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RIFM fragrance ingredient safety assessment, 2,4,6-trimethyl-4-phenyl-1,3-dioxane, CAS Registry Number 5182-36-5

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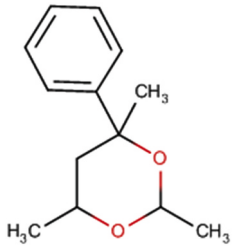
CAS Registry Number: 5182-36-5



Abbreviation/Definition List:
2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

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AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
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 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.

2,4,6-Trimethyl-4-phenyl-1,3-dioxane was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2,4,6-trimethyl-4-phenyl-1,3-dioxane is not genotoxic. Data on 2,4,6-trimethyl-4-phenyl-1,3-dioxane provide a calculated margin of exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data show that 2,4,6-trimethyl-4-phenyl-1,3-dioxane does not present a concern for skin sensitization under the current, declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2,4,6-trimethyl-4-phenyl-1,3-dioxane is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to 2,4,6-trimethyl-4-phenyl-1,3-dioxane is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 2,4,6-trimethyl-4-phenyl-1,3-dioxane was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 1999a; RIFM, 2015b)
Repeated Dose Toxicity: NOAEL = 33.33 mg/kg/day. (RIFM, 2015e)
Reproductive Toxicity: NOAEL = 300 mg/kg/day. (RIFM, 2015e)
Skin Sensitization: Not a concern for skin sensitization under the current, declared levels of use. (RIFM, 2016b; RIFM, 2016c; RIFM, 2017; RIFM, 1979; RIFM, 1972a; RIFM, 1972b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV/Vis spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Critical Measured Value: 13% (OECD 301F) RIFM (2000)
Bioaccumulation:
Screening-level: 52.1 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: 48-h *Daphnia magna* LC50: 10.8 mg/L (ECOSAR; US ECHA, 2012b)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards
Risk Assessment:

(*continued on next page*)

(*continued*)

Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 10.8 mg/L	(ECOSAR; US ECHA, 2012b)
RIFM PNEC is: 1.0805 μg/L	
• Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1	

1. Identification

1. **Chemical Name:** 2,4,6-Trimethyl-4-phenyl-1,3-dioxane
2. **CAS Registry Number:** 5182-36-5
3. **Synonyms:** 1,3-Dioxane, 2,4,6-trimethyl-4-phenyl-; 4-Phenyl-2,4,6-trimethyl-1,3-dioxane; Floropal; 2,4,6-ﾄﾘﾒﾁﾙ-4-ﾌｪﾆﾙ-1,3-ｼﾞｵｷｻﾝ; Vertacetal; Lorexan; Vertacetal coer; 2,4,6-Trimethyl-4-phenyl-1,3-dioxane
4. **Molecular Formula:** $C_{13}H_{18}O_2$
5. **Molecular Weight:** 206.28
6. **RIFM Number:** 5324
7. **Stereochemistry:** Isomer not specified. Three chiral centers and a total of 8 enantiomers possible.

2. Physical data

1. **Boiling Point:** 246.7 °C at 1013 hPa (RIFM, 2014b), 279.16 °C (EPI Suite), 243.2 °C at 1013 hPa (RIFM, 2015c)
2. **Flash Point:** Half-time for pH 4 at 20 °C and 30 °C was >40 days (slow hydrolysis) and at 50 °C was = 30 days (moderate hydrolysis) (RIFM, 2015a), 98 °C (average corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2014a), >93 °C (Globally Harmonized System), 105.5 °C (corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2015d)
3. **Log K_{OW}:** 3.1 and 3.4 at 35 °C (RIFM, 2001b), 3.11 (EPI Suite), 2.94 and 3.09 for isomer 1 and 2, respectively, at 22.8 °C (RIFM, 2014c)
4. **Melting Point**: 56.2 °C at 1013 hPa (RIFM, 2014b), 57.4 °C (EPI Suite), −55.9 °C at 1024 hPa (RIFM, 2015c)
5. **Water Solubility:** 90.26 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.00161 mm Hg at 20 °C (EPI Suite v4.0), 0.00293 mm Hg at 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} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. 10–100 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.22% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00088 mg/kg/day or 0.066 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0041 mg/kg/day (RIFM, 2019)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

7. Metabolism

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

8. Natural occurrence

2,4,6-Trimethyl-4-phenyl-1,3-dioxane 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.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 9/23/21.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 2,4,6-trimethyl-4-phenyl-1,3-dioxane does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 2,4,6-Trimethyl-4-phenyl-1,3-dioxane was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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, 2,4,6-trimethyl-4-phenyl-1,3-dioxane was not mutagenic in the Ames test.

The clastogenic activity of 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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 2,4,6-trimethyl-4-phenyl-1,3-dioxane in DMSO at concentrations up to 2030 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 662.9 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 20 h 2,4,6-Trimethyl-4-phenyl-1,3-dioxane did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2015b). Under the conditions of the study, 2,4,6-trimethyl-4-phenyl-1,3-dioxane was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 2,4,6-trimethyl-4-phenyl-1,3-dioxane does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/23/21.

11.1.2. Repeated dose toxicity

The MOE for 2,4,6-trimethyl-4-phenyl-1,3-dioxane is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 2,4,6-trimethyl-4-phenyl-1,3-dioxane. In an OECD 422/GLP study, 12 Sprague Dawley (Crl:CD [SD]), SPF rats/sex/dose were administered Floropal (2,4,6-trimethyl-4-phenyl-1,3-dioxane) through oral gavage at doses of 0, 30, 100, or 300 mg/kg/day. Recovery groups of 6 non-mated rats/sex were maintained for 2 weeks for the control and high-dose treatment groups. During the study, no animal mortality was reported, and no treatment-related adverse effects were reported for urinalysis, hematology, blood chemistry, and sensory function. In females, a treatment-related temporary loss of locomotor activity was reported in the high-dose group during gestation as well as during study days 25–37 in the recovery group. In addition, food consumption increased without any effect on the body weight in high-dose males and recovery group females. Although relative liver weights were significantly increased in animals of the high-dose group, absolute liver weight changes were only observed in males. While the increased liver weights were reversible in the recovery group, liver weights between controls and males treated with 300 mg/kg/day were 36% greater in high-dose treated males; these changes were not accompanied by alterations in liver enzyme levels. Furthermore, because centrilobular hepatocellular hypertrophy at 100 mg/kg/day (1/6 males) and at 300 mg/kg/day (5 animals/sex) was reversed during recovery, the liver hypertrophy was considered to be an adaptive response (Hall et al., 2012). Based on the loss of locomotor activity in females and changes in liver weights in both sexes at 300 mg/kg/day, the NOAEL for repeated dose toxicity was considered to be 100 mg/kg/day for the repeated dose toxicity endpoint (RIFM, 2015e).

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 100/3 or 33.33 mg/kg/day.

Therefore, the 2,4,6-trimethyl-4-phenyl-1,3-dioxane MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2,4,6-trimethyl-4-phenyl-1,3-dioxane NOAEL in mg/kg/day by the total systemic exposure to 2,4,6-trimethyl-4-phenyl-1,3-dioxane, 33.33/0.0041, or 8129.

*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: 04/15/21.

11.1.3. Reproductive toxicity

The MOE for 2,4,6-trimethyl-4-phenyl-1,3-dioxane is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on 2,4,6-trimethyl-4-phenyl-1,3-dioxane.

In an OECD 422/GLP study, groups of 12 Sprague Dawley (Crl:CD [SD]), SPF rats/sex/dose were administered Floropal (2,4,6-trimethyl-4-phenyl-1,3-dioxane) through oral gavage at doses of 0, 30, 100, or 300 mg/kg/day. Males were treated for 6 weeks (2 weeks prior to, during, and after mating), and females were similarly treated for up to 6 weeks (2 weeks prior to mating, throughout gestation, and for 4 days after delivery). Additional non-mated groups of 6 rats/sex were assigned to recovery groups that were treated with doses of 0 or 300 mg/kg/day for 6 weeks followed by 2 weeks of recovery. In addition to systemic toxicity parameters, reproductive toxicity parameters also were assessed. No significant changes were observed on the effects of fertility or on the

development of pups; thus, the NOAEL for reproductive toxicity was considered to be 300 mg/kg/day, the highest dose tested (RIFM, 2015e). **Therefore, the 2,4,6-trimethyl-4-phenyl-1,3-dioxane MOE for the reproductive toxicity endpoint can be calculated by dividing the 2,4,6-trimethyl-4-phenyl-1,3-dioxane NOAEL in mg/kg/day by the total systemic exposure to 2,4,6-trimethyl-4-phenyl-1,3-dioxane, 300/0.0041 or 73171.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/15/21.

11.1.4. Skin sensitization

Based on the existing data, 2,4,6-trimethyl-4-phenyl-1,3-dioxane does not present a concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Based on the existing data, 2,4,6-trimethyl-4-phenyl-1,3-dioxane is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react directly with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). 2,4,6-Trimethyl-4-phenyl-1,3-dioxane was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens, but positive in the human cell line activation test (h-CLAT) (RIFM, 2016b; RIFM, 2016c; RIFM, 2017). In guinea pigs, the Buehler test using 2,4,6-trimethyl-4-phenyl-1,3-dioxane did not present reactions indicative of sensitization when tested up to 2% (RIFM, 1979). In a guinea pig open epicutaneous test (OET), no sensitization reactions were observed in any of the animals up to 100% (RIFM, 1972b). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 2% of 2,4,6-trimethyl-4-phenyl-1,3-dioxane in dimethyl phthalate (DMP), no reactions indicative of sensitization were observed in any of the 52 volunteers (RIFM, 1972a). The dose per unit area could not be calculated because the size of the patch was not specified.

Based on the weight of evidence (WoE) from structural analysis, *in vitro* studies, and animal and human studies, 2,4,6-trimethyl-4-phenyl-1,3-dioxane does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment: 12/09/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2,4,6-trimethyl-4-phenyl-1,3-dioxane would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 2,4,6-trimethyl-4-phenyl-1,3-dioxane in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 2,4,6-trimethyl-4--phenyl-1,3-dioxane does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/04/20.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2,4,6-trimethyl-4-phenyl-1,3-dioxane is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.7. Risk assessment

No inhalation data are available on 2,4,6-trimethyl-4-phenyl-1,3-dioxane. Based on the Creme RIFM Model, the inhalation exposure is 0.066 mg/day. This exposure is 7.1 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, exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/16/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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 of Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity (Salvito et al., 2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class−specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA VoU survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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 is > 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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 $\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.

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), 2,4,6-trimethyl-4-phenyl-1,3-dioxane presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. **RIFM, 2000:** The ready biodegradability of the test material was determined by the manometric respirometry test according to the OECD 301F method. Under the conditions of the study, 13% biodegradation was observed after 38 days.

RIFM, 2001a: The inherent biodegradability of the test material was determined by the manometric respirometry test according to the OECD 302C method. Under the conditions of the study, no biodegradation was observed after 31 days.

RIFM, 1999b: The biodegradability of the test material was determined by the closed bottle test according to the Council Directive 92/96 EEC, method C.4-E. Under the conditions of the study, no biodegradation was observed after 28 days.

RIFM, 1996: The biodegradability of the test material was determined by the BODIS test. Under the conditions of the study, biodegradation of 15.3% was observed after 28 days.

11.2.1.3. Ecotoxicity. **RIFM, 2016a:** A fish (*Danio rerio*) acute toxicity study was conducted according to the OECD 203 method under static conditions. The 96-h LC50 value based on the nominal concentration of the test material was reported to be 42.4 mg/L.

RIFM, 1999b: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC0/EC100 value based on the arithmetic mean of analytical values was reported to be 52.0 mg/L.

11.2.1.4. Other available data. 2,4,6-Trimethyl-4-phenyl-1,3-dioxane has been preregistered for REACH with no additional data at this time.

11.2.1.5. Risk assessment refinement. Since 2,4,6-trimethyl-4-phenyl-1,3-dioxane 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.4	3.4
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional 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 further assessment is necessary.

The RIFM PNEC is 1.0805 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 11/24/20.

12. 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:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** 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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	16.84			1000000	0.01684	
ECOSAR Acute Endpoints **(Tier 2) v1.11**	17.18	10.80	12.28	10000	1.08	Neutral Organics

- **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 2,4,6-trimethyl-4-phenyl-1,3-dioxanes.

*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 09/22/21.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

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