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Short Review

RIFM fragrance ingredient safety assessment, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine, CAS Registry Number 18096-62-3

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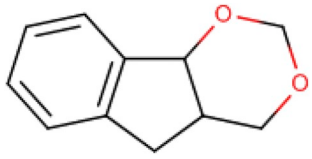
Version: 111618. This version replaces any previous versions.
Name: 4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine
CAS Registry Number: 18096-62-3



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
GLP - Good Laboratory Practice

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

4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine is not genotoxic. Data on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine provide a calculated MOE > 100 for the repeated dose and reproductive toxicity endpoints. Data on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine and read-across analog 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (CAS # 27606-09-3) show that there are no safety concerns for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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, 1999a; RIFM, 2015a)
Repeated Dose Toxicity: NOAEL = 6.7 mg/kg/day.	RIFM (2017)
Reproductive Toxicity: Developmental toxicity NOAEL = 500 mg/kg/day. Fertility NOAEL = 4.37 mg/kg/day.	(RIFM, 2015f; RIFM, 2017)
Skin Sensitization: No safety concerns at current, declared use levels.	RIFM (2001)
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: 5% (OECD 301D)	RIFM (1999b)
Bioaccumulation: Screening-level: 7.5 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 384.2 mg/L	(RIFM Framework; Salvito et al., 2002)
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: Fish LC50: 384.2 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.3842 µg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** 4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine
2. **CAS Registry Number:** 18096-62-3
3. **Synonyms:** 1-Hydroxy-2-(hydroxymethyl)indan methylene ether; Indeno[1,2-d]-1,3-dioxin, 4,4a,5,9b-tetrahydro-; 4,5-Indeno-1,3-dioxan; Indoxan; 4,4a,5,9b-ﾃﾄﾗﾋﾄﾞﾛｲﾝﾃﾞﾉ[1,2-d]-1,3-ｼﾞｵｷｼﾝ; 4,4a,5,9b-Tetrahydroindeno[1,2-d][1,3]dioxine; Indoflor; Indoflor® cryst.; Indolarome; 4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine
4. **Molecular Formula:** $C_{11}H_{12}O_2$
5. **Molecular Weight:** 176.15
6. **RIFM Number:** 5434
7. **Stereochemistry:** Isomer not specified. Two stereocenters and 4 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 276.1 °C (RIFM, 2014a), 264.36 °C (EPI Suite)
2. **Flash Point:** Loss of test item at 127 h and 50 °C for pH 4, 7, and 9 is 0.42%, 0.20%, and 0.41%, respectively (RIFM, 2015h), > 100 °C (GHS)
3. **Log K_{ow}:** 1.84 (EPI Suite), 1.76 at 22.8 °C (RIFM, 2014b)
4. **Melting Point**: 36.4 °C (RIFM, 2014a), 52.85 °C (EPI Suite)
5. **Water Solubility:** 1549 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.00393 mm Hg @ 20 °C (EPI Suite v4.0), 0.00702 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic*:** A white crystalline solid with a high animal, fecal, indole, earthy, jasmin odor.

*http://www.thegoodscentscompany.com/data/rw1033251.html#toorgano, retrieved 2/27/2018.

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** 10–100 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.021% (RIFM, 2015g)
3. **Inhalation Exposure*:** 0.000087 mg/kg/day or 0.0063 mg/day (RIFM, 2015g)
4. **Total Systemic Exposure**:** 0.00061 mg/kg/day (RIFM, 2015g)

*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::** 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno [1,2-d]-1,3-dioxin (CAS # 27606-09-3)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

6. Metabolism

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

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

4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine is not reported to occur in food 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 11/16/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. The mutagenic activity of 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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, 1999a). Under the conditions of the study, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine was not

mutagenic in the Ames test.

The clastogenic activity of 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine in DMSO at concentrations up to 1760 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 20 h 4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine did not induce binucleated cells with micronuclei when tested up to the maximum recommended concentration by OECD TG 487 in either the presence or absence of an S9 activation system (RIFM, 2015a). Under the conditions of the study, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine was considered to be non-clastogenic in the in vitro micronucleus test.

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

Additional References: El-Seedy et al., 2005; RIFM, 2015b.

Literature Search and Risk Assessment Completed On: 2/20/2018.

10.1.2. Repeated dose toxicity

The margin of exposure for 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine. An OECD 422/GLP combined repeated dose with reproduction and developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered daily via oral gavage test material 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine at doses of 0, 20, 100, or 500 mg/kg/day in corn oil. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose group to serve as 14-day treatment-free recovery groups. Males of the main group and both sexes of the recovery group were dosed for 2 weeks prior to, during, and post-mating (total of 6 weeks), while females of the main group were dosed for 2 weeks prior to mating, throughout gestation, and for 5 days after delivery. All males of the main group and all animals of the recovery group survived the duration of the study. Three females at 20 mg/kg/day, 2 females at 100 mg/kg/day, and 5 females at 500 mg/kg/day died during the treatment period (prior to, during, or after parturition). Histopathology showed test material-related changes in the kidneys, liver, lung, thymus, spleen, and submandibular lymph node among the dead animals. The cause of death could not be established based on the absence of a common lesion in the dead dams, but it was assumed that the test material contributed significantly to these deaths considering the histopathological alterations. Among surviving animals, the body weights decreased significantly among high-dose males for treatment and recovery groups and among mid- and high-dose females of the main group only. Food consumption among high-dose females decreased significantly, which corresponded to a decrease in body weight. Decreases in hindlimb grip strength were noted in females at 20 mg/kg/day and in both sexes at 500 mg/kg/day. In the main and recovery groups, there were no test material-related effects in spontaneous motor activity tests in both sexes at 20, 100, and 500 mg/kg/day when compared to the control group. The relative organ weights of the brain, liver, kidneys, and testes in males and liver in females were significantly increased at 500 mg/kg/day when compared to the control group. However, these changes were associated with the low body weights of the high-dose animals. Therefore, these changes were not considered to be toxicologically significant. In surviving animals, an accumulation of hyaline droplets in the kidneys was evident in cortical tubules in males at 20, 100, and 500 mg/kg/day. Tubular degeneration in the kidney cortex was observed in 1 mid-dose female. Centrilobular vacuolation of hepatocytes in the liver was noted in both sexes at 500 mg/kg/day. These lesions were not observed in the recovery groups. Based on the conditions of the study, the authors of the study report considered the NOAEL for systemic toxicity to be lower than 20 mg/kg/day for males and 20 mg/kg/day for females (RIFM, 2015f).

A subsequent OECD 422/GLP combined repeated dose with reproduction and developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered daily via oral gavage test material 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine at doses of 0, 1, 5, or 20 mg/kg/day in corn oil. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose groups to serve as the 14-day treatment-free recovery groups. Males of the main group were dosed for 2 weeks prior to mating, during 2 weeks of mating, and for 22 days post-mating (total of 50 days), while females of the main group were dosed for 2 weeks prior to mating, throughout gestation, and for 13 days after delivery. Males and females of the recovery groups were also dosed for 50 days. All males of the main group and all animals of the recovery groups survived the duration of the study. One low-dose and 3 high-dose pregnant females from the main groups died during parturition. In spontaneous motor activity, vertical count decreased dose-dependently in main group females at 1, 5, and 20 mg/kg/day. In the recovery groups, there were no treatment-related effects in the grip strength test and spontaneous motor activity of both sexes when compared to the control group. The absolute and relative thyroid weights were significantly increased among males of the high-dose group; however, there were no histopathological changes in the thyroid. The authors of the study report considered the NOAEL for systemic toxicity to be 20 mg/kg/day (RIFM, 2017).

Incidences of mortality and alterations in neurobehavioral activity were observed among treated and mated females; this was considered to be due to dystocia among the pregnant females and hence would be considered under the reproductive toxicity endpoint. The most conservative NOAEL for repeated dose toxicity was considered to be 20 mg/kg/day based on the results obtained from both OECD 422 studies on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine.

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 20/3 or 6.7 mg/kg/day.

Therefore, the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine MOE for the repeated dose toxicity endpoint can be calculated by dividing the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine NOAEL in mg/kg/day by the total systemic exposure to 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine, 6.7/0.00061 or 10984.

In addition, the total systemic exposure to 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine (0.61 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: 02/26/18.

10.1.3. Reproductive toxicity

The margin of exposure for 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 developmental toxicity data on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine. An OECD 422/GLP combined repeated dose with a reproduction and developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered daily via oral gavage test material 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine at doses of 0, 20, 100, or 500 mg/kg/day in corn oil. Additional groups of 6 rats/sex/dose

Model[a]	Goodness of fit		BMD_{10Pct} ()	$BMDL_{10Pct}$ ()	Basis for model selection
	p-value	AIC			
Gamma	**0.358**	**26.635**	**17.4**	**4.37**	**Individual models: global goodness-of-fit: all p-values > 0.1 scaled residuals: all < 2 across models: BMDL range deemed "sufficiently close" to use lowest AIC instead of lowest BMDL in viable models (less than 3-fold)**
Logistic	0.301	26.991	13.5	8.84	
LogLogistic	0.152	28.635	18.5	3.77	
Probit	0.297	27.058	12.8	8.11	
LogProbit	0.152	28.635	17.2	7.11	
Weibull	0.152	28.635	18.6	4.37	
Multistage 3°	0.351	26.730	14.9	4.30	
Multistage 2°	0.332	26.981	13.2	4.12	
Quantal-Linear	0.291	27.527	9.38	3.80	

[a] Selected model in bold; scaled residuals for selected model for doses 0, 1, 5, and 20 were -0.59, 1.17, -0.59, 0, respectively.

Fig. 1.

were assigned to the control and high-dose groups to serve as the 14-day treatment-free recovery groups. Males of the main group and both sexes of the recovery group were dosed for 2 weeks prior to, during, and post-mating (total of 6 weeks), while females of the main group were dosed for 2 weeks prior to mating, throughout gestation, and for 5 days after delivery. In addition to systemic toxicity, reproductive toxicity parameters were also assessed. There were no treatment-related effects in live birth index, mean litter size, body weights, sex ratio, viability index, and external examination of pups for postnatal days 0 and 4. The NOAEL for the development of offspring was considered to be 500 mg/kg/day, the highest dose tested (RIFM, 2015f). **Therefore, the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine MOE for the developmental toxicity endpoint can be calculated by dividing the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine NOAEL in mg/kg/day by the total systemic exposure to 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine, 500/0.00061 or 819672.**

There are sufficient fertility data on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine. An OECD 422/GLP combined repeated dose with a reproduction and developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered daily via oral gavage test material 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine at doses of 0, 20, 100, or 500 mg/kg/day in corn oil. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose group to serve as the 14-day treatment-free recovery groups. Males of the main group and both sexes of the recovery group were dosed for 2 weeks prior to, during, and post-mating (total of 6 weeks), while females of the main group were dosed for 2 weeks prior to mating, throughout gestation, and for 5 days after delivery. In addition to systemic toxicity, reproductive toxicity parameters were also assessed. Dystocia (death during parturition) was observed in 2 and 1 females at 20 and 500 mg/kg/day, respectively. The NOAEL for fertility was considered to be 500 mg/kg/day for males and less than 20 mg/kg/day for females (RIFM, 2015f). Since dystocia was observed in dams of the lowest dose group, a subsequent OECD 422 was conducted at lower dose levels. An OECD 422/GLP combined repeated dose with a reproduction and developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered daily via oral gavage 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine at doses of 0, 1, 5, or 20 mg/kg/day in corn oil. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose groups to serve as the 14-day treatment-free recovery groups. Males of the main group were dosed for 2 weeks prior to mating, during 2 weeks of mating, and for 22 days post-mating (total of 50 days), while females of the main group were dosed for 2 weeks prior to mating, throughout gestation, and for 13 days after delivery. Males and females of the recovery groups were also dosed for 50 days. In addition to systemic toxicity, reproductive toxicity parameters were also assessed. One low-dose and 3 high-dose pregnant females from the main groups died during parturition. Dystocia was observed. All animals that died showed retained fetuses in the uterus at necropsy. No test material-related adverse effects were observed in estrous cycling, mating period, mating index, gestation period, male and female fertility indexes, gestation index, post-implantation loss rate, or live birth index. The NOAEL for fertility was considered to be 20 mg/kg/day for males and lower than 1 mg/kg/day for females. (RIFM, 2017).

A benchmark dose (BMD v2.1) analysis was conducted on the incidences of dystocia among the dams, as shown in Fig. 1. The most conservative BMDL10 value of 4.37 mg/kg/day was considered for results obtained on the incidences of dystocia among the treated dams. **Therefore, the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine MOE for the fertility endpoint can be calculated by dividing the 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine NOAEL in mg/kg/day by the total systemic exposure to 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine, 4.37/0.00061 or 7164.**

In addition, the total systemic exposure to 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine (0.61 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: 02/26/18.

10.1.4. Skin sensitization

Based on the existing data and read-across material 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (CAS # 27606-09-3), 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 and read-across material 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (CAS # 27606-09-3; See Section V), 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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). 4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine was found to be negative in the

in vitro direct peptide reactivity assay (DPRA) and the LuSens assay (RIFM, 2015d). In a guinea pig maximization test, neat read-across material 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin did not present reactions indicative of sensitization (RIFM, 2001).

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

Additional References: RIFM, 1975.

Literature Search and Risk Assessment Completed On: 11/16/2018.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of significant absorbance in the critical range, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine does not present a concern for phototoxicity or photoallergenicity.

10.1.6. UV spectra analysis

UV/Vis absorption spectra (OECD TG 101) for 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L $mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/07/17.

10.1.7. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.7.1. Risk assessment. There are no inhalation data available on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine. Based on the Creme RIFM Model, the inhalation exposure is 0.0063 mg/day. This exposure is 74.6 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine 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, 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine was identified as a fragrance material with no 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 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine as possibly being either 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 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 $\geq$ 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), 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. RIFM, 1999b: The biodegradability of the test material was determined using the Closed Bottle test according to the OECD 301D method. 2.9 mg/L test material was suspended in a mineral medium, inoculated with a mixed population of aquatic microorganisms (activated sludge), and incubated for 28 days under aerobic conditions in the dark. Under the conditions of the study, biodegradation of 5% was observed.

10.2.3.2. Ecotoxicity. RIFM, 1999b: A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC0 was reported to be 94.9 mg/L (arithmetic mean of analytical values).

RIFM, 2015c: An algae growth inhibition test was conducted according to the OECD 201 method. The 72-h EC50 was reported to be greater than 100 mg/L based on the growth rate and yield inhibition.

RIFM, 2015e: A Fish (*Danio rerio*) acute toxicity study was conducted according to the OECD 203 method under static conditions. The 96-h LC50 was reported to be greater than 100 mg/L.

10.2.4. Other available data

4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine has been registered for REACH with no additional data at this time.

10.2.5. Risk assessment refinement

Since 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC calculation.

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>384.2</u>			1,000,000	0.3842	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	1.76	1.76
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 0.3842 μg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at the screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

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.

Declaration of interests

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 A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110725.

Appendix

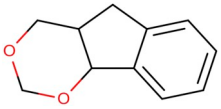
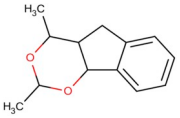
Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2016).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).

- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v3.4 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 (OECD, 2018).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	4,4a,5,9b-Tetrahydroindeno[1,2-d]-1,3-dioxine	2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin
CAS No.	18096-62-3	27606-09-3
Structure		
Similarity (Tanimoto Score)		0.7
Read-across Endpoint		• Skin sensitization
Molecular Formula	$C_{11}H_{12}O_2$	$C_{13}H_{16}O_2$
Molecular Weight	176.15	204.69
Melting Point (°C, EPI Suite)	36.4	66.56
Boiling Point (°C, EPI Suite)	276.1	287.22
Vapor Pressure (Pa @ 25 °C, EPI Suite)	0.35	0.00156
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	1.76	2.43
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	3890	1600
J_{max} (μg/cm^2/h, SAM)	29.74	17.96
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.63E-006	2.04E-006
Skin Sensitization		
Protein Binding (OASIS v1.1)	• No alert found	• No alert found
Protein Binding (OECD)	• No alert found	• No alert found
Protein Binding Potency	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• No alert found	• No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No skin sensitization reactivity domains alerts identified.	• No skin sensitization reactivity domains alerts identified.
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v3.4)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine (CAS # 18096-62-3). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, 2,4-dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (CAS # 27606-09-3) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- 2,4-Dimethyl-4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxin (CAS # 27606-09-3) was used as a read-across analog for the target material 4,4a,5,9b-tetrahydroindeno[1,2-d]-1,3-dioxine (CAS # 18096-62-3) for skin senzitization.
  - o The target substance and the read-across analog are structurally similar and belong to the class of dioxanes.
  - o The target substance and the read-across analog share a 1,3-dioxane structure.
  - o The key difference between the target substance and the read-across analog is that the analog has 2 methyl substitutions at positions 2 and 4, while the target substance does not. This structural difference is toxicologically insignificant.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o There are no alerts for the target substance and the read-across analog for the skin sensitization endpoint. Data are consistent with *in silico* alerts.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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