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RIFM fragrance ingredient safety assessment, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde, CAS registry number 52475-86-2

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Name: 1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
CAS Registry Number: 52,475-86-2
Additional CAS*
52,474-60-9
Methyl-3-(4-methyl-3-pentenyl)cyclohex- 3-ene-1-

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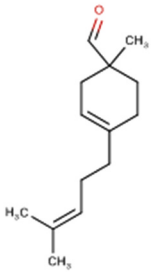
carbaldehyde
*Included because the materials are isomers



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., 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; Safford et al., 2015a, 2017; Comiskey 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
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, 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

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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.

1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde is not genotoxic. Data provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from the target material and read-across analog isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8) provided 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde a No Expected Sensitization Induction Level (NESIL) of 5900 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde 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 I material, and the exposure to 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde 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, 2006b; RIFM, 2015b)
Repeated Dose Toxicity: NOAEL = 25 mg/kg/day.	RIFM (2015c)
Reproductive Toxicity: Developmental toxicity and Fertility NOAEL = 775 mg/kg/day.	RIFM (2015c)
Skin Sensitization: NESIL = 5900 μg/cm^2.	RIFM (2018b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra; RIFM Database)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 70% day 70 (OECD 301F)	RIFM (2014c)
Bioaccumulation: Critical Measured Value: Fast metabolized (Fish S9 Liver Fractions) for CAS # 52,475-86-2	RIFM (2010)
Ecotoxicity: Critical Ecotoxicity Endpoint: 7-day *Daphnia magna* NOEC: 0.28 mg/L CAS # 52,475-86-2	RIFM (2006a)

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: 7-day *Daphnia magna* NOEC: 0.28 mg/L CAS # 52,475-86-2	RIFM (2006a)

RIFM PNEC is: 5.6 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1

1. Identification

Chemical Name: 1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde	Chemical Name: 1-Methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
CAS Registry Number: 52,475-86-2	**CAS Registry Number:** 52,474-60-9
Synonyms: 3-Cyclohexene-1-carboxaldehyde, 1-methyl-4-(4-methyl-3-pentenyl)-; Precyclemone B; 1-ﾒﾁﾙ-4-(4-ﾒﾁﾙ-3-ﾍﾟﾝﾃﾆﾙ)-3-ｼｸﾛﾍｷｾﾝ-1-ｶﾙﾎﾞｱﾙﾃﾞﾋﾄﾞ; 1-Methyl-4-(4-methylpent-3-en-1-yl)cyclohex-3-ene-1-carbaldehyde; 3-Cyclohexene-1-carboxaldehyde, 1-methyl-4-(4-	**Synonyms:** 1-Methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde; 1-Methyl-3-(4-methylpent-3-en-1-yl)cyclohex-3-ene-1-carbaldehyde; 3-Cyclohexene-1-carboxaldehyde, 1-methyl-3-(4-methyl-3-penten-1-yl)-; 3-Cyclohexene-1-carboxaldehyde, 1-

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methyl-3-penten-1-yl)-; 1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde	methyl-3-(4-methyl-3-pentenyl)-; Myrmac aldehyde; Precyclemone B
Molecular Formula: $C_{14}H_{22}O$	**Molecular Formula:** $C_{14}H_{22}O$
Molecular Weight: 206.29	**Molecular Weight:**206.29
RIFM Number: 5718	**RIFM Number:** 5717
Stereochemistry: Isomer not specified. One chiral center is present, and 2 total enantiomers are possible.	**Stereochemistry:** Isomer not specified. One chiral center is present, and 2 total enantiomers are possible.

2. Physical data*

1. **Boiling Point:** 285.5 °C (EPI Suite), 275 °C (548 K) at 1023 ± 1 hPa (RIFM, 2016a)
2. **Flash Point:** >93 °C (Globally Harmonized System), >200 °F; CC (Givaudan), estimated half-life at 25 °C was >1 year at pH 7 and 9 and ≥ 54 days at pH 4 (RIFM, 2016a), 114 °C (RIFM, 2016a)
3. **Log K_{OW}:** 4.8 (RIFM, 2014b), 4.69 (RIFM, 2005b), 5.19 (EPI Suite)
4. **Melting Point**: 47.47 °C (EPI Suite), less than −80 °C (<193 K) (RIFM, 2016a)
5. **Water Solubility:** 1.512 mg/L (EPI Suite)
6. **Specific Gravity:** 0.92 (Givaudan)
7. **Vapor Pressure:** 0.00143 mm Hg at 20 °C (EPI Suite v4.0), 0.00262 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** A colorless to pale yellow liquid with a floral, spicy, aldehydic odor

*Physical data is the same for both materials.

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 v3.1.4)*

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.24% (RIFM, 2018a)
2. **Inhalation Exposure**:** 0.00092 mg/kg/day or 0.067 mg/day (RIFM, 2018a)
3. **Total Systemic Exposure***:** 0.0072 mg/kg/day (RIFM, 2018a)

*When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in Hydroalcoholics, inhalation exposure, and total exposure.

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer Classification

Class I, Low

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

6.2. Analogs selected

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** Isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. Read-across Justification

See Appendix below

7. Metabolism

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

8. Natural occurrence

1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde and the additional material are not reported to occur in foods by the VCF*.

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

No dossiers available as of 11/11/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.047
2	Products applied to the axillae	0.14
3	Products applied to the face/body using fingertips	0.38
4	Products related to fine fragrances	2.5
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.64
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.24

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.33
5D	Baby cream, oil, talc	0.079
6	Products with oral and lip exposure	0.047
7	Products applied to the hair with some hand contact	0.33
8	Products with significant ano-genital exposure (tampon)	0.079
9	Products with body and hand exposure, primarily rinse-off (bar soap)	1.1
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.57
10B	Aerosol air freshener	3.0
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.079
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	64

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde, the basis was the reference dose of 0.25 mg/kg/day, a skin absorption value of 40%, and a skin sensitization NESIL of 5900 μg/cm^2.

[b]For a description of the categories refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf; December 2019).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.4.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 1-Methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) without metabolic activation, negative for cytotoxicity with metabolic activation, and negative for 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 and clastogenic effects of the target material.

The mutagenic activity of 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde in dimethyl sulfoxide (DMSO) at concentrations up to 78.1 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (RIFM, 2006b). Under the conditions of the study, 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was not mutagenic in the Ames test.

The clastogenic activity of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was assessed in an *in vitro* micronucleus assay conducted in compliance with GLP regulation and in accordance with OECD TG 487. Human peripheral blood lymphocytes (HPBL) were treated with 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde in DMSO at concentrations ranging between 0.21 and 2060 μg/mL in the presence and absence of S9. The percentage of cells with micronucleated binucleated cells in the test material-treated groups was not statistically significantly increased relative to vehicle control at any dose level (RIFM, 2015b). Under the conditions of the study, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was negative for induction of micronuclei in human cells.

Based on the available data, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde does not present a concern for genotoxic potential.

Additional References: RIFM, 1999a.

Literature Search and Risk Assessment Completed On: 01/27/21.

11.1.2. Repeated dose toxicity

The MOE for 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde 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 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde. In an OECD 422/GLP-compliant study, groups of 10 Wistar Han rats/sex/dose were administered 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde at doses of 0, 1000, 3000, and 10,000 ppm (mg/kg/day equivalency in males: 0, 75–80, 214–219, and 775–840, respectively; in females: 0, 86–118, 245–364, and 826–1048, respectively) through the diet. Males were treated for 33 days (2 weeks prior to mating, during mating, and until study completion), and females were treated for 41–57 days (2 weeks prior to mating, during mating, and up to lactation day 4). No animal mortality was reported at any dose level during the study. Overall, there were no alterations in functional parameters such as hearing, pupillary reflex, static righting reflex, and grip strength. Male body weights were unaffected at all tested doses; however, bodyweight gain in males that received 10,000 ppm was decreased during weeks 1 and 3 compared to controls. In female animals of the 10,000-ppm group, animals demonstrated a trend of decreased body weight during the mating period followed by a significant decrease in body weight during lactation. Bodyweight gain was significantly lowered during week 2 of the mating period in groups that received 1000 and 10,000 ppm doses. Due to palatability issues of the test diet, there was an initial decrease in food consumption in both sexes at the 3000 and 10,000 ppm dose groups that was restored within 2–3 days. Absolute and relative food consumption was significantly lower for females at 10,000 ppm than controls during lactation. Conversely, food consumption was significantly increased in females at 1000 ppm during the post-coitum (days 0–2) period. Altered food consumption was not dose-dependent and therefore was not considered to be toxicologically relevant. Hematological changes in male mean corpuscular hemoglobin (1000 ppm) and volume (1000 and 3000 ppm) were not considered toxicologically relevant due to the absence of a dose-response. In females, the 10,000-ppm dose increased blood levels of alkaline phosphatase, chloride, and sodium combined with lowered total blood bilirubin levels. Decreased blood bilirubin and increased chloride in females were also observed at the 3000-ppm dose. In males, there was an increase in chloride levels at the 10,000-ppm dose; inorganic phosphate (blood) was decreased at the 1000 ppm dose. Macroscopic examinations revealed several incidental findings (observed in lymph nodes, preputial gland, spleen, and uterus) that were not considered treatment-related adverse events; these species- and age-specific findings lacked a dose-response and/or were within the historical control range. Absolute and relative organ weights were evaluated for all dose groups during necropsy. In males, relative kidney weights

were increased at the 3000-ppm dose while the 10,000-ppm dose group demonstrated significantly increased liver (absolute and relative), epididymis (relative), and kidney (relative) weights. In females, adrenal weights were significantly decreased at 3000 ppm (relative) and 10,000 ppm (absolute and relative) doses. Additionally, relative liver and kidney weights were significantly increased in females that received the 1000 ppm dose. Since organ weight changes were observed in both sexes at the 3000 ppm as well as the 10,000 ppm dose groups, these findings were considered treatment-related adverse effects. Microscopic findings revealed treatment-related effects in both sexes. In males, the liver and kidneys were significantly affected, whereas, in females, alterations of the urinary bladder, thyroid gland, and spleen were more pronounced. Variable degrees of hepatocellular hypertrophy were observed in males and females at all dose levels. In both sexes, treatment-related hepatocellular hypertrophy (minimal) was observed at 1000 (1/5 females), 3000 (3/5 females and 1/5 males), and 10,000 (3/5 females and 4/5 males) ppm. More pronounced hepatocellular hypertrophy was observed in females (1/5) and males (1/5) at 10,000 ppm. In all males that received the highest dose, species-specific α-2-globulin related nephropathy was confirmed by the presence of hyaline droplets in the kidneys. In females (3/5), hypertrophy of the urothelium was reported (minimal: 2, slight: 1) at 10,000 ppm. Minimal follicular cell hypertrophy in the thyroid gland was observed at 3000 (2/5 females) and 10,000 (3/5 females) ppm. A dose-dependent decrease in extramedullary hematopoiesis (spleen) was observed in females at 1000 ppm (minimal: 1/5, slight: 2/5, moderate: 2/5), 3000 (slight: 2/6, moderate: 4/6), and 10,000 (minimal: 1/5, slight: 2/5) ppm doses. Based on the changes in organ weights and observed effects in microscopic findings for both sexes at the 3000 and 10,000 ppm doses, the NOAEL for repeated dose toxicity was considered to be 1000 ppm (corresponding to 75–80 and 86–118 mg/kg/day for males and females, respectively). The more conservative NOAEL of 75 mg/kg/day was selected for the repeated dose toxicity endpoint (RIFM, 2015c).

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 75/3 or 25 mg/kg/day.

Therefore, the 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde MOE for the repeated dose toxicity endpoint can be calculated by dividing the 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde NOAEL in mg/kg/day by the total systemic exposure to 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde, 25/0.0072, or 3472.

In addition, the total systemic exposure to 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde (7.2 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020b) and a reference dose of 0.25 mg/kg/day.

Derivation of reference dose (RfD)

The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The RfD for 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 25 mg/kg/day by the uncertainty factor, 100 = 0.25 mg/kg/day.

*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: 12/15/20.

11.1.3. Reproductive toxicity

The MOE for 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient repeated dose toxicity data on 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde. In an OECD 422/GLP-compliant study, groups of 10 Wistar Han rats/sex/dose were administered 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde at doses of 0, 1000, 3000, and 10,000 ppm (mg/kg/day equivalency in males: 0, 75–80, 214–219, and 775–840, respectively; in females: 0, 86–118, 245–364, and 826–1048, respectively) through the diet. Males were treated for 33 days (2 weeks prior to mating, during mating, and until study completion), and females were treated for 41–57 days (2 weeks prior to mating, during mating, and up to lactation day 4). No animal mortality was reported at any dose level during the study. No treatment-related effects were seen on reproductive parameters such as mating, fertility and conception indices, precoital time, and numbers of corpora lutea and implantation sites at any dose levels. With respect to developmental toxicity, pups at 10,000 ppm (both sexes) had lower body weights than controls on day 1 and day 4 of lactation. This was considered treatment-related but secondary to maternal toxicity and was not considered to be adverse. No treatment-related effects were seen for gestation index and duration, parturition, and early postnatal pup development, including mortality, clinical signs, and macroscopy. Thus, the NOAEL for developmental toxicity and fertility was considered to be 10,000 ppm (equivalent to 826 and 775 mg/kg/day for males and females, respectively), the highest dose tested. The most conservative NOAEL of 775 mg/kg bw/day was selected for the developmental toxicity and fertility endpoints (RIFM, 2015c).

Therefore, the 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde MOE for the reproductive toxicity endpoint can be calculated by dividing the 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde NOAEL in mg/kg/day by the total systemic exposure to 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde, 775/0.0072, or 107,639.

In addition, the total systemic exposure to 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde (7.2 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/10/21.

11.1.4. Skin sensitization

The target material is a mixture of 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde. Based on the existing data on the target and the read-across material isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8), the target mixture is considered a skin sensitizer with a defined NESIL of 5900 μg/cm^2.

11.1.4.1. Risk assessment. The target material is a mixture of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde. Based on the read-across material isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8; see Section VI), the target mixture is a skin sensitizer. The chemical structure indicates that these materials would be expected to react with skin proteins (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), a mixture of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde did not induce sensitization reactions when tested up to 25%. Higher

concentrations were not tested (RIFM, 2014a). In another LLNA, the read-across material isohexenyl cyclohexenyl carboxaldehyde was found to be sensitizing with an EC3 value of 24.0% (6000 μg/cm^2) (RIFM, 2014d). In a guinea pig maximization test, using the target mixture of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde, no skin sensitization reactions were observed (RIFM, 1999b). When tested in an open epicutaneous test (OET), the read-across material did not induce skin sensitization in guinea pigs (RIFM, 1982). In a Confirmation of No Induction in Humans test (CNIH), the read-across material did not induce sensitization in any of the 108 subjects when 5905 μg/cm^2 of in 1:3 ethanol:diethylphthalate (EtOH:DEP) was used for induction and challenge (RIFM, 2018b). In a human maximization test with the read-across material, no skin sensitization reactions were observed when 3% (2070 μg/cm^2) isohexenyl cyclohexenyl carboxaldehyde was used (RIFM, 1974).

Based on the available data and read-across to isohexenyl cyclohexenyl carboxaldehyde summarized in Table 1, the mixture of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde is considered to be a weak skin sensitizer with a defined NESIL of 5900 μg/cm^2. Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020b) and a reference dose of 0.25 mg/kg/day.

Additional References: RIFM, 1964a; RIFM, 1964b; Klecak (1985).

Literature Search and Risk Assessment Completed On: 01/18/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde 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 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde 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 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, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/09/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde. Based on the Creme RIFM Model, the inhalation exposure is 0.067 mg/day. This exposure is 20.9 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/12/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11(US EPA, 2012a) did not identify 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's

Table 1
Data summary for isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8), used as a read-across analog for mixture of 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde and 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[b] (Induction) μg/cm^2	WoE NESIL μg/cm^2
6000 [1]	**Weak**	5905	2070	NA	5900

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

* EC3 values from LLNA studies with ethanol:DEP vehicle are reported.

[3] WoE NESIL limited to 2 significant figures.

[a] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

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.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. For CAS # 52,475-86-2.

RIFM, 2014c: Ready biodegradability of the test material was evaluated in a manometric respirometry test according to the OECD 301F method. Under these conditions, biodegradation of 41%, 60%, and 70% was observed on days 28, 60, and 70, respectively.

RIFM, 2005a: The biodegradability of the test material was evaluated using the manometric respirometry test according to OECD guideline 301F. 1-Methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (35 mg/L) was added to flasks containing mineral medium inoculated with activated sludge and incubated for 28 days. The mean biodegradation rate was 23% on day 28.

RIFM, 2009: The ready biodegradability of 1-methyl-3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde was evaluated using the Headspace test according to the OECD 310 guidelines. No biodegradation was observed on days 28 and 56.

RIFM, 2010: The *in vitro* stability of the test material was determined in fish S9 liver fractions. Metabolic stability was determined by monitoring the disappearance (GC-MS) of 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde as a function of incubation time (0, 5, 10, 20, 40, and 60 min). The test material was categorized as fast metabolized.

11.2.2.1.2. Ecotoxicity. For CAS # 52,475-86-2.

RIFM, 2006a: A short-term chronic toxicity study was conducted with fathead minnow (*Pimephales promelas*) following the EPA-821-R-02-013 guidelines. The 7-day NOEC values based on nominal test concentration were reported to be 2.27 mg/L and 1.13 mg/L for survival and growth, respectively.

RIFM, 2006a: A short-term chronic study was conducted with *Daphnia magna* following the EPA-821-R-02-013 method. The 7-day NOEC values based on nominal test concentration were reported to be 1.13 mg/L and 0.28 mg/L for survival and reproduction, respectively.

RIFM, 2016b: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guideline under semi-static conditions. The 48-h EC50 value based on average exposure concentration was reported to be 0.15 mg/L (95% CI: −0.12–0.18 mg/L).

RIFM, 2017: The algae growth inhibition test was conducted according to the OECD 201 guideline under static conditions. The 72-h EC50 values based on time-weighted average concentration for growth rate and yield were reported to be 1.8 mg/L (95% CI: 1.6–2.1 mg/L) and 0.26 mg/L (95% CI: 0.18–0.40 mg/L).

11.2.2.1.3. Other available data. 1-Methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde has been pre-registered for REACH with no additional data at this time.

11.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

Exposure information and PEC calculation (following RIFM

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1.271			1000000	0.001271	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.253	0.083	0.262	10000	0.0083	Aldehydes (mono)
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.233	0.178	0.447			Neutral Organic SAR (Baseline Toxicity)
Tier 3: Measured Data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish			1.13			
Daphnia			0.28	50	5.6	
Algae						

Framework: Salvito, 2002)

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.69	4.69
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	10–100	10–100
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 5.6 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: 01/12/21.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **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
- **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 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:** 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 11/11/21.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113029.

Appendix

Read-across Justification

Methods

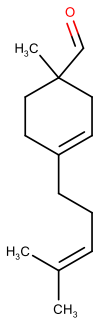
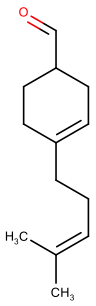
The read-across analog was identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent 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, 2017).

- 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
Principal Name	1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde	Isohexenyl cyclohexenyl carboxaldehyde
CAS No.	52,475-86-2	37,677-14-8

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Structure		
Similarity (Tanimoto Score)		1.00 • Skin sensitization
Molecular Formula	$C_{14}H_{22}O$	$C_{13}H_{20}O$
Molecular Weight	206.329	192.302
Melting Point (°C, EPI Suite)	47.47	27.71
Boiling Point (°C, EPI Suite)	285.50	278.05
Vapor Pressure (Pa @ 25°C, EPI Suite)	3.49E-01	7.83E-01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.51E+00	4.35E+00
Log KOW	5.19	4.73
J_{max} (μg/cm^2/h, SAM)	0.24	0.68
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	9.79E+01	7.38E+01
Skin Sensitization		
Protein Binding (OASIS v1.1)	Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes	Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes
Protein Binding (OECD)	Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls	Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Schiff base formation identified	Alert for Schiff base formation identified
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on the target material 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52,475-86-2). Hence, *in silico* evaluation was conducted to determine a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8) was identified as read-across material with data for their respective toxicity endpoints.

Conclusions

- Isohexenyl cyclohexenyl carboxaldehyde (CAS # 37,677-14-8) was used as a read-across analog for 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52,475-86-2) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of cyclohexene aldehydes.
  - o The target material and the read-across analog share a cyclohexene ring and exocyclic carboxaldehyde structural features.
  - o The key difference between the target material and the read-across analog is that the target material has methyl substitution on the β carbon, which is not present in the read-across analog. Methyl substitution will offer steric hindrance to the reactivity of the target material. Therefore the read-across analog is more reactive compared to the target material.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. The Tanimoto score is mainly driven by the aliphatic portion of the structure. Differences between the structures that affect the Tanimoto score do not affect consideration of the toxicity endpoint.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.

- o The CAESAR model for skin sensitization predicts the target material and the read-across analog to be a sensitizer with good reliability. The data on the read-across analog confirms that the substance is a sensitizer. Therefore 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.

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