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Short Review

RIFM fragrance ingredient safety assessment, 2,3,3-trimethylindanone, CAS registry number 54440-17-4

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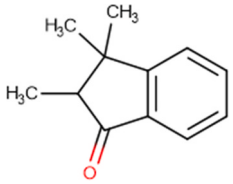
Name: 2,3,3-Trimethylindanone

CAS Registry Number: 54440-17-4

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., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic

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estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; Comiskey et al., 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 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 material has not been fully evaluated for photoallergenic potential.

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. This material has not been fully evaluated for photoallergenic potential.

2,3,3-Trimethylindanone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Target data show that 2,3,3-trimethylindanone is not genotoxic and provide a calculated Margin of Exposure (MOE) >100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material, and the exposure to 2,3,3-trimethylindanone is below the TTC (0.009 mg/kg/day and 0.47 mg/day, respectively). Data show that there are no safety concerns for 2,3,3-trimethylindanone for skin sensitization under the current declared levels of use. The photoirritation endpoint was evaluated based on data; 2,3,3-trimethylindanone does not present a concern for photoirritation under the current, declared levels of use. 2,3,3-Trimethylindanone was not evaluated for photoallergenicity. The environmental endpoints were evaluated; 2,3,3-trimethylindanone 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 (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2004d; RIFM, 2006b; RIFM, 2006a)
Repeated Dose Toxicity: NOAEL = 150 mg/kg/day. (RIFM, 2007)
Reproductive Toxicity: No NOAEL available. Exposure is below TTC.
Skin Sensitization: No concern for skin sensitization under the current declared levels of use. (RIFM, 2004e)
Photoirritation/Photoallergenicity: Not photoirritating. Not evaluated for photoallergy. (RIFM, 2020a)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Critical Measured Value: 0% (OECD 301F)	(RIFM, 2004c)

Bioaccumulation:

Screening-level: 27 L/kg	(EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:

Screening-level: 48-hour *Daphnia magna* LC50: 5.142 mg/L	(EPI Suite v4.11; US EPA, 2012a)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) >1	(RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: 48-hour *Daphnia magna* LC50: 5.142 mg/L	(EPI Suite v4.11; US EPA, 2012a)

RIFM PNEC is: 0.5142 μg/L

- Revised PEC/PNECs (2019 IFRA VoU): North America and Europe: <1

1. Identification

1. **Chemical Name:** 2,3,3-Trimethylindanone
2. **CAS Registry Number:** 54440-17-4
3. **Synonyms:** 2,3,3-Trimethyl-2,3-dihydro-1H-inden-1-one; 2,3-Dihydro-2,3,3-trimethyl-1H-inden-1-one; Safraleine; Saffron indenone; 2,3,3-Trimethylindanone
4. **Molecular Formula:** $C_{12}H_{14}O$
5. **Molecular Weight:** 174.24 g/mol
6. **RIFM Number:** 6788
7. **Stereochemistry:** No isomer specified. One stereocenter is present, and 2 total stereoisomers are possible.

2. Physical data

1. **Boiling Point:** 267.71 °C (EPI Suite v4.11)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** 3.40 (EPI Suite v4.11)
4. **Melting Point**: 60.64 °C (EPI Suite v4.11)
5. **Water Solubility:** 73.7 mg/L (EPI Suite v4.11)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.00496 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm with a peak at 290 nm and returning to the baseline by 320 nm; molar absorption coefficient (2762 L mol^{-1} • cm^{-1} for unspecified condition) is above the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. 1–10 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.10% (RIFM, 2020b)
2. **Inhalation Exposure*:** 0.000087 mg/kg/day or 0.0066 mg/day (RIFM, 2020b)
3. **Total Systemic Exposure**:** 0.0012 mg/kg/day (RIFM, 2020b)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class II*, Intermediate (Expert Judgment)

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.5
II*	I	I

*See the Appendix below for details.

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Photoirritation/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

2,3,3-Trimethylindanone is not reported to occur in foods by the VCF*.

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

9. Reach dossier

Available; accessed on 10/21/22 (ECHA, 2012a).

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, 2,3,3-trimethylindanone does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 2,3,3-trimethylindanone 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 2,3,3-trimethylindanone in ethanol 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, 2004d). Under the conditions of the study, 2,3,3-trimethylindanone was not mutagenic in the Ames test.

The clastogenicity of 2,3,3-trimethylindanone was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster lung cells were treated with 2,3,3-trimethylindanone in ethanol at concentrations up to 1750 μg/mL in the dose range finding (DRF) study; the main study was conducted at concentrations up to 250.0 μg/mL in the presence and absence of metabolic activation. Statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with and without S9 metabolic activation (RIFM, 2006b). Without S9 metabolic activation, dose-dependent increases in cells with chromosome aberrations (0.5%, 2.5%, and 3.5%) were observed at the concentrations scored (31.3, 62.5, and 125.0 μg/mL), but these increases were within the historical control range (0.0%–4.0%) and therefore considered not biologically relevant. However, with S9 metabolic activation, dose-dependent increases in cells with chromosome aberrations (2.5%, 3.5%, and 25%) were observed at the concentrations scored (7.8, 15.6, and 31.3 μg/mL). The 25% increase at 31.3 μg/mL was statistically significant, was outside the historical control range, and the number of cells carrying exchanges (9.5%) was significantly increased compared to the respective solvent control (0.5%). Therefore, this result was considered to be biologically relevant. Under the conditions of the study, 2,3,3-trimethylindanone was considered to be clastogenic in the *in vitro* chromosome aberration assay.

To further investigate the results observed in the *in vitro* chromosome aberration assay, the clastogenic activity of 2,3,3-trimethylindanone was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in corn oil via a single oral administration to groups of male and female NMRI mice. Doses of 312.5, 625, or 1250 mg/kg body weight were administered. Mice from each dose level were euthanized at 24 or 48 h and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2006a). Under the conditions of the study, 2,3,3-trimethylindanone was considered not to be clastogenic in the *in vivo* micronucleus test.

Based on the data available, 2,3,3-trimethylindanone does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/14/22.

11.1.2. Repeated dose toxicity

The MOE for 2,3,3-trimethylindanone 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 2,3,3-trimethylindanone. In a GLP and OECD 407-compliant study, groups of 5 SPF Wistar rats/sex/dose were administered 2,3,3-Trimethylindanone via gavage at doses of 0, 50, 150, and 450 mg/kg/day for 28 days. No mortality occurred throughout the study period. No treatment-related adverse effects were observed in clinical signs, food consumption, body weights, hematology, clinical chemistry, urinalysis, organ weights, or macroscopic or microscopic pathology. Based on no treatment-related adverse effects seen up to the highest dose, the repeated dose NOAEL for this study was considered to be 450 mg/kg/day (RIFM, 2007).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 407 study (ECHA, 2012b). The safety factor has been approved by The Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 450/3 or 150 mg/kg/day.

Therefore, the MOE for 2,3,3-trimethylindanone is equal to the 2,3,3-trimethylindanone NOAEL in mg/kg/day divided by the total systemic exposure for 2,3,3-trimethylindanone, 150/0.0012 or 125000.

Additionally, the total systemic exposure to 2,3,3-trimethylindanone (1.2 μg/kg/day) is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material (9 μg/kg/day; Kroes et al., 2007) 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: None.

Literature Search and Risk Assessment Completed On: 03/17/22.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2,3,3-trimethylindanone or any read-across materials. The total systemic exposure to 2,3,3-trimethylindanone is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 2,3,3-trimethylindanone or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2,3,3-trimethylindanone (1.2 μg/kg/day) is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/13/22.

11.1.4. Skin sensitization

Based on the existing data, 2,3,3-trimethylindanone does not present a concern for skin sensitization.

11.1.4.1. Risk assessment. Based on the existing data, 2,3,3-trimethylindanone is not considered a skin sensitizer. The data are summarized in Table 1. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). In a murine local lymph node assay (LLNA), 2,3,3-trimethylindanone was found to be non-sensitizing when tested up to 100% (25000 μg/cm^2) (RIFM, 2004e). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 5000 μg/cm^2 of 2,3,3-trimethylindanone in 1:3 ethyl alcohol:diethyl phthalate, no reactions indicative of sensitization were observed in any of the 97 volunteers (RIFM, 2006c).

Based on the weight of evidence (WoE) from structural analysis, animal studies, and human studies, 2,3,3-Trimethylindanone does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/09/22.

11.1.5. Photoirritation/photoallergenicity

Based on *in vitro* study data, 2,3,3-trimethylindanone does not present a concern for photoirritation. 2,3,3-Trimethylindanone has not been evaluated for photoallergy; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 2,3,3-trimethylindanone.

11.1.5.1. Risk assessment. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate significant absorbance, with a peak absorbance at 290 nm and returning to baseline by 320 nm. The corresponding molar absorption coefficient is above the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). In a 3T3-Neutral Red Uptake photoirritation test, 2,3,3-Trimethylindanone was not predicted to have photoirritating potential (RIFM,

Table 1
Summary of existing data on 2,3,3-Trimethylindanone.

WoE Skin Sensitization Potency Category[a]	Human Data				Animal 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	LLNA[d] Weighted Mean EC3 Value μg/cm^2	GPMT[e]	Buehler[e]
No evidence of sensitization[g]	5000	N/A	N/A	N/A	Negative up 25000 (100%)	N/A	N/A
	***In vitro* Data[f]**			***In silico* protein binding alerts (OECD Toolbox v4.5)**			
	KE 1	**KE 2**	**KE 3**	**Target Material**	**Autoxidation simulator**	**Metabolism simulator**	
	N/A	N/A	N/A	No alert found	No alert found	No alert found	

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

[a] WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

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

[e] Studies conducted according to the OECD TG 406 are included in the table.

[f] Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

[g] Determined based on Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients (Api et al., 2015).

2020a). Based on *in vitro* study data, 2,3,3-Trimethylindanone does not present a concern for photoirritation. 2,3,3-Trimethylindanone has not been evaluated for photoallergy; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 2,3,3-trimethylindanone.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were generated for 2,3,3-Trimethylindanone. Significant absorbance was observed under unspecified conditions, with peak absorbance within the range of 290–700 nm seen at 290 nm and returning to baseline by 320 nm. The corresponding molar absorption coefficient was 2762 L mol^{-1} • $cm^{-1,}$ which is above 1000 L mol^{-1} • cm^{-1}, the benchmark of concern for photoirritating and photoallergenic effects (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/13/22.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2,3,3-trimethylindanone 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 2,3,3-trimethylindanone. Based on the Creme RIFM Model, the inhalation exposure is 0.0066 mg/day. This exposure is 71.2 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: 10/12/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2,3,3-trimethylindanone 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 of 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 2,3,3-trimethylindanone 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 2,3,3-trimethylindanone as possibly being persistent but not bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2017). 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

Based on the current VoU (2019), 2,3,3-trimethylindanone presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. **RIFM, 2004b:** The inherent biodegradability of the test material was determined by the manometric respirometry test according to the OECD 301C method. Under the conditions of the study, no biodegradation was observed after 28 days.

RIFM, 2004c: The ready biodegradability of the test material was determined by the manometric respirometry test according to the OECD 301F method. No biodegradation was observed after 28 days.

11.2.2.1.2. Ecotoxicity. **RIFM, 2004a:** A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC50 was 27 mg/L based on nominal exposure concentrations (95% confidence interval between 24 and 32 mg/L).

11.2.2.1.3. Other available data. 2,3,3-Trimethylindanone has been registered under REACH, with no additional data at this time.

11.2.3. Risk assessment refinement

Since 2,3,3-trimethylindanone has passed the screening criteria, measured data are included for completeness only and have 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.4	3.4
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	1–10	<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 0.5142 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: 10/03/22.

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/

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	14.20			1000000	0.01420	
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	7.96	5.142	6.53	10000	0.514	Neutral Organics

- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **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 ChemView:** https://chemview.epa.gov/chemview/
- **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 03/03/23.

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, and divalent S? No.
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No.
Q6. Benzene derivative with certain substituents? No.
Q7. Heterocyclic? No.
Q17. Readily hydrolyzed to a common terpene? No.
Q19. Open chain? No.
Q23. Aromatic? Yes.
Q27. Rings with substituents? Yes.
Q28. More than one aromatic ring? No.
Q30. Aromatic ring with complex substituents? Yes.
Q31. Is the substance an acyclic acetal or ester of substances defined in Q30? No.
Q32. Does it contain only the functional groups listed in Q30 or Q31 and either: a) a single fused non-aromatic carbocyclic ring; b) aliphatic substituent chains longer than 5 carbon atoms; or c) a polyoxyethylene ($n \geq 4$) on the aromatic or aliphatic side chain? Yes.
Class Intermediate (Class II).

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