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RIFM fragrance ingredient safety assessment, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene, CAS Registry Number 66327-54-6

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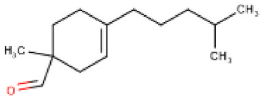

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Name: 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene

CAS Registry Number: 66327-54-6

Abbreviation/Definition List:

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

AF - Assessment Factor

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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 (Safford et al., 2015a; Safford et al., 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 et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

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

1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is not genotoxic. Data on read-across analog 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog 2,4-dimethyl-3-cyclohexen-1-carboxaldehyde (CAS # 37677-14-8) provided 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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 data and ultraviolet (UV) spectra; 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 2003a; RIFM, 2016a)
Repeated Dose Toxicity: NOAEL = 25 mg/kg/day.	RIFM (2015b)
Reproductive Toxicity: Developmental toxicity and Fertility NOAEL = 775 mg/kg/day.	RIFM (2015b)
Skin Sensitization: NESIL = 5900 μg/cm^2.	RIFM (2018)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database; RIFM, 1980a; RIFM, 1980b)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 65% day 60 (OECD 302C)	RIFM (2009a)
Bioaccumulation: Critical Measured Value: Fast metabolized (Fish S9 Liver Fractions)	RIFM (2010)
Ecotoxicity: Critical Ecotoxicity Endpoint: 7-day *Ceriodaphnia dubia* NOEC: 0.47 mg/L	RIFM (2006)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 7-day *Ceriodaphnia dubia* NOEC: 0.47 mg/L	RIFM (2006)

RIFM PNEC is: 9.4 μg/L

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

1. Identification

1. **Chemical Name:** 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene
2. **CAS Registry Number:** 66327-54-6
3. **Synonyms:** 3-Cyclohexene-1-carboxaldehyde, 1-methyl-4-(4-methylpentyl)-; 1-Methyl-4-(4-methylpentyl)-3-cyclohexene-1-carboxaldehyde; 1-Methyl-4-(4-methylpentyl)cyclohex-3-ene-1-carbaldehyde; Vernaldehyde; 1-メチル-1-ホルミル-4-(4′-メチル-ペンチル)-3-シクロヘキセン; 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene
4. **Molecular Formula:** $C_{14}H_{24}O$
5. **Molecular Weight:** 208.34 g/mol
6. **RIFM Number:** 1018
7. **Stereochemistry:** Stereoisomer not specified. One chiral center and a total of 2 enantiomers possible.

2. Physical data

1. **Boiling Point:** 275.39 °C (EPI Suite), 274 °C (547 K) at 1008 ± 4 hPa; reaction and/or decomposition was observed in combination with boiling (RIFM, 2017)
2. **Flash Point:** 78 °C (Givaudan), 78 °C (Globally Harmonized System), 112 °C (RIFM, 2017), half-life at 25, 40, 50, and 60 °C = 9.1, 11, 11, and 14 days for pH 4, ≥24, 25, 25, and 19 days for pH 7 and 55, 36, 25, and 21 days for pH 9, respectively (RIFM, 2017)
3. **Log K_{OW}:** 5.9 (RIFM, 2007), 5.27 (EPI Suite), 5.3 (RIFM, 2014b)
4. **Melting Point**: 46.54 °C (EPI Suite), less than −80 °C (<193 K) (RIFM, 2017)
5. **Water Solubility:** 1.248 mg/L (EPI Suite)
6. **Specific Gravity:** 0.8934 at 25 °C (RIFM), 0.888–0.895 at 25 °C (Givaudan)
7. **Vapor Pressure:** 0.00252 mm Hg at 20 °C (EPI Suite v4.0), 0.00454 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 500 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 natural, fresh, green, aldehydic odor

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:** 0.028% (RIFM, 2020a)
2. **Inhalation Exposure*:** 0.00010 mg/kg/day or 0.0076 mg/day (RIFM, 2020a)
3. **Total Systemic Exposure**:** 0.0012 mg/kg/day (RIFM, 2020a)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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:** 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2)
c. **Reproductive Toxicity:** 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2)
d. **Skin Sensitization:** Isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8)
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 available for inclusion in this safety assessment.
Additional References: None.

8. Natural occurrence

1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is not reported to occur in foods by the VCF*.

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

9. REACH dossier

Pre-Registered for 2010; no dossier available as of 11/11/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.20
2	Products applied to the axillae	0.14
3	Products applied to the face/body using fingertips	0.10
4	Products related to fine fragrances	1.6
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.35
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.050
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.10
5D	Baby cream, oil, talc	0.017
6	Products with oral and lip exposure	0.50
7	Products applied to the hair with some hand contact	0.15
8	Products with significant ano-genital exposure (tampon)	0.017
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.71
10A		0.25

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
	Household care products with mostly hand contact (hand dishwashing detergent)	
10B	Aerosol air freshener	0.40
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.017
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	28

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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene, the basis was the sub-chronic reference dose of 0.25 mg/kg/day, a predicted 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).
[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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene does not present a concern for genetic toxicity.

11.1.1.1. Risk assessment. 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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 mutagenicity of 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene was assessed in an Ames study conducted in compliance with GLP regulations and in accordance with OECD TG 471 using both the standard plate incorporation and modified preincubation methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102 were treated with 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene at concentrations up to 5000 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, 2003a). Based on the criteria of the assay, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is considered non-mutagenic in the Ames assay.

The clastogenic activity of 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene in dimethylformamide (DMF) at concentrations up to 2000 μg/mL in the presence and absence of metabolic activation (S9) at the 3-h and 24-h timepoints. The highest evaluated concentration in the 3-h treatment without S9 induced statistically significant increases in micronucleated cells; however, this induction was within the laboratory historical control range and was considered to be biologically irrelevant. 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene did not induce binucleated cells with micronuclei in any other test condition (RIFM, 2016a). 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene does not present a concern for genotoxic potential.

Additional References: None.

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

11.1.2. Repeated dose toxicity

The MOE for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene. Read-across material 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2; see Section VI) has sufficient repeated dose toxicity data. 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 10000 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 10000 ppm were decreased during weeks 1 and 3 compared to controls. In female animals of the 10000-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 10000-ppm doses. Due to palatability issues with the test diet, there was an initial decrease in food consumption in both sexes at the 3000- and 10000-ppm dose groups that was restored within 2–3 days. Absolute and relative food consumption was significantly lower for females at 10000 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 10000-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 10000-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 10000-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 10000 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 10000 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 10000 (3/5 females and 4/5 males) ppm. More pronounced hepatocellular hypertrophy was observed in females (1/5) and males (1/5) at 10000 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 10000 ppm. Minimal follicular cell hypertrophy in the thyroid gland was observed at 3000 (2/5 females) and 10000 (3/5 females) ppm. A dose-dependent decrease in extramedullary hematopoiesis (spleen) was observed in females at 1000 (minimal: 1/5, slight: 2/5, moderate: 2/5), 3000 (slight: 2/6, moderate: 4/6), and 10000 (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 10000-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, 2015b).

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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene, 25/0.0012, or 20833.

In addition, the total systemic exposure to 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene (1.2 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Derivation of subchronic reference dose (RfD):

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, 2020c) and a subchronic RfD of 0.25 mg/kg/day.

The subchronic RfD for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene. Read-across material 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2; see Section VI) has sufficient reproductive toxicity data. 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 10000 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 like mating, fertility and conception indices, precoital time, and numbers of corpora lutea and implantation sites at any dose levels. In female animals of the 10000-ppm group, animals demonstrated a trend of decreased body weight during the mating period followed by a significant decrease in body weight during lactation. With respect to developmental toxicity, pups at 10000 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 10000 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 endpoint (RIFM, 2015b).

Therefore, the 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene, 775/0.0012, or 645833.

In addition, the total systemic exposure to 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene (1.2 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 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

Based on the existing data and the read-across to 2,4-dimethyl-3-cyclohexen-1-carboxaldehyde (CAS # 37677-14-8), 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is considered a skin sensitizer with a defined NESIL of 5900 μg/cm^2.

11.1.4.1. Risk assessment. Based on the available data and read-across material isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8; see Section VI), 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene is a skin sensitizer. The chemical structure indicates that these materials would be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), the target material 1-formyl-1-methyl-4-(4-methyl-pentyl)-3--cyclohexene did not induce sensitization at the maximum tested concentration of 25% (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 contrast, 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 (CNIH) test, 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 (1:3 EtOH:DEP) was used for induction and challenge (RIFM, 2018). In a CNIH with 2% target material in dimethyl phthalate, no sensitization

was induced in 52 subjects who completed the test. The dose per unit area could not be calculated for this study, as the patch size was not specified in the report (RIFM, 1964a). In a human maximization test, no sensitization reactions were observed in 24 subjects in response to the target material at 4% (2760 μg/cm^2) (RIFM, 1977). Similarly, 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 isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8) summarized in Table 1, 1-formyl-1-methyl-4-(4--methyl-pentyl)-3-cyclohexene 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, 2020c) and a subchronic RfD of 0.25 mg/kg/day.

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

Literature Search and Risk Assessment Completed On: 02/08/21.

11.1.5. Phototoxicity/photoallergenicity

Based on available data and UV absorbance spectra, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene does not present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. UV absorption spectra indicate no significant absorption between 290 and 500 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). In *in vivo* studies, 3% and 10% 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene showed no phototoxic or photoallergenic reactions, respectively, when applied to guinea pigs (RIFM, 1980a; RIFM, 1980b). Based on lack of UV absorbance and available *in vivo* study data, 1-formyl-1-methyl-4-(4-methyl--pentyl)-3-cyclohexene does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV spectra for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene demonstrate no absorbance between the wavelengths of 290 and 500 nm. Molar absorption coefficient for the same range is below the benchmark of concern for phototoxicity, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

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

Table 1
Data summary for isohexenyl cyclohexenyl carboxaldehyde, used as a read-across for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene read-across.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data1	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL2 (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; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

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

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

[2] Data derived from CNIH or HMT.

[3] WoE NESIL limited to 2 significant figures.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene. Based on the Creme RIFM Model, the inhalation exposure is 0.0076 mg/day. This exposure is 184.2 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

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

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene 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, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. **RIFM, 2004:** The inherent biodegradability of the test material was evaluated by the manometric respirometry test according to the OECD 302C method. The test material at 30 mg/L had biodegradation of 53.8% observed after 42 days.

RIFM, 2003b: The ready biodegradability of the test material was evaluated by the manometric respirometry test according to the OECD 301F method. At 100 mg/L, biodegradation of 33% was observed after 35 days.

RIFM, 2009a: The inherent biodegradability of the test material was determined by the manometric respirometry test following the OECD 302C guidelines. Under conditions of the study, the test material underwent 45% biodegradation after 28 days (65% biodegradation after 60 days).

RIFM, 2009b: The purpose of this study was to determine the ready biodegradability of the test material using a manometric respirometry test according to the OECD 301F method. The test material undergoes 27% biodegradation after 28 days and 50% after 60 days.

RIFM, 2014c: The ready biodegradability of the test material was evaluated by the manometric respirometry test according to the OECD 301F method. At 30 mg/L, biodegradation of 65% was observed after 60 days.

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-formyl-1-methyl-4-(4-methyl--pentyl)-3-cyclohexene 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. **RIFM, 2016b:** The *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 0.17 mg/L (95% CI: 0.099–0.25 mg/L).

RIFM, 2006: 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 3.74 mg/L and 0.47 mg/L for survival and growth, respectively.

RIFM, 2006: A short-term chronic study was conducted with *Ceriodaphnia dubia* following the EPA-821-R-02-013 method. The 7-day NOEC value based on nominal test concentration was reported to be 0.47 mg/L for both survival and reproduction.

11.2.2.1.3. Other available data. 1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene has been pre-registered for REACH with no additional data at this time.

12.1.3. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	0.11			1000000	0.00011	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.231	0.073	0.233	10000	0.0073	Aldehydes (Mono)
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.197	0.152	0.394			Neutral Organic SAR (Baseline Toxicity)
Tier 3: Measured Data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish			0.47			
Daphnia	0.17		0.47	50	9.4	
Algae						

mg/L; PNECs in μg/L).

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	5.9	5.9
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	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 9.4 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. 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.2022.113072.

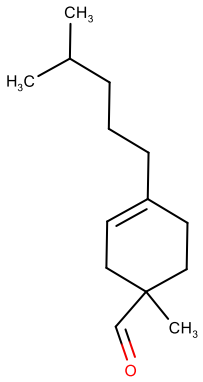
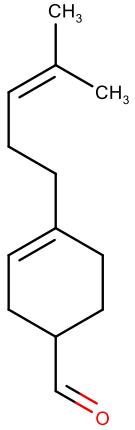
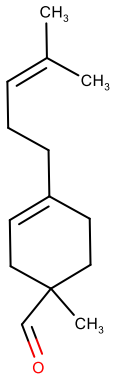
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020b). 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	Read-across Material
Principal Name	1-Formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene	Isohexenyl cyclohexenyl carboxaldehyde	1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
CAS No.	66327-54-6	37677-14-8	52475-86-2
Structure			
Similarity (Tanimoto Score)		0.88 • Skin sensitization	0.88 • Repeated dose toxicity • Reproductive toxicity
Molecular Formula	$C_{14}H_{24}O$	$C_{13}H_{20}O$	$C_{14}H_{22}O$
Molecular Weight (g/mol)	208.345	192.302	206.329
Melting Point (°C, EPI Suite)	46.54	27.71	47.47
Boiling Point (°C, EPI Suite)	275.39	278.05	285.50
Vapor Pressure (Pa @ 25 °C, EPI Suite)	6.05E-01	7.83E-01	3.49E-01
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	1.25E+00	4.35E+00	1.51E+00
Log K_{OW}	5.27	4.73	5.19
J_{max} (μg/cm^2/h, SAM)	0.20	0.68	0.24
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	9.43E+01	7.38E+01	9.79E+01
Repeated Dose Toxicity			
Repeated Dose (HESS)	Not categorized		Not categorized
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	Non-binder, without OH or NH_2 group		Non-binder, without OH or NH_2 group
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (moderate reliability)		Toxicant (low reliability)
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	See Supplemental Data 3

Summary

There are insufficient toxicity data on the target material, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene (CAS # 66327-54-6). 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 # 37677-14-8) and 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2) were identified as read-across the material with data for the skin sensitization endpoint.

Conclusions

- Isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8) was used as a read-across analog for the target material, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene (CAS # 66327-54-6), for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of alkyl-substituted cyclohexene carboxaldehyde.
  - o The target material and the read-across analog share cyclohexene with carboxaldehyde and alkyl substituents.
  - o The key difference between the target material and the read-across analog is that the target material has methyl substitution at the β carbon, which is not present in the read-across analog. The methyl substitution reduces the reactivity of aldehyde by steric hindrance compared to the read-across analog.
  - 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 compare 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. Other alerts for the skin sensitization endpoint are the same for both of the materials. The data on the read-across analog confirms that the material is a skin sensitizer. Therefore, the alerts are consistent with the data.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- 1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2) was used as a read-across analog for the target material, 1-formyl-1-methyl-4-(4-methyl-pentyl)-3-cyclohexene (CAS # 66327-54-6), for the repeated dose and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of alkyl-substituted cyclohexene carboxaldehydes.
  - o The target material and the read-across analog share cyclohexene with carboxaldehyde and alkyl substituents.
  - o The key difference between the target material and the read-across analog is that the read-across analog possesses a monosubstituted vinylene group in the alkyl chain to the cyclic ring. The target material has a saturated alkyl chain. The read-across analog contains the structural features of the target material relevant to this endpoint and is expected to have an equal or greater potential for toxicity than 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 compare 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. Other alerts for the skin sensitization endpoint are the same for both of the materials. The data on the read-across analog confirms that the material is a skin sensitizer. Therefore, the alerts are consistent with the 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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