000 (F0)/1400 (F1) ppm (equivalent to 0, 235, 469, and 939 (F0)/1314 (F1) mg/kg/day, respectively, using standard minute volume and body weight values for male and female Sprague Dawley rats) for 6 h/day. Parental

(F0) rats were mated 1:1 to produce F1 generation pups that were labeled as F1a. F1 generation rats were subsequently mated to produce F2 generation progeny that were separated into F2a and F2b litters. Examinations of F0 parental animals and F1a pups did not reveal any treatment-related adverse effects, and no microscopic changes were observed in the reproductive organs of rats exposed to 1000 ppm. No treatment-related adverse effects on the development of F0 progeny (F1a litter) were observed at any dose level. Due to the absence of toxicity at the highest dose of 1000 ppm in F0-treated animals, the highest dose was increased to 1400 ppm for F1 generation-treated animals (parental animals to F2a and F2b litters). Mortality of F1 animals that were exposed to 1400 ppm was observed in 2 males and 1 female during the first week of exposure. Body weights were statistically significantly decreased in males exposed to 1400 ppm during weeks 31 and 34 of treatment. Body weight for F1 dams during gestation and lactation of F2a and F2b litters were similar for treated animals at all tested doses and control groups. F1 animals exposed to 1000 ppm (post-weaning and prior to F1 parental animal selection and subsequent increase of the highest dose to 1400 ppm) exhibited clinical signs of ataxia, lacrimation, irregular breathing, and urine-soaked fur that continued for approximately 3 months after the highest dose was increased to 1400 ppm. Starting at week 16, F1 generation rats that were exposed to 1400 ppm adapted to treatment and lethargy was observed as a post-exposure reaction. During lactation days 1–4, statistically significant decreases in the number of viable F2 pups that were born to F1 rats exposed to 1400 ppm were observed. In F2a pups, body weights were statistically significantly decreased at doses of 250 and 500 ppm during the latter half of lactation, while the highest dose of 1400 ppm caused body weight decreases in the pups throughout the lactation period. Statistically significant decreases in F2b body weights were only observed at the highest dose. In all paired F1 males exposed to 1400 ppm, male fertility indices were 20% less than controls. Male fertility for males that were paired with fertile females and exposed to 1400 ppm was 24%–29% less than the controls. Male fertility data for intergroup differences revealed statistically significant decreases among 1400 ppm males compared to 250 ppm males during mating for F2a and F2b litters, and at 500 ppm during mating for F2b litters. Additionally, mating indices for rats exposed to 1400 ppm were statistically significantly less compared to 250 ppm during mating for F2a and F2b litters. A subsequent study on F1 parental males during the post-exposure recovery period found that in high-dose group males, the reproductive effects were reversible after males were unexposed for 2 days following the last exposure and prior to mating for 8 weeks. Necropsy examinations of all F1 parental animals and F2a and F2b weaned litters did not show any treatment-related lesions, and microscopic examinations of reproductive organs from control and F1 parental animals exposed to 1400 ppm did not reveal any treatment-related effects. The NOAEL for F0 parental toxicity was considered to be 1000 ppm or 939 mg/kg/day, the highest dose tested. The NOAEL for F1 parental toxicity was considered to be 500 ppm or 469 mg/kg/day, based on mortality, toxicologically relevant clinical signs, and decreased body weight observed in F1 generation animals exposed to 1400 ppm. The NOAEL for reproductive toxicity was also considered to be 500 ppm or 469 mg/kg/day, based on decreased male fertility and decreased mating of F1 parents from the highest dose of 1400 ppm. The NOAEL for developmental toxicity was considered to be 500 ppm or 469 mg/kg/day, based on decreased viability and survival of F2 generation litters born from F1 parental animals that were exposed to 1400 ppm (US EPA, 2019; OECD SIDS on Cyclohexanone; ECHA, 2011).

An OECD 414/GLP prenatal developmental toxicity study was conducted in pregnant female Himalayan rabbits (15/group), where cyclohexane was administered through oral gavage at doses of 0, 50, 250, or 500 mg/kg/day in distilled water from gestation days (GDs) 7–19. Rabbits that received 500 mg/kg/day had statistically significantly decreased food consumption, body weight, and bodyweight gain. There were no treatment-related adverse effects reported for gestational parameters or on the development of fetuses up to 500 mg/kg/day. Therefore, the NOAEL for maternal toxicity was considered to be 250 mg/kg/day, based on decreases in food consumption, body weight, and bodyweight gain among the highest-dose group dams. The NOAEL for developmental toxicity was considered to be 500 mg/kg/day, the highest dose tested (ECHA, 2011).

Multiple prenatal developmental toxicity studies were also conducted in rats (US EPA, 1984; OECD SIDS on Cyclohexanone), in which the NOAELs supported the results of the OECD 414 study in rabbits. The developmental toxicity NOAEL of 500 mg/kg/day was selected from the more robust OECD 414 study for the developmental toxicity endpoint. **Therefore, the cyclohexanone MOE for the developmental toxicity endpoint can be calculated by dividing the cyclohexanone NOAEL in mg/kg/day by the total systemic exposure to cyclohexanone, 500/0.000012 or 41666667.**

The reproductive toxicity NOAEL of 469 mg/kg/day was selected from the multi-generational study for the reproductive toxicity endpoint. **Therefore, the cyclohexanone MOE for the reproductive toxicity endpoint can be calculated by dividing the cyclohexanone NOAEL in mg/kg/day by the total systemic exposure to cyclohexanone, 469/0.000012 or 39083333.**

In addition, the total systemic exposure to cyclohexanone (0.012 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental and reproductive toxicity endpoints of a Cramer Class II material at the current level of use.

Duration in detail	GLP/ Guideline	No. of animals/ dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/ NOEL	Justification of NOAEL/LOAEL/NOEL	Reference
Gestation days 6–19, 6 h/ day	GLP/guideline not mentioned	Pregnant CD (Sprague Dawley derived) rats (26 animals/dose)	Inhalation (vehicle: air)	0, 303, 657, or 1410 ppm (equivalent to 0, 298, 646, and 1385 mg/kg/day)	NOAEC for maternal and developmental toxicity = 657 ppm or 646 mg/kg/day	Based on maternal toxicity (reduced body weight, increased incidence of lacrimation, lethargy, nasal discharge), and fetal toxicity (reduce fetal weight, increase in ossification variation) at 1410 ppm	US EPA, 1984; US EPA, 2010; OECD SIDS on Cyclohexanone
Gestation days 5–20, 7 h/ day	GLP/guideline not mentioned	Pregnant Sprague Dawley rats (10 animals/dose)	Inhalation	0, 100, 250, or 500 ppm (equivalent to 0, 98, 246, and 491 mg/kg/day) (purity: 99.8%)	NOAEC for maternal toxicity = 100 ppm or 98 mg/kg/day NOAEC for developmental toxicity = 500 ppm or 491 mg/kg/day	Based on the grey mottling of lungs in dams treated at 250 and 500 ppm No effects observed in highest dose tested in pups	OECD SIDS on Cyclohexanone; Health Canada, 2018; US EPA, 2010

Additional References: Seidenburg and Becker, 1987; Hall et al., 1974; US EPA, 2010 (accessed 11/02/18)

Literature Search and Risk Assessment Completed On: 11/21/18.

Table 1
Maximum acceptable concentrations for cyclohexanone that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	NRU[b]
2	Products applied to the axillae	0.0015%	6.6×10^{-5}%[b]
3	Products applied to the face using fingertips	0.029%	NRU[b]
4	Fine fragrance products	0.027%	0.0013%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	1.7×10^{-4}%[b]
6	Products with oral and lip exposure	0.016%	NRU[b]
7	Products applied to the hair with some hand contact	0.056%	NRU[b]
8	Products with significant ano-genital exposure	0.0029%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054%	1.7×10^{-5}%[b]
10	Household care products with mostly hand contact	0.19%	NRU[b]
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	NRU[b]

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

10.1.4. Skin sensitization

Based on the existing data and the application of dermal sensitization threshold (DST), cyclohexanone does not present a concern for skin sensitization under current, declared levels of use.

10.1.4.1. Risk assessment. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v4.2). Cyclohexanone was found to be negative in an *in vitro* glutathione depletion assay. (OECD toolbox v4.2) In a confirmatory human repeat insult patch test (HRIPT) with cyclohexanone of an unspecified concentration in petrolatum, 1 out of 10 subjects showed skin reactions comprised of definite edema and erythema (RIFM, 1977). The study stated that the re-challenge was performed on this subject, and no reactions indicative of skin sensitizations were observed. However, limited details were provided for the conditions of the re-challenge, and the data describing the subject's reaction in response to the re-challenge was not provided in the report.

Acting conservatively, due to the positive data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008, 2011, 2015b; Roberts et al., 2015). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for cyclohexanone that present no appreciable risk for skin sensitization based on the reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

10.1.4.2. Additional References. None.

10.1.4.3. Literature Search and Risk Assessment Completed On. 11/15/18.

10.1.5. Phototoxicity/photoallergenicity

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

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

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for cyclohexanone were obtained. The spectra indicate minor 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 et al., 2009).

10.1.5.3. Additional References. None.

10.1.5.4. Literature Search and Risk Assessment Completed On. 10/18/18.

10.1.6. Local respiratory toxicity

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

10.1.6.1. Risk assessment. There are insufficient inhalation data available on cyclohexanone. Based on the Creme RIFM Model, the inhalation exposure is 0.0000057 mg/day. This exposure is 82456 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.

10.1.6.2. Additional References. Gupta et al., 1979; Carpenter et al., 1949; Smyth et al., 1969; DeCeaurriz et al., 1983; Brondeau et al., 1989; Treon et al., 1943; Pinching and Doving, 1974; Specht et al., 1940; Frantik et al., 1994; Mraz et al., 1994; Klimisch et al. (1988); Mitran et al., 1997; Silver (1992); Mraz et al., 1998; Major and Silver, 1999; US EPA, 1987a; US EPA, 1987b; US EPA, 1984; NIOSH, 1980; Frederick et al., 2009; Willis et al., 2011; Lim et al., 2018a,b

10.1.6.3. Literature Search and Risk Assessment Completed On. 11/13/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of cyclohexanone was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, cyclohexanone 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) did not identify cyclohexanone as possibly persistent nor bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on the current VoU (2015), cyclohexanone presents no risk to the aquatic compartment in the screening-level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. No data available.

10.2.3.2. Ecotoxicity. No data available.

10.2.4. Other available data

Cyclohexanone ether has been registered for REACH, and the following data is available:

A Fish (Fathead minnow) acute toxicity study was conducted according to the OECD 203 method. The 96-h LC50 was reported to be 527–732 mg/L.

10.2.5. Risk assessment refinement

Since cyclohexanone has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>756.39</u>			1,000,000	0.756	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	1.13	1.13
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
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 0.756 μg/L. The revised PEC/PNECs for EU and North America are: not applicable. The material was cleared at the screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 11/19/18.

11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/

- **IARC:** http://monographs.iarc.fr
- **OECD SIDS:** http://webnet.oecd.org/hpv/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 05/20/19.

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.

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