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RIFM fragrance ingredient safety assessment, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-, CAS Registry Number 211299-54-6

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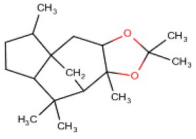
Version: 080618. This version replaces any previous versions.

Name: 4H-4a,9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-

CAS Registry Number: 211299-54-6



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
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect

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Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test.
TTC - Threshold of Toxicological Concern
UV/Vis Spectra - Ultraviolet/Visible Spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe under the limits 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.

Summary: The use of this material under current conditions is supported by existing information.

4H-4a,9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) (CAS # 211299-54-6) was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) show that it is not genotoxic, and that there are no safety concerns for skin sensitization under the current, declared levels of use. Data from 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) provide a calculated MOE > 100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class III material, and the exposure to 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) is below the TTC (0.0015 mg/kg/day and 0.47 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) is not expected to be phototoxic/photoallergenic. For the environmental endpoints, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,-5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R) is not PBT as per the IFRA Environmental Standards, and its risk quotients (i.e., PEC/PNEC) for the aquatic environment based on its current volume of use in Europe and North America are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 2013; RIFM, 2014c; RIFM, 1998f)
Repeated Dose Toxicity: NOAEL = 67 mg/kg/day.	(RIFM, 1998e)
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.	
Skin Sensitization: No safety concerns at current, declared use levels.	(RIFM, 1997a; RIFM, 2008)
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: 8.4% (BODIS)	RIFM (1997b)
Bioaccumulation: Screening-level: 747 L/kg	(EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Critical Ecotoxicity Endpoint: 96-h Fish LC50: > 2.77 mg/L	RIFM (1998b)
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-h Fish LC50: > 2.77 mg/L	RIFM (1998b)
RIFM PNEC is: 2.77 µg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: < 1	

1. Identification

1. **Chemical Name:** 4H-4a,9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-
2. **CAS Registry Number:** 211299-54-6
3. **Synonyms:** 4H-4a, 9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R E; Ambrocenide 10; Ambrocenide; Creden-AC; Ambrocenide krist; 4H-4a,9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-
4. **Molecular Formula:** $C_{18}H_{30}O_2$
5. **Molecular Weight:** 278.4
6. **RIFM Number:** 6404
7. **Stereochemistry:** 4aR,5R,7aS,9R isomer specified. Six stereocenters and 64 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** > 553.7 K (280.5 °C) (RIFM, 1998k)
2. **Flash Point:** 150 °C at 1013 hPa (RIFM, 2014b)
3. **Log K_{OW}:** 4.865 @ pH 7 and 22 °C (RIFM, 1998h)
4. **Melting Point:** 325.7–331.5 K (52.6–58.3 °C) (RIFM, 1998i)
5. **Water Solubility:** 4.28 mg/L ± 0.22 mg/L @ 20 °C (RIFM, 1998g)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 6.0 ± 0.5 × 10(-2) Pa or 4.5 ± 0.4 × 10(-4) mm Hg at 20 °C (RIFM, 1998j)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** A colorless clear liquid with a woody odor.*

*http://www.thegoodscentscompany.com/data/rw1612511.html, 12/05/17.

3. Exposure

1. **Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.016% (RIFM, 2014a)
3. **Inhalation Exposure*:** 0.000017 mg/kg/day or 0.0012 mg/day (RIFM, 2014a)
4. **Total Systemic Exposure**:** 0.00021 mg/kg/day (RIFM, 2014a)

*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 IV. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

1. **Cramer Classification:** Class III, High

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

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

6. Metabolism

No relevant data available for inclusion in this safety assessment.

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

4H-4a,9-Methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- is not reported to occur in nature 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.

8. IFRA standard

None.

9. REACH dossier

Available, accessed 12/05/17.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. The mutagenic activity of 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- 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 method. At concentrations up to 5000 μg/plate, *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated with 4 h-4a,9-methanoazuleno [5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS, 9R)- in acetone, and 95% ethanol was used for *Escherichia coli* strain WP2uvrA. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2013; RIFM, 2014c). Under the conditions of the study, 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-was not mutagenic in the Ames test.

The clastogenicity of 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- 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 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- in ethanol at concentrations up to 120 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 item, either with or without S9 metabolic activation (RIFM, 1998f). Under the conditions of the study, 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, 4 h-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/17/2017.

10.1.2. Repeated dose toxicity

The margin of exposure for 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-. An OECD 407/GLP oral gavage subchronic study was conducted in SPF-bred Wistar rats. Groups of 5 rats/sex/dose were administered 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-(Cedren-AC) at doses of 0, 50, 200, or 1000 mg/kg/day for 28 days. Additional groups of 5 rats/sex/dose were assigned to the control and high-dose groups with a 15-day recovery period. At 1000 mg/kg/day, 1 female was found dead on day 19 with clinical signs of hunched posture and piloerection. The cause of death was considered to be accidental due to the absence of any corroborative findings. Macroscopic examination of the high-dose group animals revealed thickening/irregular surface of forestomach or limiting ridge, dark red discolored renal medulla in the kidney, and accentuated lobular pattern or red brown discoloration of the liver. However, no treatment-related macroscopic findings were observed in any of the recovery group animals after week 6. There was an increase in the absolute and relative liver weights after 4 and 6 weeks, reaching statistical significance in most cases. There was a statistically significant decrease in the absolute thymus weights among high-dose males after 4 weeks. Microscopic examination of the high-dose group animals revealed vascular congestion in the kidney, hepatocellular hypertrophy (which was not found in recovery group animals), and inflammation of the forestomach accompanied by hyperplasia of the squamous epithelium noted after 4 weeks (only minor degrees of squamous hyperplasia persisted in 1 male and 1 female after 6 weeks). At 200 mg/kg/day, thickening/irregular surface of forestomach or limiting ridge, inflammation of the forestomach accompanied by hyperplasia of the squamous epithelium, and hepatocellular hypertrophy were observed. Alterations in the stomach were considered to be a response to the irritating properties of the test material. Alterations in the liver (hypertrophy and increased liver weight) were considered to reflect a higher metabolic activity of the test material—an adaptive response. The NOAEL was considered to be 200 mg/kg/day, based on decreased thymus weight and microscopic effects in the kidney among high-dose group animals (RIFM, 1998e). A default safety factor of 3 was used when deriving a NOAEL from a 28-day OECD 407 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. The derived NOAEL for the repeated dose toxicity data is 200/3 or 67 mg/

kg/day.

The 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-MOE for the repeated dose toxicity endpoint can be calculated by dividing the 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- NOAEL in mg/kg/day by the total systemic exposure to 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R), 67/0.00021 or 319048.

In addition, the total systemic exposure to 4H-4a,9-methanoazuleno [5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- (0.21 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class III 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: None.

Literature Search and Risk Assessment Completed On: 04/24/18.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- or on any read-across materials. The total systemic exposure to 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- is below the TTC for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- or on any read-across material that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- (0.21 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/22/17.

10.1.4. Skin sensitization

Based on the existing data, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. Based on the existing data, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Toxtree 2.6.13; OECD toolbox v3.4). In guinea pigs, a maximization test did not present reactions indicative of sensitization (RIFM, 1997a). Additionally, in a confirmatory human repeat insult patch test (HRIPT) with 1377 μg/cm^2 of 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- in 10% triethylcitrate and 1:3 ethanol:diethyl phthalate, no reactions indicative of sensitization was observed in any of the 105 volunteers (RIFM, 2008).

Based on the weight of evidence from structural analysis as well as animal and human studies, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a safety concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/24/17.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of absorbance, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. 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: 10/12/17.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-, is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)-. Based on the Creme RIFM Model, the inhalation exposure is 0.0012 mg/day. This exposure is 392 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/30/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- 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, 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- 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 v4.1 identified 4H-4a,9-methanoazuleno[5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- 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 v4.1). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

in the dark at a constant temperature. The degradation was determined by oxygen measurements over a 28-day period. No biodegradation was observed.

RIFM, 1997b: Biodegradability was determined in a Biological Oxygen Demand (BOD) test for insoluble substances over 28 days. Percent biodegradation was calculated by dividing the cumulative BOD by the Theoretical Oxygen Demand (ThOD) × mg test substance and then multiplying by 100. Biodegradation of 8.4% was observed after 28 days.

10.2.2.2. Ecotoxicity. RIFM, 1998b: An acute toxicity study was conducted with rainbow trout (*Oncorhynchus mykiss*) according to the OECD 203 method under semi-static conditions. The 96-h LC50 of the test material to rainbow trout was reported to be greater than 2.77 mg/L based on measured concentration.

RIFM, 1998c: A *Daphnia magna* immobilization test was conducted according to the OECD 202 I method under semi-static conditions. The 48-h E50 was reported to be greater than 3.12 mg/L based on measured concentration.

RIFM, 1998d: An algal growth inhibition test was conducted according to the OECD 201 method. The 72-h EbC50 (biomass) and ErC50 (growth rate) were reported to be greater than 4.3 mg/L.

10.2.2.3. Other available data. No additional data is available.

10.2.3. 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)**	1.22			1,000,000	0.00122	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.239	0.184	0.487	10,000	0.0184	Neutral Organics
Tier 3: Measured Data including REACH						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	>2.77			1000	2.77	
Daphnia		>3.12				
Algae		>4.3				

10.2.2. Risk assessment

Based on the current Volume of Use (2015), 4H-4a,9-methanoazuleno [5,6-d]-1,3-dioxole, octahydro-2,2,5,8,8,9a-hexamethyl-, (4aR,5R,7aS,9R)- presents a risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Biodegradation. RIFM, 1998a: The ready biodegradability of the test material was evaluated in a closed bottle test according to the OECD 301D method. The solution was inoculated with a small number of micro-organisms from a mixed population and kept in closed bottles

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.86	4.86
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 2.77 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 volumes of use.

Literature Search and Risk Assessment Completed On: 11/28/17.

11. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 06/12/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the research institute for fragrance materials, inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.

IFRA (International Fragrance Association), 2015. Volume of Use Survey, February 2015.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.

RIFM (Research Institute for Fragrance Materials, Inc), 1997a. Guinea Pig Maximization Test of Skin Sensitization with 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Cedren-ac). Unpublished report from Symrise. RIFM report number 55538. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1997b. 4H-4a,9-Methanoazuleno [5,6-d]-1,3-dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide): Assessment of the Biodegradability with the BOD Test for Insoluble Substances (BODIS). Unpublished report from Symrise. RIFM report number 67623. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998a. Assessment of the Ready Biodegradability of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- with the Closed Bottle Test. Unpublished report from Dragoco Gerberding & Co. GmbH. RIFM report number 39049. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998b. Acute Toxicity Testing of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- in Rainbow Trout (Oncorhyncus Mykiss) (Teleostei, Salmonidae). Unpublished report from Dragoco Gerberding & Co. GmbH. RIFM report number 39050. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998c. Assessment of Toxic Effects of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- on Daphnia Magna Using the 48 H Acute Immobilisation Test. Unpublished report from Dragoco Gerberding & Co. GmbH. RIFM report number 39051. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998d. Testing of Toxic Effects of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- on the Single Cell Green Alga Scenedesmus Subspicatus. Unpublished report from Dragoco Gerberding & Co. GmbH. RIFM report number 39052. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998e. Subacute 28-day Oral Toxicity with 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Cedren-AC) by Daily Gavage in the Rat Followed by a 15-day Recovery Period. Unpublished report from Symrise. RIFM report number 55530. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998f. Chromosome Aberration Test in Chinese Hamster Ovary Cells in Vitro with 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Cedren-ac). Unpublished report from Symrise. RIFM report number 55541. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998g. 4H-4a,9-Methanoazuleno [5,6-d]-1,3-dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide): Water Solubility. Unpublished report from Symrise. RIFM report number 67625. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998h. 4H-4a,9-Methanoazuleno [5,6-d]-1,3-dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide): Partition Coefficient. Unpublished report from Symrise. RIFM report number 67626. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998i. Melting Temperature of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide). Unpublished report from Symrise. RIFM report number 70136. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998j. Determination of the Vapour Pressure of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide). Unpublished report from Symrise. RIFM report number 70138. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1998k. Boiling Temperature of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide). Unpublished report from Symrise. RIFM report number 70140. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008. 4H-4a,9-Methanoazuleno [5,6-d]-1,3-dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)-: Repeated Insult Patch Test. Unpublished report from Symrise. RIFM report number 55532. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2013. Bacterial Reverse Mutation Test of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide Krist). Unpublished report from Symrise. RIFM report number 69343. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2014a. Exposure Survey 05, September 2014.

RIFM (Research Institute for Fragrance Materials, Inc), 2014b. 4H-4a,9-Methanoazuleno [5,6-d]-1,3-dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide): Burning Rate. Unpublished report from Symrise. RIFM report number 69354. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2014c. Bacterial Reverse Mutation Test of 4H-4a,9-Methanoazuleno[5,6-D]-1,3-Dioxole, Octahydro-2,2,5,8,8,9a-Hexamethyl-, (4aR,5R,7aS,9R)- (Ambrocenide Krist). Unpublished report from

Symrise. RIFM report number 69564. RIFM, Woodcliff Lake, NJ, USA.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.