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Short Review

RIFM fragrance ingredient safety assessment, p,α-dimethylstyrene, CAS Registry Number 1195-32-0

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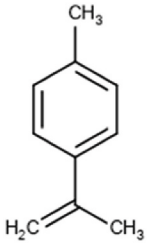
Name: p,α-Dimethylstyrene

CAS Registry Number: 1195-32-0



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
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This 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

(*continued on next column*)

(*continued*)

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.

p,α-Dimethylstyrene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that p,α-dimethylstyrene is not genotoxic. Data on read-across analog styrene (CAS # 100-42-5) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint. The reproductive toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure is below the TTC (0.03 mg/kg/day). The skin sensitization endpoint was completed using the Dermal Sensitization Threshold (DST) for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra; p,α-dimethylstyrene is not expected to be phototoxic/photoallergenic. For the local respiratory endpoint, a calculated MOE >100 was provided by read-across analog α-methylstyrene (CAS # 98-83-9). The environmental endpoints were evaluated; p,α-dimethylstyrene 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, 2016a; RIFM, 2016c)

Repeated Dose Toxicity: NOAEL = 242 mg/kg/day. (EU Risk Assessment Report; Syrene; EU RAR, 2008)

Reproductive Toxicity: No NOAEL available. Exposure is below TTC.

Skin Sensitization: Not a concern for skin sensitization under the declared use levels; exposure is below the DST.

Phototoxicity/Photoallergenicity: Not phototoxic/Not expected to be photoallergenic. (UV/Vis Spectra; RIFM Database; RIFM, 2016b)

Local Respiratory Toxicity: NOAEC = 48.34 mg/m^3 (NTP, 2007)

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Screening-level: 2.83 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation:

Screening-level: 199.2 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:

Screening-level: Fish LC50: 3.31 mg/L (RIFM Framework; Salvito, 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, 2002)

Critical Ecotoxicity Endpoint: Fish LC50: 3.31 mg/L (RIFM Framework; Salvito, 2002)

RIFM PNEC is: 0.00331 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** p,α-Dimethylstyrene
2. **CAS Registry Number:** 1195-32-0
3. **Synonyms:** Benzene, 1-methyl-4-(1-methylethenyl)-; p-Isopropenyltoluene; 1-Methyl-4-isopropenylbenzene; 2-p-Tolylpropene; 1-Isopropenyl-4-methylbenzene; α-para-Dimethylstyrene; para-Cymenene; p,α-Dimethylstyrene
4. **Molecular Formula:** $C_{10}H_{12}$
5. **Molecular Weight:** 132.2 g/mol
6. **RIFM Number:** 6182

7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 183.63 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** 3.99 (EPI Suite)
4. **Melting Point**: −26.96 °C (EPI Suite)
5. **Water Solubility:** 35.29 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.515 mm Hg at 20 °C (EPI Suite v4.0), 1.8 mm Hg at 20 °C (Fragrance Materials Association), 0.746 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm under biologically relevant neutral conditions. Molar absorption coefficient under neutral conditions (431 L mol^{-1} • cm^{-1}) is below the benchmark (1000 L mol^{-1} • cm^{-1}). Absorbance was demonstrated under the acidic condition, with a molar absorption coefficient (2147 L mol^{-1} • cm^{-1}) above the benchmark. There was no absorbance demonstrated under basic conditions.
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. 0.1–1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v2.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.00066% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.0000002 mg/kg/day or 0.000012 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0000050 mg/kg/day (RIFM, 2019)

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

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

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
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2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** Styrene (CAS # 100-42-5)
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** α-Methylstyrene (CAS # 98-83-9)
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

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

8. Natural occurrence

p,α-Dimethylstyrene is reported to occur in the following foods by the VCF*:
Citrus fruits.
Curry (*Bergera koenigii* L.)
Eucalyptus oil (*Eucalyptus globulus* Labill).
Mace (*Myristica fragrans* Houttuyn).
Mastic (*Pistacia lentiscus*).
Nutmeg (*Myristica fragrans* Houttuyn).
Pistachio oil (*Pistacia vera*).
Pistacia atlantica.
Salvia species.
Turpentine oil (*Pistacia terebinthus*).
*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. This is a partial list.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 02/07/22.

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 and use levels, p,α-dimethylstyrene does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. p,α-Dimethylstyrene 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, 2014). 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 p,α-dimethylstyrene 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 *Escherichia coli* strain WP2uvrA were treated with p,α-dimethylstyrene 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, 2016). Under the conditions of the

study, p,α-dimethylstyrene was not mutagenic in the Ames test.

The clastogenic activity of p,α-dimethylstyrene 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 p,α-dimethylstyrene in DMSO. The micronuclei analysis was conducted at concentrations up to 10000 μM (1323 μg/mL) in the presence and absence of metabolic activation. p,α-Dimethylstyrene did not induce binucleated cells with micronuclei when tested up to the cytotoxic concentration in either the presence or absence of an S9 activation system (RIFM, 2016c). Under the conditions of the study, p,α-dimethylstyrene was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, p,α-dimethylstyrene does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/30/21.

11.1.2. Repeated dose toxicity

The MOE for p,α-dimethylstyrene is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on p,α-dimethylstyrene. Read-across material styrene (CAS # 100-42-5; see Section VI) has sufficient data to support the repeated dose toxicity endpoint. Styrene has been extensively studied globally by several organizations and expert panels, such as NTP, EPA, OEHHA, ECHA, ATSDR. Moreover, styrene-mediated toxicity has been studied in animals as well as humans.

A variety of repeated inhalation exposure studies in different animal species are available; however, the rat and mouse have been investigated the most extensively. Some of the well-characterized target sites are the nasal epithelium (rats and mice), lung (rats and mice), and liver (mice). Nasal epithelial damage involving chronic inflammation of olfactory epithelium, olfactory nerve atrophy, and effects of Bowman's gland were reported, and a NOAEC has been identified. The nasal lesions induced by styrene exposure are more severe in mice than in rats. Over the years, a number of investigative studies have been undertaken to characterize and explain these species differences and to investigate the relevance of these findings to humans. The results of these investigations have demonstrated that the extent of styrene metabolism to styrene oxide is similar in rats and mice. However, the balance of bioactivation to detoxification of styrene oxide is protective in humans compared to rodents due to lack of CYP4502F2 and CYP4502F4 isoforms in human nasal tissues and increased presence (and activity) of epoxide hydrolase. This ultimately results in a reduced human capacity to form styrene oxide and an enhanced capacity to detoxify it. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which results in significantly different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is highly unlikely that high levels will be deposited in the olfactory area. Human investigations have shown that exposure up to 77 ppm (8 h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function (ECHA, 2011b).

Most of the available repeated oral exposure studies have been performed in rats and mice. A carcinogenicity bioassay in rats has demonstrated no treatment-related toxicity in animals receiving 1000 mg/kg/day dose of styrene for 2 years, contrary to a marked increase in mortality at 2000 mg/kg/day (NTP, 1979). In another 2-year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level tested. However, it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity was reported in 1 study where rats were exposed via gavage to 800 mg/kg/day styrene for 2 weeks (ECHA, 2011b). Similarly, a mouse cancer bioassay reported increased mortality and hepatic necrosis at the highest dose of 300 mg/kg/day; a NOAEL of 150 mg/kg/day was identified from this study. Furthermore, 1 significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol) (NTP, 1979; ECHA, 2011b). No repeated dose dermal studies are available, although low systemic toxicity would be predicted in most conventional experimental species with the possible exception of some mice strains (EU RAR, 2008).

Mice are more sensitive than rats to the respiratory toxicity of styrene. Exposure to 50 ppm styrene for 13 weeks resulted in atrophy of the nasal olfactory epithelium and dilatation, hypertrophy, and hyperplasia of the Bowman's gland (Cruzan et al., 1997). At 100 ppm, atrophy of the nasal olfactory nerve fibers was observed; focal crowding of non-ciliated epithelial cells in the bronchioles was observed at 150 ppm. Chronic exposure resulted in respiratory metaplasia of the nasal olfactory epithelium and dilatation, respiratory metaplasia, and epithelial hyperplasia of the Bowman's gland in mice exposed to ≥20 ppm for 2 years (Cruzan et al., 2001). Decreased eosinophilia of epithelial cells and bronchiolar epithelial hyperplasia were observed in the lungs of mice exposed to ≥20 ppm. The carcinogenicity of styrene has been examined in studies in rats and mice (Conti et al., 1988; Maltoni et al., 1982; Cruzan et al., 2001; Cruzan et al., 1998). No significant increases in the incidence of neoplastic lesions were observed in rats exposed through whole-body inhalation to styrene concentrations as high as 1000 ppm, 6 h/day, 5 days/week for 2 years (Cruzan et al., 1998). There was a dose-dependent increase in mammary tumors. Similarly, exposure of female rats to 600 or 1000 ppm styrene, 6 h/day, 5 days/week for 21 months did not result in styrene-related increases in the incidence of neoplastic tumors (ASTDR, 2010). There was a significant trend in increased incidences of malignant mammary tumors in female rats exposed to styrene; no additional increases in specific tumors were observed in this study (Conti et al., 1988). The findings of the Conti et al. (Conti et al., 1988) study conflict with those of Cruzan et al. (Cruzan et al., 1997) that reported a concentration-related decrease in mammary tumors in female rats exposed to similar or higher styrene concentrations for a longer duration. However, the decrease in body weight in the female rats exposed to ≥200 ppm may have influenced the lower occurrence of mammary tumors. Contrary to the results of the rat studies, significant increases in the incidence of bronchioloalveolar carcinoma were observed in female mice exposed to 160 ppm, 6 h/day, 5 days/week for approximately 2 years (Cruzan et al., 2001). Significant trends for increasing incidences of bronchioloalveolar adenoma were also observed in mice of both sexes. The incidence of adenoma was significantly higher than controls in males exposed to 40, 80, or 160 ppm and in females exposed to 20, 40, or 160 ppm. Due to the increased ability to detoxify styrene in humans, the rodent carcinogenicity reported following styrene exposure is not relevant to human health.

The effects of repeated styrene exposure in humans have been studied extensively but the value of many of the studies, for regulatory purposes, is limited due to lack of precision regarding the styrene exposures experienced in affected individuals or the failure to compare the exposed subjects with a suitable control group. Styrene is reported to have potential CNS effects (producing mild narcotic symptoms) following single exposures. There has been a particular emphasis on investigating its potential to produce other neurological and psychological (neurobehavioral) effects on long-term exposure (EU RAR, 2008).

Several studies in rats have reported ototoxicity resulting in hearing loss among weanling and adult F344 rats exposed to ≥800 ppm styrene by inhalation for 3 weeks. The effects were more pronounced at higher frequencies. It has been suggested that the reported data were consistent with an enhanced response in the weanling rats. Similarly, adult Wistar

rats exposed to styrene by inhalation for 4 weeks exhibited hearing loss at 600 ppm but not at 100 or 300 ppm. Morphologic changes in the cochlea of styrene-exposed rats were reported in these studies. Alterations in the inner and outer hair cells have been reported in 2 independent studies. In another study involving male Long-Evans rats, styrene administration through gavage at various doses for different time periods led to cochlear outer hair cell loss at the 800-ppm dose. Their microscopic analysis suggested that supporting cells in the cochlea known as Deiter's cells might be the proximal target for styrene toxicity (OEHHA, 2010). In 3 independent studies reviewed in the EU Risk Assessment, ototoxicity in rats was observed at doses of 600 ppm and above. However, no such effects were reported at 200 ppm (13 weeks), 300 ppm (4 weeks), or 500 ppm (4 weeks). 1 study (4 weeks) suggests that active rats are more susceptible to styrene-induced ototoxicity at lower exposure concentrations in comparison to their sedentary/ordinary counterparts due to the increased styrene uptake, as a consequence of the increased ventilation rate during periods of increased physical activity. Despite the lack of clearly elucidated toxicological mechanism, ototoxicity is considered to be of potential relevance to human health because a) the histopathological evaluation confirms the destruction of the outer hair cells (especially of row 3) of the cochlea, b) it is accompanied by an elevation of the hearing thresholds in the mid-frequency range (10–20 kHz), and c) the irreversible destruction of the hair cells occurs at slightly lower exposure concentrations than those elevating the hearing threshold. Furthermore, the severity of ototoxicity was not found to be dependent on the treatment duration. Hence, from the available NOAEC values the most conservative value of 200 ppm was considered to be the NOAEC for repeated dose toxicity endpoint as clear evidence of ototoxicity (both functional and histological) reported was reported at concentrations ≥600 ppm. In fact, the EU risk assessment concludes that ototoxicity is the most accurate endpoint for assessing the risk of styrene exposure in humans (EU RAR, 2008).

Additional studies on styrene are presented in Table 1 below.

Based on the observed ototoxicity, the 200-ppm dose was determined to be the NOAEC for the repeated dose toxicity endpoint. Using standard minute volume and body weight values for mice, the calculated NOAEL for repeated dose toxicity is 33 mg/kg/day.

$$mg \Big/ L = \frac{ppm \times Molecular\ weight}{24.45 \times 1000} = \frac{200 \times 104.15}{24.45 \times 1000} = 0.852\ mg \Big/ L$$

$$NOAEL\ (mg/kg/day) = \frac{NOAEC\ (mg/L) \times UF \times MV \times (T/day)}{Body\ weight\ (kg)} = \frac{0.852 \times 1 \times 0.12 \times 360}{0.152} = 242 mg \Big/ kg \Big/ day$$

Where Uncertainty factor (UF) is 1.

Minute volume (MV) is 0.12 L/min for mice (Subchronic) (default values taken from Bide et al., 1997).

Exposure time (T/day) is 360 min (6 h/day for 5 days a week).

Body weight is 0.152 kg (average for mice) (default values taken from Bide et al., 1997).

Therefore, the p,α-dimethylstyrene MOE for the repeated dose toxicity endpoint can be calculated by dividing the styrene NOAEL in mg/kg/day by the total systemic exposure to p,α-dimethylstyrene, 242/0.005, or 48400.

In addition, the total systemic exposure to p,α-dimethylstyrene (0.005 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: NAP, 2014; ATSDR, 2010; NTP, 1979; WHO, 1984; NTP, 2006; Savolainen and Pfaffli (1978); WHO, 1994; ECHA, 2008; Health Canada, 1993; #24107 ECHA, 2011a.

Literature Search and Risk Assessment Completed On: 06/18/21.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are no reproductive toxicity data on p,α-dimethylstyrene or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure (0.005 μg/kg/day) is below the TTC for p,α-dimethylstyrene (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012).

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/18/21.

11.1.4. Skin sensitization

Based on the application of DST, p,α-dimethylstyrene does not present a safety concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. No skin sensitization studies are available for p,α-dimethylstyrene. The chemical structure of this material indicates that it would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0). Acting conservatively due to the lack of data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008; Safford et al., 2011; Roberts et al., 2015; Safford et al., 2015b). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 2 provides the maximum acceptable concentrations for p,α-dimethylstyrene that present no appreciable risk for skin sensitization based on the reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/26/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis absorption spectra and *in vitro* study data, p,α-dimethylstyrene would not be expected to present a concern for phototoxicity. Based on the available UV/Vis absorption spectra p,α-dimethylstyrene does not present a concern for photoallergenicity.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate minor absorption between 290 and 700 nm under the biologically relevant neutral condition. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Absorbance under the acidic condition between 290 and 700 nm was demonstrated, and the corresponding molar absorption coefficient was above the benchmark of concern. There was no absorbance under basic conditions. Although the molar absorbance coefficient for peak absorbance under acidic conditions was above the benchmark of concern for phototoxic effects, it should be

Table 1
Additional studies on styrene.

Duration in detail	GLP/ Guideline	No. of animals/dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/ NOEL	Justification of NOAEL/ LOAEL/NOEL	Reference
8 weeks	Non-guideline and non-GLP	Rats, male SD (10/ group)	Inhalation	0, 30, and 800 ppm (equivalent to 32.7 and 873 mg/kg/day, assumed 360 min as duration)	LOAEL- 30 ppm	Based on increase in number of dense bodies in the nasal mucosa above ≥30 ppm	US EPA, 1987
32 weeks, 8 h/ day, 5 days/ week	Non-guideline and non-GLP	Rats, male Wistar (8/ group)	Inhalation	0 (control), 200, 2000 ppm to (equivalent to 265.6 and 2655.4 mg/kg/day)	NOAEL was considered to be 200 ppm (equivalent to 265.6 mg/kg/day)	Based on decrease in body weight and decrease in sensory nerve conduction velocity in 2000 ppm groups (started at 4 weeks of age)	Yamamoto et al. (1997); ATSDR, 2010
3 weeks, 5 days/week	Non-guideline and non-GLP	Rats, male F344 (12/ dose)	Inhalation	0 (control), 800 ppm for 14 h/day (equivalent to 2226 mg/kg/day) (99.8%)	LOAEL for ototoxicity was considered to be 800 ppm	Based on auditory brainstem response (ABR) at 8, 16, 30 kHz were severely affected in the treatment group	Yano et al. (1992); ECHA, 2011a; ATSDR, 2010
4 weeks, 5 days/week	Non-guideline and non-GLP	Long Evans rat	Inhalation	0 (control), 750 ppm (3248 mg/m^3) 6 h/day (equivalent to 1484 mg/ kg/day)	LOAEL for ototoxicity was 750 ppm (1484 mg/kg/day)	Based on hearing loss accompanied by histological damage	ATSDR, 2010
4-weeks, 12 h/ day, 5 days/ week	Non-guideline and non-GLP	Male Wistar rat (10–12/ group)	Inhalation (whole body)	0 (control), 100, 300, and 600 ppm (433, 1299, 2598 mg/m^3) (equivalent to 226.15, 678.4, 1357 mg/ kg/day respectively)	NOAEL reported as 300 ppm (678 mg/kg/day)	Based on hearing impairment and loss of outer hair cells at the high dose	ECHA, 2011a; ATSDR, 2010
4-weeks	Non-guideline and non-GLP	Long Evans rat (3 month and 4 months old)	Inhalation	700 ppm (3031 mg/m^3) 6 h/day (1385 mg/kg/day), 5 days/week (not clear about dose levels used in the study)	LOAEL was reported as 700 ppm (1385 mg/ kg/day)	Based on hearing loss	ECHA, 2011a; ATSDR, 2010
3-weeks, 14 h/ day	Non-guideline and non-GLP	12 male weanling Fischer 344 rats	Inhalation	0 (filtered air), 800, 1000, and 1200 ppm (equivalent to 2226, 2783, 3339 mg/ kg/day)	LOAEL was considered to be 800 ppm (2226 mg/kg/day)	Based on increased auditory thresholds at and above rats exposed to 800 ppm	Pryor et al., 1987; ATSDR, 2010; ECHA, 2011a; WHO, 1994
13-weeks, 5 days/week	Non-guideline and non-GLP	Sprague Dawley rats (10/sex/dose)	Inhalation	565 mg/m^3 for 7 h/day (equivalent to 170.94 mg/ kg/day),	NOAEL derived as 565 mg/m^3 (170.94 mg/kg/ day)	No functional and morphological renal changes	Viau et al., 1987; ATSDR, 2010
180 days	Non-guideline and non-GLP	50 rats	Inhalation	0 (control), 6–6.3 mg/L (1958.4–2056.3 mg/kg/ day); 50 rats were given repeated 7–8 h exposures to styrene, 5 days/week for 6 months; in total 137–139 exposures	NOAEL was considered to be 6 mg/L	No systemic toxicity was reported, except local effects like eye and nose irritation were observed.	Spencer et al., 1942
180 days, 7–8 h exposure to styrene, 5 days/week	Non-guideline and non-GLP	94 guinea pigs	Inhalation	0 (control), 6–6.3 mg/L (equivalent to 1710–1796 mg/kg/day)	LOAEL was considered to be 6 mg/L	10% of animals dosed died, decrease in bodyweight gain, microscopic examination of lungs revealed lung irritation, congestion, hemorrhage, edema, and exudation	Spencer et al., 1942
Duration not mentioned but styrene-exposed for up to 264 exposures (5 days/week)	Non-GLP and non-guideline	12 rabbits	Inhalation	0 (control),6–6.3 mg/L (1199–1258 mg/kg/day)	NOAEL was considered to be 6 mg/L	No significant changes were reported.	Spencer et al., 1942
12 months for females and 7 months for males (about 52 weeks), 7–8 h, 5 days/week	Non-GLP and non-guideline	4 monkeys (2 male and 2 females in treatment group and 3 in control)	Inhalation	0 (control), 6–6.3 mg/L (1106–1161 mg/kg/day) (1300 ppm)	NOAEL was considered to be 6 mg/L	Based on no significant effects reported	Spencer et al., 1942
26 weeks, 7 h/ day	non-GLP and non-guideline	Guinea pigs (24/group)	Inhalation	0 (control), 3 mg/L, (equivalent to 748.44 mg/ kg/day)	NOAEL- 3 mg/L (equivalent to 748.44 mg/kg/ day)	Based on no significant effects reported	Spencer et al., 1942
28 days, 5 days/week		Rats	Oral (olive oil emulsified in	0 (control), 100, 500, 1000 and 2000 mg/kg/day	NOAEL-2000 mg/ kg	No systemic effects were seen. At 500 mg/kg/day-	Spencer et al., 1942

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Table 1 (*continued*)

Duration in detail	GLP/ Guideline	No. of animals/dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/ NOEL	Justification of NOAEL/ LOAEL/NOEL	Reference
	Non-GLP and non-guideline		gum arabic solution)			irritant effects and slight local reaction in esophagus and stomach perhaps resulting in diminished weight gain; 1000 and 2000 mg/kg/day - lethal and irritant effects, esophagus and stomach irritation was pronounced, often resulting in death of animal.	
13 weeks, 6 h/day, 5 days/week	GLP study	Sprague Dawley rat (10/sex/dose), a separate group of 15 males exposed for 2, 5, 13 weeks	Inhalation (whole body)	0.94, 2,27, 4.23, and 6.31 mg/L (purity is >99.4%) (equivalent to 243.7, 588.7, 1096.3, and 1636.3 mg/kg/day)	NOAEL for systemic toxicity was considered to be 6.31 mg/L (equivalent to 1636.3 mg/kg/day)	Based on no treatment-related systemic effects reported	ECHA (2011b)
13 weeks, 6 h/day 5 days/week	GLP study	CD-1 mouse (10/sex/dose) 5/sex/group as satellite group for examination of clinical chemistry and liver histopathology at week 1 and additional 30 males were used for 5-bromo-deoxyuridine labeling	Inhalation	0 (control), 0.22, 0.44, 0.65, and 0.84 mg/L (purity ->99.4%) (equivalent to 85.2, 170.4, 251.8, 325.4 mg/kg/day) CD-1 mice is not available for conversion of doses so mice values were used	NOAEL was considered to be 0.22 mg/L (equivalent to 85.2 mg/kg/day)	Based on mortality (2 females were dead) at 0.84 mg/L, abnormalities in the lungs and liver (inflammation, fibrosis, hepatocyte loss) at higher doses >0.44 mg/L.	ECHA (2011b)
180 days	Non-GLP and non-guideline	Rats (female)	Inhalation	200, 2000 mg/m^3 (equivalent to 49, 489.6 mg/kg/day) Assumed duration of exposure as 6 h	LOAEL derived as 49 mg/kg/day	Female rats exhibited spontaneous activity, and long-term memory was impaired in male rats after 4 months of exposure in both doses.	IPCS (1984)
360 days, 7 h/day	Non-GLP and non-guideline	monkeys (1–2/sex/dose)	Inhalation	0 (control), 1300 ppm (equivalent to 893.6 mg/kg/day)	NOAEL- 1300 ppm (893.6 mg/kg/day)	No adverse effects were reported.	Wolf et al., 1956
100 days, 6 days/week	Non-GLP and non-guideline	Groups of 5 male albino rats	Oral (Groundnut oil)	0 (control), 200, and 400 mg/kg/day	NOAEL-200 mg/kg/day	Based on significant increase in SGOT, SGPT, microscopic changes like focal necrosis comprised of a few degenerated hepatocytes and inflammatory cells at the high dose	Srivastava et al., 1982
4 weeks	Non-GLP and non-guideline	Mice (75 animals)	Not reported	550 mg/kg/day	NOAEL derived as 550 mg/kg/day	No effect on the growth of the treated animals	WHO (1984)
2–11 weeks, 6 h/day 5 days/week	Non-GLP and non-guideline	Male Wistar rats (40/group)	Inhalation	0 (control), 300 ppm (339.23 mg/kg/day)	Derived LOAEL was 300 ppm (339.2 mg/kg/day)	Increase in the serum creatine kinase activity during weeks 4 and 6 and decrease in cholinesterase activity during 4 and 6 weeks. Decrease in the protein content in brain, increased acid proteinase activity at dose tested.	Savolainen, 1977
13 weeks, 6 h/day 5 days/week	Non-GLP and non-guideline	Male Sprague Dawley rats	Inhalation	50 ppm, (equivalent to 54.6 mg/kg/day) (purity is 99%)	Derived LOAEL is 50 ppm (equivalent to 54.6 mg/kg/day)	MAO B activity was decreased in all areas of the brain examined (from 38% to 50% compared to control)	Coccini et al., 1999
13 weeks, 5 days/week	Non-GLP and non-guideline	Male Sprague Dawley rats (12/group)	Oral (Corn oil)	0 (corn oil), 250, 500 mg/kg/day	LOAEL for neurotoxicity was 250 mg/kg/day	Based on significant decrease in DOPAC/DA, HVA/DA ratios in striatum at the high dose level, decrease in motor activity and grip strength, and lack of coordination	Chakrabarti (1995); WHO, 1994
13 weeks, 6 h/day, 5 days/week	Non-GLP and non-guideline	Sprague Dawley rats (10/sex/dose, 15 male rats/dose)	Inhalation	0 (control), 200, 500, 1000, and 1500 ppm (equivalent to 220.9, 552.3, 1104.7, and 1657	NOAEL for nasal tract effects-200 ppm; NOAEL for systemic toxicity	At 500, 1000, and 1500 ppm- focal disorganization of the olfactory epithelium of the nasal passages,	Cruzan et al., 1997

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Table 1 (*continued*)

Duration in detail	GLP/ Guideline	No. of animals/dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/ NOEL	Justification of NOAEL/ LOAEL/NOEL	Reference
				mg/kg/day) (purity is >99.4%)	was considered to be 1500 ppm (equivalent to 1657 mg/kg/day)	rosette formation, focal hyperplasia of basal cells, single-cell necrosis, and apparent cell loss noted at this dose levels and higher	
58 days, 5 days/week	Non-GLP and non-guideline	Immunized Rabbits (12 animals)	Oral (gavage) (vegetable oil and potato starch)	250 mg/kg/day	NOAEL derived as 250 mg/kg/day	Acute variations were reported in titer of complement and antibody, decrease in phagocytic activity	WHO (1984)
216 days, 5 days/week	Non-GLP and non-guideline	Immunized Rabbits (12 animals)	Oral (gavage) (vegetable oil and potato starch)	5 mg/kg/day	NOAEL derived as 5 mg/kg/day	No changes were reported.	WHO (1984)
202 days, 5 days/week	Non-GLP and non-guideline	Immunized Rabbits (12 animals)	Oral (gavage) (vegetable oil and potato starch)	0.5 mg/kg/day	NOAEL derived as 0.5 mg/kg/day	No changes were reported.	WHO (1984)
13 weeks, 6 h/ day 5 days/ week	Non-GLP and non-guideline	F344 rats (14/sex/dose)	Inhalation (whole body)	0 (control), 50, 200, 800 ppm (equivalent to 60.54, 242.15, 968.53 mg/kg/ day)	NOAEL was considered as 200 ppm (equivalent to 242.15 mg/kg/ day)	Based on evidence of damage and impairment in the auditory system at the next highest dose of 800 ppm	ECHA (2011b)
7-weeks, 5 days/week	Non-GLP and non-guideline	B6C3F1 mice (5/sex/ dose)	Oral (corn oil)	0 (control), 147, 215, 316, 464, 681 mg/kg/day	Derived NOAEL was considered to be 464 mg/kg/day	Based on mortality reported at the high dose level	NTP (1979)
7-weeks, 5 days/week	Non-GLP and non-guideline	Fischer 344 rats; (5/sex/ dose) 40/sex /dose in control	Oral (corn oil)	0 (control), 681, 1000, 1470, 2150, and 3160 mg/ kg/day	Derived NOAEL was considered to be 2150 mg/kg/ day	Based on mortality reported at the high dose level	NTP (1979)
52 weeks, 4 h/ day, 5 days/ week	Non-GLP and non-guideline	Sprague Dawley rats (30/sex/dose in treatment groups, 60/ sex in control)	inhalation	0 (control), 25, 50, 100, 200, and 300 ppm (16.61, 33.21, 66.42, 132.84 and 199.26 mg/kg/day) (purity is 99.8%)	Derived LOAEL- 25 ppm (equivalent to 16.61 mg/kg/day)	Increased incidence of total (benign and malignant) and malignant mammary tumors in females	Conti et al., 1988
52 weeks, 4–5 days/week	Non-GLP and non-guideline	Groups of 80 (40/sex) Sprague Dawley rats	Oral (olive oil)	0 (control), 50 or 250 mg/ kg/day (purity −99.8%)	Derived LOAEL- 50 mg/kg/day	Increase in leukemias and total and malignant tumors, and high mortality at the high dose	Conti et al., 1988
52 weeks, 4 h/ day 5 days/ week	Non-GLP and non-guideline	Sprague Dawley rats (30/sex/dose)	Inhalation	0 (control), 25, 50, 100, 200, 300 ppm (equivalent to 0, 16.6, 33.2, 66.4, 132.8, or 199.3 mg/kg/ day) (purity: 99.8%)	NOAEL- 300 ppm (equivalent to 199.3 mg/kg/day)	No effects were seen at any dose level.	Maltoni et al., 1982
52 weeks, 4–5 days/week	Non-GLP and non-guideline	Sprague Dawley rats (40/sex/dose)	Oral (olive oil)	0 (control), 50, and 250 mg/kg (purity is 99.8%)	NOAEL-250 mg/ kg/day	No effects were seen at low- and high-dose levels.	Maltoni et al., 1982
18 weeks, 16 h/day, 5 days/week	Non-GLP and non-guideline	Rats	Inhalation	0 (control), 350, 700, or 1400 ppm (equivalent to 973.2, 1946.5, and 3893.0 mg/kg/day)	LOAEL was considered to be 350 ppm (equivalent to 973.2 mg/kg/day)	Based on neurological effects, mild reductions in spontaneous activity and grip strength. A marked reduction in response speed and accuracy was noted on day 1 with tolerance rapidly developed at higher dose levels	Kulig, 1988; WHO, 1994
90 days	Non-GLP and non-guideline	Sprague Dawley rats (32 male)	Inhalation	0 (control), 90, and 320 ppm (equivalent to 98.2, 349.2 mg/kg/day) Assuming 6 h as duration of exposure	NOAEL- 98.2 mg/ kg/day	Based on increase in glial fibrillary acidic protein concentration, increase in the sensory and motor cortex and in the hippocampus at the high dose	Rosengren, 1989; ATSDR, 2010; ECHA, 2011a
18 weeks, 16 h/day, 5 days/week	Non-GLP and non-guideline	8 WAG/Rij Rats	Inhalation	0 (control), 350, 700, and 1400 ppm (equivalent to 1055.3, 2110.6, 4221 mg/ kg/day)	LOAEL was considered to be 350 ppm	Based on reversible changes: small decrease in forelimb grip strength was reported on week 9 and hindlimb grip strength on week 12 and 15, significant decrease in spontaneous movement at higher dose levels	Kulig (1989)
		Male Wistar rats	Inhalation				

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Table 1 (*continued*)

Duration in detail	GLP/ Guideline	No. of animals/dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/ NOEL	Justification of NOAEL/ LOAEL/NOEL	Reference
11 weeks, 6 h/ day, 5 days/ week	Non-GLP and non-guideline			300 ppm (equivalent to 339.2 mg/kg/day)	LOAEL derived as 339.2 mg/kg/day	Based on increased fat levels for 4 weeks and increased brain proteolysis at 300 ppm	Savolainen, 1978; IPCS, 1984
4 weeks, 6 h/ day; 5 days/ week, 6 weeks recovery period	Non-GLP and non-guideline	Male Long Evans rats	Inhalation	0 (control- filtered air), 4.29 ± 0.12 mg/L, 4.32 ± 0.14 mg/L, 4.32 ± 0.10 mg/L (average of 4.26 mg/ L; equivalent to 1113.1 mg/kg/day)	LOAEL derived as 1113.1 mg/kg/ day	Ototoxicity (hearing loss accompanied by histological damage) in rats exposed repeatedly to 4.26 mg/L styrene does not worsen with duration of exposure (from 1 to 4 weeks). Hearing loss seems to progress after the end of the exposure period, reaching its maximum at around 6 weeks post-exposure.	ECHA (2011b)
4 weeks, 6 h/ day; 5 days/ week	Non-GLP and non-guideline	Groups of 8 male Long Evans rats	Inhalation	0, 500, 650, 850, 1000, and 1500 ppm (equivalent to 0, 556.5, 723.5, 946.1, 1113.0, 1669.5 mg/kg/ day)	NOAEL derived as 556.5 mg/kg/day	Based on audiometry and were accompanied by damage to the outer hair cells in dose groups >650 ppm	Loquet et al., 1999; ATSDR, 2010

noted that the acidic condition in this assay is defined as pH 2 or less and may not be biologically relevant for our purposes, where the route of exposure is topical. In an *in vitro* 3T3 Neutral Red Uptake phototoxicity test, p,α-dimethylstyrene was not predicted to be phototoxic (RIFM, 2016b). Based on the lack of absorbance under neutral conditions and the *in vitro* study data, p,α-dimethylstyrene does not present a concern for phototoxicity. Based on the lack of absorbance under neutral conditions, p,α-dimethylstyrene does not present a concern for photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm under biologically relevant neutral conditions. The molar absorption coefficient under neutral conditions (431 L mol^{-1} • cm^{-1}) is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009). Absorbance was demonstrated under the acidic condition, with a molar absorption coefficient (2147 L mol^{-1} • cm^{-1}) above the benchmark. There was no absorbance demonstrated under basic conditions.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/28/21.

11.1.6. Local respiratory toxicity

There are insufficient inhalation data available on p,α-eimethylstyrene; however, in a chronic inhalation study for the analog α-methyl styrene (CAS # 98-83-9; see Section VI), a LOAEC of 483.35 mg/m^3 was reported (NTP, 2007).

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In a 2-year inhalation exposure carcinogenicity study, 50 male and 50 female F344/N rats were exposed to α-methyl styrene at 0, 483.35, 1450.04, and 4833.46 mg/m^3 for 6 h plus 12 min/ day and 5 days/week (NTP, 2007). Standard observations in the respiratory tract included microscopic evaluations of nasal cavities, larynx, trachea, and lungs. Effects observed in the trachea and lungs were sporadic and were observed in all the exposure groups including control. Treatment-related effects were observed only in the nasal cavities, in the form of olfactory epithelium basal cell hyperplasia in all treatment groups in both males and females, and olfactory epithelium degeneration in the animals from 4833.46 mg/m^3 group. Based on these observations, the local respiratory toxicity LOAEC is identified as 483.35 mg/m^3. Using a safety factor of 10, a NOAEC is calculated at 48.34 mg/m^3.

This NOAEC expressed in mg/kg lung weight/day is:

- (48.34 mg/m^3) × (1 m^3/1000 L) = 0.04834 mg/L
- MV of 0.21 L/min for a Fisher rat × duration of exposure of 372 min per day (min/day) (according to GLP study guidelines) = 78.12 L/ day
- (0.04834 mg/L) × (78.12 L/day) = 3.78 mg/day
- (3.78 mg/day)/(0.0016 kg lung weight of rat*) = 2362.5 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.000012 mg/day; this value was derived from the concentration survey data in the Creme RIFM exposure model (Comiskey, 2015; Safford, 2015abib_Safford_et_al_2015a). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 0.000018 mg/kg lung weight/day resulting in a MOE of 131250000 (i.e., [2362.5 mg/kg lung weight of rat/day]/[0.000018 mg/kg lung weight of human/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to inter-species and intra-species variation, the material exposure by inhalation at 0.000012 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by, Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: "Comparative Physiology and Anatomy", subsection, "Comparative Airway Anatomy."

Additional References: Duchamp (1982); Revial et al., 1982; Helmig et al., 1999a; Helmig et al., 1999b.

Literature Search and Risk Assessment Completed On: 07/15/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of p,α-dimethylstyrene was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1,

Table 2
Maximum acceptable concentrations for p,α-dimethylstyrene that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	1.6×10^{-6}%
2	Products applied to the axillae	0.0015%	2.0×10^{-4}%
3	Products applied to the face using fingertips	0.029%	7.1×10^{-5}%
4	Fine fragrance products	0.027%	6.6×10^{-4}%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	1.4×10^{-4}%
6	Products with oral and lip exposure	0.016%	6.7×10^{-4}%
7	Products applied to the hair with some hand contact	0.056%	3.0×10^{-5}%
8	Products with significant ano-genital exposure	0.0029%	No Data[b]
9	Products with body and hand exposure, primarily rinse-off	0.054%	1.0×10^{-4}%
10	Household care products with mostly hand contact	0.19%	5.1×10^{-4}%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[b]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	0.0098%

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

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, p,α-dimethylstyrene 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 p,α-dimethylstyrene 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 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, 2017a). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), p,α-dimethylstyrene presents no risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. No data available.

11.2.1.3. Ecotoxicity. RIFM, 2000: The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guideline, under static conditions. The 48-h EC50 value based on the mean measured concentration was reported to be 2.58 (95% CI: 1.79–3.73 mg/L).

11.2.1.4. Other available data. p,α-Dimethylstyrene has been pre-registered for REACH with no additional information available at this time.

11.2.1.5. Risk assessment refinement. Since p,α-dimethylstyrene has passed the screening criteria, measured data are included for completeness and have not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.99	3.99
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
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 0.00331 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 07/19/21.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	3.31			1000000	0.00331	

- **ECHA:** https://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
  - **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
  - **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
  - **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
  - **IARC:** https://monographs.iarc.fr
  - **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
  - **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
  - **US EPA ChemView:** https://chemview.epa.gov/chemview/
  - **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 02/07/22.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113390.

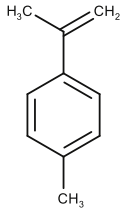
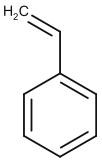
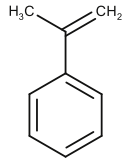
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (Date et al., 2020). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017b).

- 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 material 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, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018) and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material
Principal Name	p,α-Dimethylstyrene	Styrene	α-Methylstyrene
CAS No.	1195-32-0	100-42-5	98-83-9
Structure			
Similarity (Tanimoto Score)		0.59	0.85
SMILES	CC(=C)c1ccc(C)cc1	C=Cc1ccccc1	CC(=C)c1ccccc1
Endpoint		Repeated dose toxicity	Local respiratory toxicity
Molecular Formula	$C_{10}H_{12}$	C_8H_8	C_9H_{10}
Molecular Weight (g/mol)	132.206	104.152	118.179
Melting Point (°C, EPI Suite)	−20.00	−30.65	−23.20
Boiling Point (°C, EPI Suite)	185.30	145.30	165.40
Vapor Pressure (Pa @ 25°C, EPI Suite)	9.95E+01	8.53E+02	2.53E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	3.53E+01	3.00E+02	1.16E+02
Log K_{OW}	3.99	2.95	3.48
J_{max} (μg/cm^2/h, SAM)	6.79	48.84	21.22
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.84E+02	2.79E+02	2.58E+02
Repeated dose toxicity			
Repeated Dose (HESS)	Not categorized	Styrene (renal toxicity) alert | Toluene (renal toxicity) alert	
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	N/A

N/A: not applicable for the endpoint under consideration.

Summary

There are insufficient toxicity data on p,α-dimethylstyrene (CAS # 1195-32-0). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, [metabolism data], physical–chemical properties, and expert judgment, styrene (CAS # 100-42-5) and α-methylstyrene (CAS # 98-83-9) were identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Styrene (CAS # 100-42-5) was used as a read-across analog for the target material, p,α-dimethylstyrene (CAS # 1195-32-0), for the repeated dose toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of aromatic hydrocarbons.
  - o The target material and the read-across analog share a benzene ring with the vinyl group.
  - o The key difference between the target material and the read-across analog is that the target material has a vinyl group on an isopropyl substitution on the benzene ring while the read-across analog has a vinyl group on an ethyl substitution on the benzene ring. The vinyl group on an ethyl substitution is more reactive compared to the one on an isopropyl substitution due to methyl group hindrance. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - o The similarity between the target material 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 material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for the target material corresponds to skin absorption $\leq$40%, and J_{max} for the read-across analog corresponds to skin absorption $\leq$80%. While the percentage of skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The read-across analog has an alert for styrene- and toluene-related renal toxicity. The data on the read-across analog confirms that the substance has adequate MOE at the current level of use. Therefore, structural alert on the read-across is superseded based on the data.
  - o The target material 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.
- α-Methylstyrene (CAS # 98-83-9) was used as a read-across analog for the target material, p,α-dimethylstyrene (CAS # 1195-32-0), for the local respiratory toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of aromatic hydrocarbons.
  - o The target material and the read-across analog share a benzene ring with the vinyl group.

- o The key difference between the target material and the read-across analog is that the target material has a para methyl substitution on the benzene ring which the read-across analog lacks. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
- o The similarity between the target material 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 material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- o Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for the target material corresponds to skin absorption $\leq$40%, and J_{max} for the read-across analog corresponds to skin absorption $\leq$80%. While the percentage of skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
- o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- o There are no *in silico* alerts for the target material and the read-across analog *In silico* alerts are consistent with data.
- o The target material 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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