Food and Chemical Toxicology 141 (2020) 111476

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short Review

RIFM fragrance ingredient safety assessment, 4-methyl-3-penten-2-one, CAS Registry Number 141-79-7

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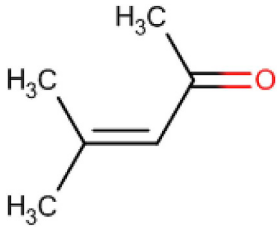
ARTICLE INFO

Keywords:
Genotoxicity
Repeated dose
Developmental, and reproductive toxicity
Skin sensitization
Phototoxicity/photoallergenicity
Local respiratory toxicity
Environmental safety

Version: 082319. This version replaces any previous versions.
Name: 4-Methyl-3-penten-2-one CAS Registry Number: 141-79-7



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https://doi.org/10.1016/j.fct.2020.111476
Received 14 February 2020; Received in revised form 18 May 2020; Accepted 27 May 2020
Available online 20 June 2020

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., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test

TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api, 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

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

4-Methyl-3-penten-2-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-methyl-3-penten-2-one is not genotoxic. Data on 4-methyl-3-penten-2-one provide a calculated margin of exposure (MOE) > 100 for the repeated dose, reproductive, and local respiratory toxicity endpoints. Data show that there are no safety concerns for 4-methyl-3-penten-2-one for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 4-methyl-3-penten-2-one is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; for the hazard assessment based on the screening data, 4-methyl-3-penten-2-one is not persistent, bioaccumulative, and toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards. For the risk assessment, 4-methyl-3-penten-2-one was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

Human Health Safety Assessment

Genotoxicity: Not genotoxic	(RIFM, 2009; RIFM, 2011)
Repeated Dose Toxicity: NOAEL = 1.07 mg/kg/day.	(ECHA REACH Dossier: 4-Methylpent-3-en-2-one; ECHA, 2011)
Reproductive Toxicity: Developmental toxicity: NOAEL = 314 mg/kg/day. Fertility: NOAEL = 107 mg/kg/day.	(ECHA REACH Dossier: 4-Methylpent-3-en-2-one; ECHA, 2011)
Skin Sensitization: Not a concern for skin sensitization under the current, declared levels of use.	(ECHA REACH Dossier: 4-Methylpent-3-en-2-one; ECHA, 2011)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra; RIFM Database)
Local Respiratory Toxicity: NOAEC = 12.4 mg/m^3.	(ECHA REACH Dossier: 4-Methylpent-3-en-2-one; ECHA, 2011)

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Critical Measured Value: 75% (OECD 301F) (ECHA REACH Dossier: 4-Methylpent-3-en-2-one; ECHA, 2011)

Bioaccumulation:

Screening-level: 3.71 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:
Screening-level: Not applicable
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; no Volume of Use in 2015 reported for Europe and North America

1. Identification

1. **Chemical Name:** 4-Methyl-3-penten-2-one
2. **CAS Registry Number:** 141-79-7
3. **Synonyms:** Isopropylidene acetone; Mesityl oxide; Methyl isobutenyl ketone; 3-Penten-2-one, 4-methyl-; 4-Methylpent-3-en-2-one; 4-Methyl-3-penten-2-one
4. **Molecular Formula:** $C_6H_{10}O$
5. **Molecular Weight:** 98.14
6. **RIFM Number:** 6133
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 130 °C @ 100 mm Hg (Fragrance Materials Association [FMA]), 119.61 °C (EPI Suite)
2. **Flash Point:** 87 °F; CC (FMA)
3. **Log K_{OW}:** 0.92 (Biobyte Corp.), 1.37 (EPI Suite)
4. **Melting Point**: -64.36 °C (EPI Suite)
5. **Water Solubility:** 8035 mg/L (EPI Suite)
6. **Specific Gravity:** 0.859 (FMA)
7. **Vapor Pressure:** 7.9 mm Hg 20 °C (FMA), 12.3 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:** Clear, colorless to pale yellow, oily, or viscous liquid with an unpleasant, pungent, grassy green or vegetable, acrylic odor

3. Volume of use (worldwide band)

1. < 0.1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1. **95**th **Percentile Concentration in Toothpaste:** 0.00048% (RIFM, 2017)

(No reported use in hydroalcoholics)

2. **Inhalation Exposure*:** < 0.0001 mg/kg/day or < 0.0001 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.0000032 mg/kg/day (RIFM, 2017)

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

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

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See the Appendix below for further details.

2. Analogs Selected:
a. Genotoxicity: None
b. Repeated Dose Toxicity: None
c. 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

7. Metabolism

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

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

4-Methyl-3-penten-2-one is reported to occur in the following foods by the VCF*:

Annatto (*Bixa orellana* L.)	Mushroom
Apple brandy (Calvados)	Rosemary
Citrus fruits	Wine
Guava and feyoa	

*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.

9. REACH dossier

Available; accessed 06/28/19 (ECHA, 2011)

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 4-methyl-3-penten-2-one does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 4-methyl-3-penten-2-one 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 4-methyl-3-penten-2-one 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, 2009). Under the conditions of the study, 4-methyl-3-penten-2-one was not mutagenic in the Ames test.

The clastogenic activity of 4-methyl-3-penten-2-one 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 4-methyl-3-penten-2-one in purified water at concentrations up to 981.4 μg/mL in the dose range finding study (DRF) study, and a micronuclei analysis was conducted at concentrations up to 981.4 μg/mL in the presence and absence of S9 for the 3-h treatment condition and up to 300 μg/mL in the absence of S9 for the 24-h treatment condition. 4-Methyl-3-penten-2-one did not induce binucleated cells with micronuclei when tested up to maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2011). Under the conditions of the study, 4-methyl-3-penten-2-one was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 4-methyl-3-penten-2-one does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/14/19.

11.1.2. Repeated dose toxicity

The MOE for repeated dose toxicity endpoint is adequate for 4-methyl-3-penten-2-one at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 4-methyl-3-penten-2-one. An OECD 422 and GLP-compliant inhalation study was conducted on groups of 12 Sprague Dawley rats/sex/dose. The animals were exposed to 4-methyl-3-penten-2-one at concentrations of 31, 103, and 302 ppm through inhalation for up to 49 days. These concentrations were equal to 0, 32, 107, and 314 mg/kg, respectively. No treatment-related mortality was reported during the study. Additionally, no treatment-related adverse effects were observed in hematology, clinical biochemistry, urinalysis, organ weights, or gross pathology. Several local respiratory effects were reported: minimal to minor sero-cellular exudate of the nasal passage and respiratory epithelium, minimal squamous metaplasia of the nasal passage and respiratory epithelium, minimal respiratory metaplasia, and minimal to minor chronic focal inflammation. The sero-cellular exudate was composed of a proteinaceous serum-like component and a cellular component that contained a small number of polymorphonuclear leukocytes. Nasal passage pathology was attributed to exposure to an irritating vapor. At all doses, body weight and food consumption were significantly lower than the controls. In addition, bodyweight gains in males were significantly decreased at the mid- and high-doses but, this effect was not seen in females. No treatment-related effects were reported for any of the investigated parameters except organ weights. Increases in relative weights of epididymides and testes were reported in males, but this effect was attributed to decreased bodyweight gains. Since body weight and food consumption were lower in all dose groups, a NOAEL could not be determined. Based on decreased body weight and food consumption, a LOAEL of 32 mg/kg/day was determined for repeated dose toxicity (ECHA, 2011).

A default safety factor of 10 was used when deriving a NOAEL from a LOAEL. The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 32/10 or 3.2 mg/kg/day.

A default safety factor of 3 was used when deriving a NOAEL from 28-day or OECD 422 studies (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 3.2/3 or 1.07 mg/kg/day.

Therefore, the MOE for the repeated dose toxicity endpoint is equal to the 4-methyl-3-penten-2-one NOAEL in mg/kg/day divided by the total systemic exposure to 4-methyl-3-penten-2-one, 1.07/0.0000032 or 334375.

In addition, the total systemic to 4-methyl-3-penten-2-one (0.0032 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

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

Additional References: Smyth (1942).

Literature Search and Risk Assessment Completed On: 07/30/19.

11.1.3. Reproductive toxicity

The MOE for 4-methyl-3-penten-2-one is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on 4-methyl-3-penten-2-one. An OECD 422/GLP combined repeated dose toxicity study with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were exposed to the test material 4-methylpent-3-en-2-one via inhalation (whole-body exposure) at concentrations of 0, 31, 103, or 302 ppm (equivalent to 0, 32, 107, and 314 mg/kg/day, respectively, as per the standard minute volume (MV) and body weight for male and female Sprague Dawley rats) for 6 h per day, 7 days per week. Rats were exposed for 14 days pre-mating, 14 days of mating, and up to lactation day 4 for females. In addition to systemic toxicity parameters, reproductive toxicity parameters were also assessed. A significant reduction in feed consumption corresponding to a reduction in mean body weight was observed in an exposure-dependent manner for all male- and female-exposed groups. Post-exposure increase in porphyrin nasal discharge was observed for all treatment groups, and post-exposure sialorrhea was observed in 3/12 high-exposure group male rats; these clinical signs indicated the irritating nature of the vaporized test material. Copulation was observed in all control and high-exposure group female rats, while only 11/12 female rats in the low- and mid-exposure groups. The mean number of dams which delivered a litter was statistically significantly lower in the 302 ppm group (7/12) when compared to the control group (12/12). For the second mating, the data for litters delivered by unexposed dams from

males of the high-exposure second mating was similar to the data collected from the litters delivered to control dams from the main study. The relative testes and epididymides weights for the high-exposure group males were significantly higher than the controls; the increased relative epididymides weights also extended for the mid-exposure group males. This difference was a result of decreased body weight for the mid- and high-exposure group male rats rather than an effect on the testes or epididymides, and there were no exposure-related changes observed during necropsy and histopathology examinations. The mean number of male pups per litter on post-partum days 0 and 4 were significantly higher in the 31 and 302 ppm exposure groups and was significantly lower for the 103 ppm exposure group when compared to the control group. Changes in the number of male and female pups per litter in all treatment groups were not concentration-dependent and hence, were not considered to be toxicologically significant. The mean litter weight was slightly lower, and the mean pup weight was slightly higher for the mid-exposure group, which was due to the lower number of pups per litter. Thus, the NOAEC for fertility was considered to be 103 ppm or 107 mg/kg/day, based on the decreased number of dams which delivered litters at 302 ppm. The NOAEC for developmental toxicity was considered to be 302 ppm or 314 mg/kg/day, the highest concentration tested (ECHA, 2011).

The 4-methyl-3-penten-2-one MOE for the fertility endpoint can be calculated by dividing the 4-methyl-3-penten-2-one NOAEL in mg/kg/day by the total systemic exposure to 4-methyl-3-penten-2-one, 107/0.0000032 or 33437500.

The 4-methyl-3-penten-2-one MOE for the developmental toxicity endpoint can be calculated by dividing the 4-methyl-3-penten-2-one NOAEL in mg/kg/day by the total systemic exposure to 4-methyl-3-penten-2-one, 314/0.0000032 or 98125000.

In addition, the total systemic exposure to 4-methyl-3-penten-2-one (0.0032 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/16/19.

11.1.4. Skin sensitization

Based on the existing data, 4-methyl-3-penten-2-one is not a concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Based on the existing data, 4-methyl-3-penten-2-one is not considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts, 2007; Toxtree 3.1.0; OECD Toolbox v4.2). 4-Methyl-3-penten-2-one was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens (RIFM, 2014; RIFM, 2015). In contrast, a guinea pig (Dunkin Hartley strain) maximization test showed no reactions indicative of skin sensitization (ECHA, 2011).

Based on weight of evidence (WoE) from structural analysis and animal studies, 4-methyl-3-penten-2-one does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/01/19.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4-methyl-3-penten-2-one would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 4-methyl-3-penten-2-one in experimental models. UV/Vis absorption spectra indicate minor absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, 4-methyl-3-penten-2-one does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) 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 $mol^{-1} \cdot cm^{-1}$ (Henry, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/22/19.

11.1.6. Local respiratory toxicity

The MOE for 4-methyl-3-penten-2-one is adequate for the local respiratory toxicity endpoint at the current level of use.

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In an OECD 422 and GLP-compliant toxicity study, 12 groups of Sprague Dawley rats/sex/dose were treated with 4-methyl-3-penten-2-one vapors at concentrations of 0, 124, 413 and 1211 mg/m^3 for 6 h/day, 7 days/week via whole-body inhalation (ECHA, 2011). Treatment duration was 49 days in males (pre-mating and during and after the mating period) and 39–49 days in females (2 weeks prior to mating and up to lactation day 4). Standard observations included mortality, cage-side observations, bodyweight changes, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. Minimal sero-cellular exudate within the olfactory epithelium in nasal passages was observed in all test groups in male and female rats. Respiratory epithelium metaplasia of minimal severity and chronic focal inflammation of minimal severity were observed in the high- and/or mid-exposure groups. Squamous metaplasia in the respiratory epithelium and sero-cellular exudate in the olfactory epithelium of minimal severity were the only local respiratory effects observed at 124 mg/m^3. Due to the common local irritation effects observed in all test groups, a NOAEC could not be established; hence a LOAEC for local respiratory toxicity was determined to be 124 mg/m^3. Therefore, by using a safety adjustment factor of 10, a NOAEC was estimated at 12.4 mg/m^3.

This NOAEC expressed in mg/kg lung weight/day is:

- (12.4 mg/m^3) × (1 m^3/1000 L) = 0.0124 mg/L
- MV of 0.17 L/min for a Sprague Dawley rat × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 61.2 L/day
- (0.0124 mg/L) × (61.2 L/d) = 0.76 mg/day
- (0.76 mg/day)/(0.0016 kg lung weight of rat*) = 475 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be < 0.0001 mg/day—this value was derived from the concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; Safford et al., 2015). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew, 2009) to give at least 0.000154 mg/kg lung weight/day resulting in an MOE of 3084416 (i.e., [475 mg/kg lung weight of rat/day]/[0.000154 mg/kg lung weight of human/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to inter-species and intra-species variation, the material exposure by inhalation at < 0.0001 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by, Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: “Comparative Physiology

and Anatomy," subsection, "Comparative Airway Anatomy."

Additional References: DeCeaurriz (1984); Carpenter (1949); Smyth (1942); Silverman (1946); Brondeau (1990); Exxon (1982); Specht (1940); Hart (1941); Johnson (2005).

Literature Search and Risk Assessment Completed On: 08/12/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 4-methyl-3-penten-2-one 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-methyl-3-penten-2-one was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 4-methyl-3-penten-2-one as possibly persistent or 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).

11.2.2. Risk assessment

Not applicable.

11.2.3. Key studies

11.2.3.1. Biodegradation. Not available.

11.2.3.2. Ecotoxicity. Not available.

11.2.4. Other available data

4-Methyl-3-penten-2-one has been registered under REACH with the following additional data available at this time:

The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guidelines. Under the conditions of the test, biodegradation of 75% was observed after 28 days.

The acute fish (*Danio rerio*) toxicity test was conducted according to the OECD 203 guidelines under static conditions. The 96-h LC50 value based on nominal concentrations was reported to be 72.93 mg/L.

The *Daphnia* acute immobilization test was conducted according to the OECD 202 guidelines under static conditions. The 48-h EC50 value based on nominal concentrations was reported to be 89.1 mg/L.

The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC50 values based on growth rate and yield were reported to be > 100 mg/L (ECHA, 2011).

Risk Assessment Refinement: Not applicable.

Literature Search and Risk Assessment Completed On: 07/25/19.

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 01/30/20.

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. Explanation of Cramer Classification

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

Q1 Normal constituent of the body? No

Q2 Contains functional groups associated with enhanced toxicity? No
Q3 Contains elements other than C, H, O, N, and divalent S? No
Q5 Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6 Benzene derivative with certain substituents? No
Q7 Heterocyclic? No
Q16 Common terpene? (see Cramer et al., 1978 for detailed explanation)? No
Q17 Readily hydrolyzed to a common terpene? No
Q19 Open chain? Yes
Q20 Aliphatic with some functional groups (see Cramer et al., 1978 for detailed explanation)? Yes
Q21 3 or more different functional groups? No
Q18 One of the list? (see Cramer et al., 1978 for a detailed explanation on the list of categories)? Yes, Intermediate (Class II)

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