Food and Chemical Toxicology 130 (2019) 110568

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

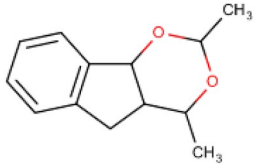
Short Review

RIFM fragrance ingredient safety assessment, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin, CAS Registry Number 27606-09-3

A.M. Api[a], D. Belsito[b], D. Botelho[a], M. Bruze[c], G.A. Burton Jr.[d], J. Buschmann[e], M.L. Dagli[f], M. Date[a], W. Dekant[g], C. Deodhar[a], M. Francis[a], A.D. Fryer[h], L. Jones[a], K. Joshi[a], S. La Cava[a], A. Lapczynski[a], D.C. Liebler[i], D. O'Brien[a], A. Patel[a], T.M. Penning[j], G. Ritacco[a], J. Romine[a], N. Sadekar[a], D. Salvito[a], T.W. Schultz[k], I.G. Sipes[l], G. Sullivan[a,*], Y. Thakkar[a], Y. Tokura[m], S. Tsang[a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member RIFM Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI 58109, USA*
[e] *Member RIFM Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member RIFM Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member RIFM Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of RIFM Expert Panel, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996-4500, USA*
[l] *Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member RIFM Expert Panel, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman, Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

Version: 111918. This version replaces any previous versions.
Name: 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin **CAS Registry Number:** 27606-09-3



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

* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

https://doi.org/10.1016/j.fct.2019.110568
Received 19 November 2018; Received in revised form 16 May 2019; Accepted 7 June 2019
Available online 08 June 2019
0278-6915/

GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api, 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.

2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin is not genotoxic. Data on 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin provide a calculated MOE > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data show that there are no safety concerns for 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class III material, and the exposure to 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2001c; RIFM, 2014a)
Repeated Dose Toxicity: NOAEL = 13.3 mg/kg/day. RIFM (2013b)
Reproductive Toxicity: Developmental toxicity NOAEL = 200 mg/kg/day. Fertility NOAEL = 40 mg/kg/day. RIFM (2013b)
Skin Sensitization: No safety concerns at current, declared use levels. RIFM (2001a)
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: 0% (OECD 301D) RIFM (2001b)
Bioaccumulation: Screening-level: 26.9 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: 96-hour Algae EC50: 24.29 mg/L (ECOSAR; US EPA, 2012b)
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: 96-hour Algae EC50: 24.29 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 2.429 µg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: < 1

1. Identification

1. **Chemical Name:** 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin
2. **CAS Registry Number:** 27606-09-3
3. **Synonyms:** 2,4-Dimethyl-5,6-indeno-1,3-dioxan; Indeno[1,2-d]-1,3-dioxin, 4,4a,5,9b-tetrahydro-2,4-dimethyl-; 2,4-ジメチル-4,4a,5,9b-テトラヒドロインデノ[1,2-d]-1,3-ジオキサン; 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d][1,3]dioxine; Magnolan; Corps 719; Ideno[1,2-d]-1,3-dioxin, 2,4-dimethyl-4,4a,9,9a-tetrahydro-; 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno [1,2-d]-1,3-dioxin
4. **Molecular Formula:** $C_{13}H_{16}O_2$
5. **Molecular Weight:** 204.69
6. **RIFM Number:** 5636
7. **Stereochemistry:** Isomer not specified. Four stereocenters and 8 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 249°C–267 °C at 1013 hPa (RIFM, 2014c), 287.22 °C (EPI Suite)
2. **Flash Point:** Half-time for pH 4 at 20 °C, 30 °C, and 50 °C was 384 (no significant hydrolysis), 78.7 (slow hydrolysis), and 13.7 (moderate hydrolysis) days, respectively (RIFM, 2015a), 136 °C (average corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2014b), > 93 °C (GHS)
3. **Log K_{OW}:** 2.67 (EPI Suite), 2.43 and 2.90 for isomer 1 and 2, respectively, at 22.8 °C (RIFM, 2014d)
4. **Melting Point**: −40.5 °C at 1013 hPa (RIFM, 2014c), 66.56 °C (EPI Suite)
5. **Water Solubility:** 217.1 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.000845 mm Hg @ 20 °C (EPI Suite v4.0), 0.00156 mm Hg @ 25 °C (EPI Suite)
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 to pale yellow clear liquid with a medium floral, green, grapefruit, narcissus, magnolia, geranium, gardenia odor*

*http://www.thegoodscentscompany.com/data/rw1007991.html#toorgano, retrieved on 02/27/2018.

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** 10–100 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.44% (RIFM, 2015d)
3. **Inhalation Exposure*:** 0.0013 mg/kg/day or 0.099 mg/day (RIFM, 2015d)
4. **Total Systemic Exposure**:** 0.013 mg/kg/day (RIFM, 2015d)

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

Additional References

None.

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

2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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.

8. IFRA standard

None.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 11/19/2018.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin does not present a concern for genotoxicity.

10.1.1.1. Risk assessment

2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation (RIFM, 2013a). BlueScreen is a screening assay that assesses genotoxic stress through alterations in

gene expressions in a human cell line. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno [1,2-d]-1,3-dioxin 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. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2001c). Under the conditions of the study, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin was not mutagenic in the Ames test.

The clastogenic activity of 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno [1,2-d]-1,3-dioxin was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin in DMSO at concentrations up to 2403 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin did not induce binucleated cells with micronuclei when tested up to cytotoxic concentrations in either the presence or absence of an S9 activation system (RIFM, 2014a). Under the conditions of the study, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin does not present a concern for genotoxic potential.

Additional References: RIFM, 2017; RIFM, 2015c.

Literature Search and Risk Assessment Completed On: 02/21/2018.

10.1.2. Repeated dose toxicity

The margin of exposure for 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin is adequate for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin to support the repeated dose toxicity endpoint. An OECD 422/GLP combined repeated dose toxicity with a reproduction and developmental toxicity screening test was conducted in Wistar rats. Groups of 11 rats/sex/dose were administered 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan) via oral gavage at doses of 0, 10, 40, or 200 mg/kg/day in corn oil. Additional groups of 6 rats/sex were added to the control and mid-dose groups, and 8 rats/sex were added to the low-dose group as a result of infertility of the dams. Male rats were dosed for at least 28 days while female rats were dosed 14 days prior to pairing, through pairing and gestation, until day 3 postpartum. One high-dose dam was found dead on day 3 of lactation, due to bacterial infection. At 200 mg/kg/day, food consumption was statistically significantly reduced in males and females during the pre-pairing period and also in the gestation period in the females. Body weight and bodyweight gain among high-dose males were statistically significantly reduced during the pre-pairing period, and body weight remained statistically significantly reduced until the end of the study. In females, body weight and bodyweight gain were reduced during the pre-pairing (statistically significant), gestation (not statistically significant), and lactation (not statistically significant) periods. The absolute and relative weights of the thymus were statistically significantly decreased in high-dose group dams. This finding was correlated with decreased lymphocytes in the thymus observed during microscopic examination, most likely related to stress. In the absence of any treatment-related findings in the other lymphoid organs (i.e., spleen, lymph nodes, or Peyer's patches), a relationship between the test material and the increased incidence of lymphocyte decreases in the thymus could not be determined. Increased hyaline droplets in the renal tubular epithelium were observed among males of the high-dose group. These kidney changes were consistent with documented changes of α-2u-globulin nephropathy in the proximal tubule, which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman, 1992; Lehman-McKeeman, 1990). The NOAEL for systemic toxicity was considered to be 40 mg/kg/day, based on decreases in body weight and food consumption among high-dose group animals (RIFM, 2013b).

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*.

The derived NOAEL for the repeated dose toxicity data is 40/3 or 13.3 mg/kg/day.

Therefore, the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin NOAEL in mg/kg/day by the total systemic exposure to 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin, 13.3/0.013 or 1023.

*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: 02/13/18.

10.1.3. Reproductive toxicity

The margin of exposure for 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin is adequate 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 sufficient reproductive toxicity data on 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin to support the reproductive toxicity endpoint. An OECD 422/GLP combined repeated dose toxicity with a reproduction and developmental toxicity screening test was conducted in Wistar rats. Groups of 11 rats/sex/dose were administered 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan) via oral gavage at doses of 0, 10, 40, or 200 mg/kg/day in corn oil. Additional groups of 6 rats/sex were added to the control and mid-dose groups, and 8 rats/sex were added to the low-dose group. Male rats were dosed for at least 28 days while female rats were dosed 14 days prior to pairing, through pairing and gestation, until day 3 postpartum. One high-dose dam was found dead on day 3 of lactation, as a result of a bacterial infection. The mean pre-coital time was dose-dependently but not statistically significantly increased in dams of the mid- and high-dose groups. Although increased in the mid-dose group, the pre-coital time was still below 4 days (regular estrus cycle in rats), while the high-dose group dams was outside this period of the historical control group range. At 200 mg/kg/day, the mean post-implantation loss was increased when compared to the controls. This was partly due to the dam, which died during the lactation period, resulting in 16 losses. This finding was not considered to be treatment-related. The birth index was statistically significantly reduced in the high-dose group, which was due to the high post-implantation loss at this dose level. The authors of the study report determined the NOAEL for fertility and developmental toxicity to be 40 mg/kg/day (RIFM, 2013b). The NOAEL for fertility was considered to be 40 mg/kg/day, based on increased pre-coital time

among high-dose group dams. Since there were no treatment-related adverse effects observed on the development of the pups, the NOAEL for developmental toxicity was considered to be 200 mg/kg/day, the highest dose tested.

Therefore, the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin MOE for the developmental toxicity endpoint can be calculated by dividing the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin NOAEL in mg/kg/day by the total systemic exposure to 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin, 200/0.013 or 15385.

Therefore, the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin MOE for the fertility endpoint can be calculated by dividing the 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin NOAEL in mg/kg/day by the total systemic exposure to 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin, 40/0.013 or 3077.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/26/2018.

10.1.4. Skin sensitization

Based on the existing data, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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 v4.1). In a guinea pig maximization test, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin neat did not present reactions indicative of sensitization (RIFM, 2001a).

Based on weight of evidence (WoE) from structural analysis and animal studies, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: RIFM, 1968.

Literature Search and Risk Assessment Completed On: 02/07/18.

10.1.5. Phototoxicity/Photoallergenicity. Based on the available UV/Vis spectra, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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, 2009). Based on lack of absorbance, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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 $mol^{-1} \cdot cm^{-1}$ (Henry, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/07/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin. Based on the Creme RIFM Model, the inhalation exposure is 0.099 mg/day. This exposure is 4.75 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/07/2018.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin 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) did not identify 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment. Based on the current Volume of Use (2015), 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin presents a risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Key studies

10.2.2.2. Biodegradation. RIFM, 2001b: The ready biodegradability of the test material was evaluated in a Closed Bottle test according to OECD 301D guidelines. Under the conditions of the study, no biodegradation was observed.

10.2.2.3. Ecotoxicity

RIFM, 2001d: A *Daphnia magna* immobilization test was conducted according to the OECD 202I method under static conditions. The 48-hour EC50 was reported to be 284 mg/L.

RIFM, 2015b: An algae growth inhibition test was conducted according to the OECD 201 method. The 72-hour ErC50 (growth rate) was reported to be 130 mg/L, and the EyC50 (yield inhibition) was reported to be 75 mg/L.

10.2.2.4. Other available data. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin has been pre-registered for REACH with no additional data.

10.2.3. Risk assessment refinement. Since 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin has passed the screening criteria measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>71.90</u>			1,000,000	0.0719	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	41.75	25.23	<u>24.29</u>	10,000	2.429	Neutral Organic

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	2.6	2.6
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	10–100	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 2.429 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: 2/8/18.

11. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 10/09/2018.

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.
Lehman-McKeeman, L.D., Caudill, D., 1992. a-2u-globulin is the only member of the lipocalin protein superfamily that binds to hyaline droplet inducing agents. Toxicol. Appl. Pharmacol. 116 (2), 170–176.
Lehman-McKeeman, L.D., Rivera-Torres, M.I., Caudill, D., 1990. Lysosomal degradation of alpha2u-globulin and alpha2u-globulin-xenobiotic conjugates. Toxicol. Appl. Pharmacol. 103 (3), 539–548.
RIFM (Research Institute for Fragrance Materials, Inc.), 1968. Human Patch Tests with Fragrance Materials and Skin Sensitization Study with Fragrance Materials with guinea Pigs. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 60420.
RIFM (Research Institute for Fragrance Materials, Inc.), 2001a. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Skin Sensitization in the guinea Pig - Magnusson and Kligman Maximisation Method. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 59764.
RIFM (Research Institute for Fragrance Materials, Inc.), 2001b. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Ready Biodegradability - Closed Bottle Test. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 59767.
RIFM (Research Institute for Fragrance Materials, Inc.), 2001c. Mutagenicity Study of 2,4-Dimethyl-4,4a,5,9b-Tetrahydroindeno[1,2-D]-1,3-Dioxin (Magnolan) in Salmonella typhimurium/mammalian Microsome Reverse Mutation Assay (Ames-Test). RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 59768.
RIFM (Research Institute for Fragrance Materials, Inc.), 2001d. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Acute Immobilisation Test (48 H) to Daphnia Magna Straus. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 59769.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013a. Report on the Testing of 2,4-Dimethyl-4,4a,5,9b-Tetrahydroindeno[1,2-D]-1,3-Dioxin in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM, Woodcliff Lake, NJ, USA RIFM report number 65555.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013b. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Combined Repeated Dose Toxicity Study with the Reproduction/developmental Toxicity Screening Test in the Han Wistar Rat. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 66409.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014a. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin: Micronucleus Test in Human Lymphocytes in Vitro. RIFM, Woodcliff Lake, NJ, USA RIFM report number 67370.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014b. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Determination of Physico-Chemical Properties Flash Point. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 69338.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014c. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Determination of Physico-Chemical Properties Melting Point and Boiling Point. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 69341.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014d. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Partition Coefficient (N-octanol/water) Using the HPLC Method. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 70419.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015a. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Hydrolysis as a Function of pH. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 69337.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015b. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Alga, Growth Inhibition Test with Pseudokirchneriella Subcapitata, 72 Hours. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 70420.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015c. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Gene Mutation Assay in Chinese Hamster V79 Cells in Vitro (V79/HPRT). RIFM, Woodcliff Lake, NJ, USA Unpublished report from Symrise. RIFM report number 70421.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015d. Exposure Survey 07, May 2015.
RIFM (Research Institute for Fragrance Materials, Inc.), 2017. 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (Magnolan): Cell Mutation Assay a the Thymidine Kinase Locus (TK+/-). RIFM, Woodcliff Lake, NJ, USA Unpublished report from RIFM report number 73005.
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.