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RIFM fragrance ingredient safety assessment, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one, CAS Registry number 81786-75-6

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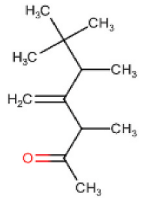
Name: 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one

CAS Registry Number: 81786-75-6

81786-74-5; (E)-3,4,5,6,6-Pentamethylhept-3-en-2-one
86115-11-9; 3,4,5,6,6-Pentamethylhept-3-en-2-one
81786-73-4; (Z)-3,4,5,6,6-Pentamethylhept-3-en-2-one

*These materials were included in this assessment because the materials are isomers.



Abbreviation/Definition List:

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2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017) compared to a deterministic aggregate approach

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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 Observed 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 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 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.

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(*continued*)

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

3,5,6,6-Tetramethyl-4-methyleneheptan-2-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is not genotoxic. Data on 3,5,6,6-tetramethyl-4-methyleneheptan-2-one provide a calculated margin of exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints. Data provided 3,5,6,6-tetramethyl-4-methyleneheptan-2-one a No Expected Sensitization Induction Level (NESIL) of 4400 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoint was completed based on ultraviolet (UV) spectra; 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the threshold of toxicological concern (TTC) for a Cramer Class II material, and the exposure to 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2009a; RIFM, 2009b)
Repeated Dose Toxicity: NOAEL = 41 mg/kg/day. RIFM (2012b)
Reproductive Toxicity: NOAEL = 129 mg/kg/day. RIFM (2012b)
Skin Sensitization: NESIL = 4400 μg/cm^2. RIFM (2012a)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Critical Measured Value: 7% (OECD 301D; day 60) for CAS # 81786-75-6 RIFM (2011a)
Bioaccumulation:
Critical Measured Value: BCF: 200 for CAS # 81786-75-6 RIFM (2014b)
Ecotoxicity:
Critical Ecotoxicity Endpoint: 96-hr Fish LC50: 4.8 mg/L for CAS # 81786-75-6 RIFM (2011d)
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: 96-hr Fish LC50: 4.8 mg/L for CAS # 81786-75-6 RIFM (2011d)
RIFM PNEC is: 4.8 μg/L
- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

Chemical Name: 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one
CAS Registry Number: 81786-75-6
Synonyms: 2-Heptanone, 3,5,6,6-tetramethyl-4-methylene-; Koavone; 3,5,6,6-Tetramethyl-4-methyleneheptan-2-one
Molecular Formula: $C_{12}H_{22}O$
Molecular Weight: 182.3
RIFM Number: 6000
Stereochemistry: Isomer not specified. Two chiral centers and one stereocenter present. A total of 4 enantiomers and 2 stereoisomers possible.

Chemical Name: (E)-3,4,5,6,6-Pentamethylhept-3-en-2-one
CAS Registry Number: 81786-74-5
Synonyms: (E)-3,4,5,6,6-Pentamethylhept-3-en-2-one; 3-Hepten-2-one, 3,4,5,6,6-pentamethyl-, (E)-; Koavone
Molecular Formula: $C_{12}H_{22}O$
Molecular Weight: 182.3
RIFM Number: 5999
Stereochemistry: E isomer specified. One chiral center and a total of 2 enantiomers possible.

Chemical Name: 3,4,5,6,6-Pentamethylhept-3-en-2-one
CAS Registry Number: 86115-11-9
Synonyms: 3,4,5,6,6-Pentamethylhept-3-en-2-one; 3-Hepten-2-one, 3,4,5,6,6-pentamethyl-; Koavone

Molecular Formula: $C_{12}H_{22}O$
Molecular Weight: 182.3
RIFM Number: 6046
Stereochemistry: Isomer not specified. Two chiral centers and one stereocenter present. A total of 4 enantiomers and 2 stereoisomers possible.

Chemical Name: (Z)-3,4,5,6,6-Pentamethylhept-3-en-2-one
CAS Registry Number: 81786-73-4
Synonyms: (Z)-3,4,5,6,6-Pentamethylhept-3-en-2-one; 3-Hepten-2-one, 3,4,5,6,6-pentamethyl-, (Z)-; Acetyl Diisoamylene; Koavone
Molecular Formula: $C_{12}H_{22}O$
Molecular Weight: 182.3
RIFM Number: 5998
Stereochemistry: Z isomer specified. One chiral center and a total of 2 enantiomers possible.

2. Physical data

CAS Registry Number: 81786-75-6	**CAS Registry Number**: 81786-74-5	**CAS Registry Number**: 86115-11-9	**CAS Registry Number**: 81786-73-4
Boiling Point: 193.77 °C (US EPA, 2012a)	**Boiling Point:** 207.93 °C (US EPA, 2012a)	**Boiling Point:** 207.93 °C (US EPA, 2012a)	**Boiling Point:** 207.93 °C (US EPA, 2012a)
Flash Point: 83 °C (Globally Harmonized System [GHS])	**Flash Point:** 83 °C (GHS)	**Flash Point:** 83 °C (GHS)	**Flash Point:** 83 °C (GHS)
Log K_{OW}: 3.85 (US EPA, 2012a)	**Log K_{OW}:** 4.19 (US EPA, 2012a)	**Log K_{OW}:** 4.19 (US EPA, 2012a)	**Log K_{OW}:** 4.19 (US EPA, 2012a)
Melting Point: -21.02 °C (US EPA, 2012a)	**Melting Point:** -18.03 °C (US EPA, 2012a)	**Melting Point:** -18.03 °C (US EPA, 2012a)	**Melting Point:** -18.03 °C (US EPA, 2012a)
Water Solubility: 27.84 mg/L (US EPA, 2012a)	**Water Solubility:** 14.27 mg/L (US EPA, 2012a)	**Water Solubility:** 14.27 mg/L (US EPA, 2012a)	**Water Solubility:** 14.27 mg/L (US EPA, 2012a)
Specific Gravity: Not Available	**Specific Gravity:** Not Available	**Specific Gravity:** Not Available	**Specific Gravity:** Not Available
Vapor Pressure: 0.695 mm Hg at 25 °C (US EPA, 2012a), 0.483 mm Hg at 20 °C (US EPA, 2012a)	**Vapor Pressure:** 0.354 mm Hg at 25 °C (US EPA, 2012a), 0.242 mm Hg at 20 °C (US EPA, 2012a)	**Vapor Pressure:** 0.354 mm Hg at 25 °C (US EPA, 2012a), 0.242 mm Hg at 20 °C (US EPA, 2012a)	**Vapor Pressure:** 0.354 mm Hg at 25 °C (US EPA, 2012a), 0.242 mm Hg at 20 °C (US EPA, 2012a)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** Not available	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
Appearance/ Organoleptic: Not available	**Appearance/ Organoleptic:** Not available	**Appearance/ Organoleptic:** Not available	**Appearance/ Organoleptic:** Not available

3. Volume of use (worldwide band)

1. 100–1000 metric tons per year (IFRA, 2015)

4. Exposure*** to fragrance ingredient (Creme RIFM Aggregate Exposure Model v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance**: 0.61% (RIFM, 2015)
2. **Inhalation Exposure*:** 0.0042 mg/kg/day or 0.30 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.014 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; 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).

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th percentile concentration in hydroalcoholics, inhalation exposure, and total exposure.

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer classification

Class II*, Intermediate (Expert Judgment)

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

*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 an explanation.

6.2. 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

6.3. Read-across justification

None

7. Metabolism

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

8. Natural occurrence

3,5,6,6-Tetramethyl-4-methyleneheptan-2-one, (E)-3,4,5,6,6-pentamethylhept-3-en-2-one, 3,4,5,6,6-pentamethylhept-3-en-2-one, and (Z)-3,4,5,6,6-pentamethylhept-3-en-2-one are not reported to occur in foods by the VCF.*

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

3,5,6,6-tetramethyl-4-methyleneheptan-2-one and the additional materials have been pre-registered for 2010; no dossiers available as of 09/28/20.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for

3,5,6,6-tetramethyl-4-methyleneheptan-2-one are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.0095
2	Products applied to the axillae	0.10
3	Products applied to the face/body using fingertips	0.019
4	Products related to fine fragrances	1.9
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.26
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.029
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0095
5D	Baby cream, oil, talc	0.0032
6	Products with oral and lip exposure	0.0095
7	Products applied to the hair with some hand contact	0.086
8	Products with significant ano-genital exposure (tampon)	0.0032
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.39
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.39
10B	Aerosol air freshener	2.6
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.0032
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For 3,5,6,6-tetramethyl-4-methyleneheptan-2-one, the basis was the reference dose of 0.41 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 4400 μg/cm^2.

[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.1.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. A mammalian cell gene mutation assay (mouse lymphoma assay) was conducted according to OECD TG 476/GLP guidelines. L5178Y mouse lymphoma cells were treated with 3,5,6,6-tetramethyl-4-methyleneheptan-2-one in dimethyl sulfoxide (DMSO) at concentrations of 500 μg/mL for 4 or 24 h. Effects were evaluated both with and without metabolic activation. No statistically significant increases in the frequency of mutant colonies were observed with any concentration of the test material, either with or without metabolic activation (RIFM, 2009b). Under the conditions of the study, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was not mutagenic to mammalian cells *in vitro*.

The clastogenicity of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster ovary cells were treated with 3,5,6,6-tetramethyl-4-methyleneheptan-2-one in DMSO at concentrations up to 5000 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (RIFM, 2009a). Under the conditions of the study, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was considered to be non-clasto genic to mammalian cells in the *in vitro* chromosome aberration assay.

Based on the data available, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/03/20.

11.1.2. Repeated dose toxicity

The MOE for 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on combined material Koavone (reaction mass of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one [main isomer; 47–75%] + (E)-3,4,5,6,6-pentamethylhept-3-en-2-one [6–15%] + (Z)-3,4,5,6,6-pentamethylhept-3-en-2-one [minor isomer; 2–12%]; CAS # 81786-75-6, CAS # 81786-74-5, and CAS # 81786-73-4, respectively [see Section I]).

In an OECD 422 GLP-compliant study, groups of 12 male and 12 female Crl:WI(Han) Wistar rats were administered Koavone via the diet at the target doses of 0, 20, 75, and 250 mg/kg/day. There was a relative decrease of 18%, 27%, and 22% for the low, mid, and high doses, respectively, in the level of the test material in the diets after storage in the animal room for 24 h. Based on this, the actual test material doses reported were as follows: the low dose was 13–16 mg/kg/day (males) and 11–15 mg/kg/day (females), the mid dose was 42–58 mg/kg/day (males) and 33–56 mg/kg/day (females), and the high dose was 158–202 mg/kg/day (males) and 129–195 mg/kg/day (females). Males were treated for a total of up to 83 days (70 days of premating + 7 days mating and until sacrifice), and females were treated for up to 103 days (70 days of premating + 1–4 days mating + 21/22 days gestation + 4/5/6 days lactation). No recovery group was included in the study. Parent male animals were euthanized after the mating period, and parent female animals/pups were euthanized at or shortly after day 4 of lactation. No treatment-related effects were reported on mortality, clinical signs, neurobehavioral observations, and motor activity among all treated male and female animals. In the high-dose group, statistically significant decreases in the mean body weights, bodyweight changes, and food consumption were reported for the entire or most part of the study; these effects were considered to be treatment-related. Statistically significant dose-dependent decreases in total white blood cells and absolute values for all types of white blood cells were reported in all treated males. The values of total white blood cells/types of white blood cells in low- and mid-dose group animals were within the historical control and hence, were considered not adverse. The changes in total white blood cells/types of white blood cells among high-dose group males were considered as adverse and related to treatment. Organ weight change was limited to an increase in relative kidney weights (statistically significant) in males at the high dose. Microscopy of the kidneys of males showed a dose-dependent increase in α-2u-microglobulin nephropathy. This effect observed in the kidney was consistent with hydrocarbon nephropathy, which occurs only in male rats and is not considered hazardous to human health (Lehman-McKeeman and Caudill, 1992 and Lehman-McKeeman et al., 1990). No other treatment-related histological effects were reported. Therefore, the NOAEL was considered to be 42 mg/kg/day for males based on effects on body weights, food consumption, and white blood cells and 41 mg/kg/day for females based on effect in body weights and food consumption. Conservatively, the lowest NOAEL of 41

mg/kg/day was considered for the risk assessment (RIFM, 2012b).

Therefore, the 3,5,6,6-tetramethyl-4-methyleneheptan-2-one MOE for the repeated dose toxicity endpoint can be calculated by dividing the Koavone NOAEL in mg/kg/day by the total systemic exposure to 3,5,6,6-tetramethyl-4-methyleneheptan-2-one, 41/0.014, or 2928.

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, 2020) and a reference dose of 0.41 mg/kg/day.

The RfD for 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 41 mg/kg/day by the uncertainty factor, 100 = 0.41 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/13/20.

11.1.3. Reproductive toxicity

The MOE for 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is adequate for the reproductive toxicity endpoints at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on combined material Koavone (reaction mass of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one [main isomer; 47–75%] + (E)-3,4,5,6,6-pentamethylhept-3-en-2-one [6–15%] + (Z)-3,4,5,6,6-pentamethylhept-3-en-2-one [minor isomer; 2–12%]; CAS # 81786-75-6, CAS # 81786-74-5, and CAS # 81786-73-4, respectively [see Section I]).

In an OECD 422 GLP-compliant study, groups of 12 male and 12 female Crl:WI(Han) Wistar rats were administered Koavone via the diet at the target doses of 0, 20, 75, and 250 mg/kg/day. There was a relative decrease of 18%, 27%, and 22% for the low, mid, and high doses, respectively, in the level of the test material in the diets after storage in the animal room for 24 h. Based on this, the actual test material doses reported were as follows: the low dose was 13–16 mg/kg/day (males) and 11–15 mg/kg/day (females), the mid dose was 42–58 mg/kg/day (males) and 33–56 mg/kg/day (females), and the high dose was 158–202 mg/kg/day (males) and 129–195 mg/kg/day (females). Males were treated for a total of up to 83 days (70 days of premating + 7 days mating and until sacrifice), and females were treated for up to 103 days (70 days of premating + 1–4 days mating + 21/22 days gestation + 4/5/6 days lactation). No recovery group was included in the study. Male parent animals were euthanized after the mating period, and female parent animals and pups were euthanized at or shortly after day 4 of lactation. No treatment-related effects were reported for pre-coital time, mating index, fertility indices (male and female), female fecundity index, gestation index, duration of gestation, pre- and post-implantation loss, and sperm-parameters (epididymal sperm motility, epididymal sperm count, epididymal sperm morphology, and testicular sperm count). Statistically significant decrease in the number of corpora lutea (lower than historical control values), corresponding to a decrease in implantation sites and the number of pups delivered was reported in the high-dose group when compared to control. Although the number of corpora lutea was lower among high-dose group females, there was no dose response, and hence, it was not considered to be of toxicological significance. The viability index in the high-dose group was higher compared to control/other treatment groups due to total litter loss of 2 litters in each of these groups (the control, low-, and mid-dose groups which amounted to 24, 19, and 21 dead pups, respectively). No treatment-related effects were reported for the liveborn index and sex ratio. No treatment-related changes in pup body weights (postnatal days 1–4) were reported. Incidences of pups that were cold or with no milk in the stomach were reported on postnatal day 1. Most of these pups were found dead. The number of pups with cold or no milk in the stomach was statistically lower in the high-dose group compared to the controls. Macroscopic examination of stillborn pups or pups found dead during lactation did not indicate any treatment-related effects. Therefore, the NOAEL for developmental toxicity was considered to be 158 mg/kg/day and 129 mg/kg/day for males and females, respectively, the highest doses tested. Since the pups evaluated following treatment of P generation did not reveal any effects on development, a NOAEL for fertility was considered to be 129 mg/kg/day, the highest dose tested among males and females. Conservatively, the lowest NOAEL of 129 mg/kg/day was considered for the reproductive toxicity endpoints (RIFM, 2012b).

Therefore, the 3,5,6,6-tetramethyl-4-methyleneheptan-2-one MOE for the developmental and reproductive toxicity endpoint can be calculated by dividing the Koavone NOAEL in mg/kg/day by the total systemic exposure to 3,5,6,6-tetramethyl-4-methyleneheptan-2-one, 129/0.014, or 9214.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/03/20.

11.1.4. Skin sensitization

Based on the existing data, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is considered a sensitizer with a defined NESIL of 4400 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is considered a sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was found to be sensitizing with an EC3 value of 64% (16000 μg/cm^2) (RIFM, 2012c). Additionally, in Confirmation of No Induction in Humans (CNIH) test with 4408 μg/cm^2 of a mixture of 47–75% 3,5,6,6-tetramethyl-4-methyleneheptan-2-one, 6–15% (E)-3,4,5,6,6-pentamethylhept-3-en-2-one, and 2–12% (Z)-3,4,5,6,6-pentamethylhept-3-en-2-one in 1:3 ethanol:diethyl phthalate, no reactions indicative of sensitization was observed in any of the 100 volunteers (RIFM, 2012a).

Based on weight of evidence (WoE) from structural analysis and animal and human studies, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is a weak sensitizer with a WoE NESIL of 4400 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 0.41 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/22/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 3,5,6,6-tetramethyl-4-

Table 1
Data summary for 3,5,6,6-tetramethyl-4-methyleneheptan-2-one.

LLNA Weighted Mean EC3 Value μg/cm^2 [No. Studies]	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2
16000 [1]	Weak	4408	NA	NA	4400

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

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

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

methyleneheptan-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 3,5,6,6-tetramethyl-4-methyleneheptan-2-one in experimental models. UV/Vis absorption spectra indicate no significant absorption 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 absorbance, 3,5,6,6-tetramethyl-4-methyleneheptan-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 no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/23/20.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 3,5,6,6-tetramethyl-4-methyleneheptan-2-one is below the Cramer Class III* TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 3,5,6,6-tetramethyl-4-methyleneheptan-2-one. Based on the Creme RIFM Model, the inhalation exposure is 0.30 mg/day. This exposure is 1.6 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/05/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 3,5,6,6-tetramethyl-4-methyleneheptan-2-one 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, 3,5,6,6-tetramethyl-4-methyleneheptan-2-one was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified 3,5,6,6-tetramethyl-4-methyleneheptan-2-one 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 BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 3,5,6,6-tetramethyl-4-methyleneheptan-2-one presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. **Biodegradation:**

CAS # 81786-75-6.

RIFM, 2011a: The ready biodegradability of the test material was determined in the closed bottle test according to the OECD 301D method. Under the conditions of the study, the test material underwent no biodegradation at day 28 and 7% at day 60.

RIFM, 2014a: The ready biodegradability of the test material was evaluated in a Modified MITI test according to the OECD 301C method. No biodegradation was observed by BOD and gas chromatography.

RIFM, 2014b: Bioconcentration potential of the test material was evaluated in fish (carp) under flow-through conditions following the Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body stipulated in the Testing Methods for New Chemical Substances Environmental Policy Bureau, Ministry of the Environment, Japan. With the test material at 0.04 mg/L and 0.004 mg/L, the steady-state BCF factors were calculated to be 81–200.

Ecotoxicity:

CAS # 81786-75-6.

RIFM, 2011c: A *Daphnia magna* acute toxicity study was conducted according to the OECD 202 method under static conditions. Under the conditions of the study, the 48-h EC50 value was reported to be 6.1 mg/L based on average exposure concentration.

RIFM, 2011d: A fish (carp) acute toxicity study was conducted according to the OECD 203 method under semi-static conditions. Under the conditions of this study, the 96-h LC50 value was reported to be 4.8 mg/L (95% CI: 3.1–7.3 mg/L) based on average exposure concentration.

For CAS # 81786-73-4.

RIFM, 2011b: An algae growth inhibition test was conducted according to the OECD 201 method. Under the conditions of the study, the ErC50 (0–72 h) of the test material for growth rate was 21.0 mg/L and the EyC50 (0–72 h) for yield was 13.0 mg/L, based on measured concentration.

Other available data:

3,5,6,6-Tetramethyl-4-methyleneheptan-2-one has been pre-registered for REACH with no additional data at this time.

11.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	3.656			1000000	0.003656	
ECOSAR Acute Endpoints **(Tier 2)** ***v1.11***	7.238	2.709	2.307			Vinyl/Allyl Ketones
ECOSAR Acute Endpoints **(Tier 2)** ***v1.11***	3.288	2.214	3.338	10000	0.2214	Neutral Organics
Tier 3: Measured Data including REACH						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	4.8			1000	4.8	
Daphnia		6.1				
Algae		13				

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.19	4.19
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	10–100	100–1000
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined Regional Volumes for all CAS #.

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

The RIFM PNEC is 4.8 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 11/08/20.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/30/21.

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

Explanation of Cramer Classification

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).

Q1. A normal constituent of the body? **No**
Q2. Contains functional groups associated with enhanced toxicity? **No**
Q3. Contains elements other than C, H, O, N, 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 explanation in Cramer et al., 1978)? **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 following categories? (a) a vicinal diketone; or a ketone or ketal of a ketone attached to a terminal vinyl group (b) secondary alcohol or ester of secondary alcohol attached to a terminal vinyl group (c) allyl alcohol or its acetal, ketal, or ester derivative (d) allyl mercaptan, an allyl sulfide, an allyl thioester or allylamine (e) acrolein, a methacrolein, or their acetals (f) acrylic or methacrylic acid (g) an acetylenic compound (h) an acyclic aliphatic ketone, ketal, or keto alcohol with no other functional groups and with 4 or more carbons on either side of the keto group (i) a substance in which the functional groups are all sterically hindered (see Cramer et al., 1978 for detailed explanation)? **Yes,** Class Intermediate (Class II)

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