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RIFM fragrance ingredient safety assessment, isohexenyl cyclohexenyl carboxaldehyde, CAS registry number 37677-14-8

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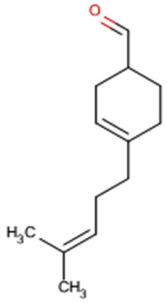
Name: Isohexenyl cyclohexenyl carboxaldehyde

CAS Registry Number: 37677-14-8

Additional CAS Numbers*:52475-89-5

Name: 3-(4-Methyl-3-pentenyl)cyclohex-3-ene-1-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., 2021)

Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2017; 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 Observable Effect Level

MOE - Margin of Exposure

MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition

NA - North America

NESIL - No Expected Sensitization Induction Level

NOAEC - No Observed Adverse Effect Concentration

NOAEL - No Observed Adverse Effect Level

NOEC - No Observed Effect Concentration

NOEL - No Observed Effect Level

OECD - Organisation for Economic Co-operation and Development

OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines

PBT - Persistent, Bioaccumulative, and Toxic

PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration

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,

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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.

Isohexenyl cyclohexenyl carboxaldehyde was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that isohexenyl cyclohexenyl carboxaldehyde is not genotoxic. Data on read-across material 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 provided isohexenyl cyclohexenyl carboxaldehyde a No Expected Sensitization Induction Level (NESIL) of 5900 □g/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; isohexenyl cyclohexenyl carboxaldehyde 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 isohexenyl cyclohexenyl carboxaldehyde is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; isohexenyl cyclohexenyl carboxaldehyde 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, 2013a; RIFM, 2016b)

Repeated Dose Toxicity: NOAEL = 25 mg/kg/day. RIFM (2015b)

Reproductive Toxicity: NOAEL = 775 mg/kg/day. RIFM (2015b)

Skin Sensitization: NESIL = 5900 □g/cm^2. RIFM (2018b)

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

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

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 71% (OECD 301D) RIFM (2009a)

Bioaccumulation: Screening-level: 616.1 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity: Critical Ecotoxicity Endpoint: 96-h Fish LC50: 0.23 mg/L (ECHA REACH Dossier: Myrac Aldehyde; ECHA, 2018)

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: 96-h Fish LC50: 0.23 mg/L (ECHA REACH Dossier: Myrac Aldehyde; ECHA, 2018)

RIFM PNEC is: 0.23 □g/L

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

1. Identification

Chemical Name: Isohexenyl cyclohexenyl carboxaldehyde	Chemical Name: 3-(4-Methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
CAS Registry Number: 37677-14-8 **Synonyms:** 3-Cyclohexene-1-carboxaldehyde, 4-(4-methyl-3-penten-1-yl)-; Empetal; 4-(4-Methyl-3-penten-1-yl)-3-cyclohexene-1-carboxaldehyde; 4-(4-Methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde; Myrac aldehyde; Vertomugal; 4-(4-ﾒﾁﾙ-3-ﾍｷｾﾝﾃﾝ-1-ｲﾙ)-3-ｼｸﾛﾍｷｾﾝ-1-ｶﾙﾎﾞｷｼｱﾙﾃﾞﾋﾄﾞ; 4-(4′-ﾒﾁﾙ-3′-ﾍｷｾﾝﾃﾝ-1-ｲﾙ)1-ﾎﾙﾐﾙ-3-ｼｸﾛﾍｷｾﾝ; 4-(4-Methylpent-3-en-1-yl)cyclohex-3-	**CAS Registry Number:** 52475-89-5 **Synonyms:** 1-Formyl-3-isohexenyl-3-cyclohexene; 3-(4-Methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde; 3-(4-Methylpent-3-en-1-yl)cyclohex-3-ene-1-carbaldehyde; 3-(4-ﾒﾁﾙ-3-ﾍｷｾﾝﾆﾙ)-3-ｼｸﾛﾍｷｾﾝ-1-ｶﾙﾊﾞﾙﾃﾞﾋﾄﾞ; 3-Cyclohexene-1-carboxaldehyde, 3-(4-methyl-3-pentenyl)-; Myrac aldehyde

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Chemical Name: Isohexenyl cyclohexenyl carboxaldehyde	Chemical Name: 3-(4-Methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
ene-1-carbaldehyde; Myraldene; Acropal; Isohexenyl cyclohexenyl carboxaldehyde	
Molecular Formula: $C_{13}H_{20}O$	**Molecular Formula:** $C_{13}H_{20}O$
Molecular Weight: 192.30 g/mol	**Molecular Weight:** 192.30 g/mol
RIFM Number: 597	**RIFM Number:** 5719
Stereochemistry: Isomer not specified. One chiral center and 2 total enantiomers possible.	**Stereochemistry:** Isomer not specified. One chiral center and 2 total enantiomers possible.

2. Physical data

CAS # 37677-14-8	CAS # 52475-89-5
Boiling Point: 549 K (275 °C) (RIFM, 2013b), 278.05 °C (EPI Suite)	**Boiling Point:** 278.05 °C (EPI Suite)
Flash Point: >200 °F; CC (Fragrance Materials Association [FMA])	**Flash Point:** >93 °C (Globally Harmonized System)
Log K_{OW}: 4.7 at 35 °C (RIFM, 1998c), 4.73 (EPI Suite)	**Log K_{OW}:** Log Pow = 4.1 (RIFM, 2014a)
Melting Point: 27.71 °C (EPI Suite)	**Melting Point:** 27.71 °C (EPI Suite)
Water Solubility: 4.348 mg/L (EPI Suite)	**Water Solubility:** 4.348 mg/L (EPI Suite)
Specific Gravity: 0.936 (FMA)	**Specific Gravity:** Not available
Vapor Pressure: 0.00329 mm Hg at 20 °C (EPI Suite v4.0), 0.005 mm Hg at 20 °C (FMA), 0.00587 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.00329 mm Hg at 20 °C (EPI Suite v4.0), 0.00587 mm Hg at 25 °C (EPI Suite)
UV Spectra: No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L • mol-1 • cm-1)	**UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L • mol-1 • cm-1)
Appearance/Organoleptic: Colorless, oily liquid	**Appearance/Organoleptic:** Not available

3. Volume of use (worldwide band)

1. **Volume of Use (worldwide band):** 100–1000 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient* (creme rifm aggregate exposure model v1.0 and v2.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.047% (RIFM, 2018a)
2. **Inhalation Exposure**:** 0.00037 mg/kg/day or 0.027 mg/day (RIFM, 2016a)
3. **Total Systemic Exposure***:** 0.0015 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 et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low

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

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:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

8. Natural occurrence

Isohexenyl cyclohexenyl carboxaldehyde has not been reported to occur in food by the VCF*.

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

9. REACH dossier

Isohexenyl cyclohexenyl carboxaldehyde and 3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde have been pre-registered for 2010; no dossiers available as of 10/13/21.

10. Conclusion

The maximum acceptable concentrationsa in finished products for isohexenyl cyclohexenyl carboxaldehyde 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)	0.040
2	Products applied to the axillae	0.14
3	Products applied to the face/body using fingertips	0.40
4	Products related to fine fragrances	1.8
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.64
5B		0.12

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.24
5D	Baby cream, oil, talc	0.040
6	Products with oral and lip exposure	0.040
7	Products applied to the hair with some hand contact	0.16
8	Products with significant ano-genital exposure (tampon)	0.040
9	Products with body and hand exposure, primarily rinse-off (bar soap)	2.9
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.36
10B	Aerosol air freshener	1.7
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.040
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	60

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 isohexenyl cyclohexenyl carboxaldehyde, the basis was the subchronic 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; December 2019).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.0.5.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the available usage and exposure data, isohexenyl cyclohexenyl carboxaldehyde does not present a concern for genotoxic potential.

11.1.1.1. Risk assessment. The mutagenic activity of isohexenyl cyclohexenyl carboxaldehyde has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and modified preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with isohexenyl cyclohexenyl carboxaldehyde in solvent dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2013a). Under the conditions of the study, isohexenyl cyclohexenyl carboxaldehyde was not mutagenic in the Ames test.

The clastogenic activity of isohexenyl cyclohexenyl carboxaldehyde 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 isohexenyl cyclohexenyl carboxaldehyde in DMSO at concentrations up to 1923 μg/mL in a dose range finding (DRF) study. Micronuclei analysis was conducted up to 143 μg/mL in the presence and absence of S9 for 3 h and the absence of S9 for 24 h. Isohexenyl cyclohexenyl carboxaldehyde did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2016b). Under the conditions of the study, isohexenyl cyclohexenyl carboxaldehyde was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, isohexenyl cyclohexenyl carboxaldehyde does not present a concern for genotoxic potential.

Additional References: RIFM, 1979; Fontaine et al., 2004.

Literature Search and Risk Assessment Completed On: 06/09/21.

11.1.2. Repeated dose toxicity

The MOE for isohexenyl cyclohexenyl carboxaldehyde is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no available repeated dose toxicity data on isohexenyl cyclohexenyl carboxaldehyde. 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 the lack of palatability of 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 while 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 the 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 isohexenyl cyclohexenyl carboxaldehyde 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 isohexenyl cyclohexenyl carboxaldehyde, 25/0.0015 or 16667.

In addition, the total systemic exposure to isohexenyl cyclohexenyl carboxaldehyde (1.5 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.

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, 2020) and a subchronic reference dose (RfD) of 0.25 mg/kg/day.

Table 1
Data Summary for isohexenyl cyclohexenyl carboxaldehyde.

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.

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

[b] Data derived from CNIH or HMT.

11.2. Derivation of subchronic RfD

The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for inter-species (10 ×) and intraspecies (10 ×) differences. The subchronic RfD for isohexenyl cyclohexenyl carboxaldehyde 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: 06/03/21.

11.2.1. Reproductive toxicity

The MOE for isohexenyl cyclohexenyl carboxaldehyde is adequate for the reproductive toxicity endpoint at the current level of use.

11.2.1.1. Risk assessment. There are no available reproductive toxicity data on isohexenyl cyclohexenyl carboxaldehyde. Read-across material 1-methyl-4-(4-methyl-3-pentenyl) cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2; see Section VI) has sufficient developmental and reproductive toxicity data.

In an OECD 422 and 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 via dietary feeding at doses of 0, 1000, 3000, or 10000 ppm. Males were treated for 33 days (2 weeks prior to mating, during mating, and until euthanasia) while females were treated for 41–57 days (2 weeks prior to mating, during mating, and up to lactation day [LD] 4). In addition to systemic toxicity parameters, the reproductive toxicity parameters were all assessed. Toxicologically relevant effects observed in the high-dose group animals included lower body weights and bodyweight gains. Food consumption was initially lower among high-dose group animals, which was attributed to the palatability of the diet. It then recovered and surpassed control levels. There were no morphological treatment-related findings in the reproductive organs of either sex, or spermatogenic staging profiles were normal for all examined males. During the first days of lactation, 3 pups each were found dead/missing (cannibalized) at 3000 and 10000 ppm. The occurrence of dead/missing pups was not considered to be toxicologically relevant since pup mortality was within the normal range for pups of this age and lacked a dose-related trend. No dead or missing pups were reported in controls or at 1000 ppm. Pup body weights at 10000 ppm were statistically significantly decreased when compared to controls in males on LD 1 and both sexes on LD 4. Since the effects on pup weights were within the historical control data range and were likely secondary to maternal toxicity, the decreased pup weights at 10000 ppm were not considered to be adverse. In macroscopic findings, 2 deceased pups did not have milk in their stomachs. Additionally, all of the pups from 1 high-dose group litter exhibited dehydration on the day of necropsy; this observation was considered to be incidental. No treatment-related adverse effects were observed on fertility (i.e., mating, fertility and conception indices, pre-coital time, and numbers of corpora lutea and implantation sites). The NOAEL for fertility was considered to be 10000 ppm (corresponding to 775–840 and 826–1048 mg/kg/day for males and females, respectively), the highest dose tested. Pups at 10000 ppm had lower body weights than controls on day 1 and day 4 of lactation, which was considered secondary to maternal toxicity; thus, the NOAEL for maternal toxicity was considered to be 3000 ppm, and the NOAEL for developmental toxicity was considered to be 10000 ppm (RIFM, 2015b). The most conservative NOAEL of 775 mg/kg/day for males was selected for the fertility and developmental toxicity endpoints.

Therefore, the isohexenyl cyclohexenyl carboxaldehyde 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 isohexenyl cyclohexenyl carboxaldehyde, 775/0.0015 or 516667.

In addition, the total systemic exposure to isohexenyl cyclohexenyl carboxaldehyde (1.5 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/24/21.

11.2.2. Skin sensitization

Based on the existing data, isohexenyl cyclohexenyl carboxaldehyde is considered a skin sensitizer with a defined NESIL of 5900 μg/cm^2.

11.2.2.1. Risk assessment. Based on the existing data, isohexenyl cyclohexenyl carboxaldehyde is considered a skin sensitizer. The chemical structure of this material indicates that it 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), isohexenyl cyclohexenyl carboxaldehyde was found to be sensitizing with an EC3 value of 24.0% (6000 μg/cm^2) (RIFM, 2014b). In a guinea pig open cutaneous test (OET), isohexenyl cyclohexenyl carboxaldehyde did not present reactions indicative of sensitization (RIFM, 1982). In a human maximization test with 3% (2070 μg/cm^2) isohexenyl cyclohexenyl carboxaldehyde, no skin sensitization reactions were observed (RIFM, 1974). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 5905 μg/cm^2 of isohexenyl cyclohexenyl carboxaldehyde in 1:3 ethanol:diethylphthalate, no reactions indicative of sensitization were observed in any of the 108 volunteers (RIFM, 2018b).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, isohexenyl cyclohexenyl carboxaldehyde is a weak sensitizer with a WoE NESIL of 5900 μg/cm^2 (Table 1). 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, 2020) and a subchronic RfD of 0.25 mg/kg/day.

Additional References: Klecak (1985).

Literature Search and Risk Assessment Completed On: 06/24/21.

11.2.3. Phototoxicity/photoallergenicity

Based on available UV/Vis absorption spectra, isohexenyl cyclohexenyl carboxaldehyde does not present a concern for phototoxicity or photoallergenicity.

11.2.3.1. Risk assessment. There are no phototoxicity studies available for isohexenyl cyclohexenyl carboxaldehyde 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 et al., 2009). Based on the lack of absorbance, isohexenyl cyclohexenyl carboxaldehyde does not present a concern for phototoxicity or photoallergenicity.

11.2.3.2. 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 et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/03/21.

11.2.4. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for isohexenyl cyclohexenyl carboxaldehyde is below the Cramer Class I TTC value for inhalation exposure local effects.

11.2.4.1. Risk assessment. There are insufficient inhalation data available on isohexenyl cyclohexenyl carboxaldehyde. Based on the Creme RIFM Model, the inhalation exposure is 0.027 mg/day. This exposure is 51.9 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: RIFM, 2017.

Literature Search and Risk Assessment Completed On: 06/24/21.

11.3. Environmental endpoint summary

11.3.1. Screening-level assessment

A screening-level risk assessment of isohexenyl cyclohexenyl carboxaldehyde 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, isohexenyl cyclohexenyl carboxaldehyde 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 did not identify isohexenyl cyclohexenyl carboxaldehyde as either being 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.

11.3.1.1. Risk assessment. Based on the current Volume of Use (2015), isohexenyl cyclohexenyl carboxaldehyde presents a risk to the aquatic compartment in the screening-level assessment.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	3.86			1000000	0.00386	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.408	0.168	0.477	10000	0.0168	Aldehydes (mono)
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.555	0.406	0.858			Neutral Organic SAR (Baseline Toxicity)
Tier 3: Measured Data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	0.23			1000	0.23	
Daphnia		0.43				
Algae		0.61				

11.3.1.2. Key studies

11.3.1.2.1. Biodegradation. RIFM, 1994: A study was conducted to determine the ultimate biodegradability of the test material using the sealed vessel test according to the OECD 301B method. Biodegradation of 47.3% was observed after 28 days.

RIFM, 1998a: The inherent biodegradability of the test material was determined by the manometric respirometry test according to OECD 302C guidelines. The test material underwent 81% biodegradation after 31 days (78% after 28 days) under the test conditions.

RIFM, 1998b: The ready biodegradability of the test material was determined by the manometric respirometry test according to the OECD 301F method. Biodegradation of 38% was observed after 28 days.

RIFM, 2009a: In order to assess the biotic degradation, a ready biodegradability test was performed in a closed bottle test performed according to slightly modified OECD 301D, EU, and ISO test guidelines. Under the conditions of the study, biodegradation of 71% was observed after 28 days.

RIFM, 2009b: The ready biodegradability of the test material was determined in the carbon dioxide sealed vessels (headspace) test following OECD 310 guidelines. The test material was biodegraded 15% at day 28 and 47% at day 56.

11.3.1.2.2. Ecotoxicity. Union Carbide Corporation Chemicals and Plastics Company, 1991: A 48-h acute toxicity test was conducted with *Daphnia magna*. The 48-h EC50 was reported to be 1.6 mg/L.

RIFM, 2018c: The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions. In this study, groups of *Daphnia magna* (4 replicates of 5) were exposed to Water Accommodated Fractions (WAFs) individually prepared at loading rates of o (control), 0.46, 1.0, 2.2, 4.6, and 10 mg/L for 48 h. The 48-h EC50 value based on mean measured concentration was determined to be 0.43 mg/L (95% C.I. 0.31–0.60 mg/L).

RIFM, 2019: The acute fish (*Danio rerio*) toxicity test was conducted according to the OECD 203 guideline (limit test), under semi-static conditions. The test was performed in a closed system without headspace. Since the test material is a liquid with low water solubility, the slow-stirring method was chosen for the preparation of the test medium. The test material was carefully applied to the surface of the test water in the stirring vessel at a loading rate of 100 mg/L. The 96-h LC50 value based on mean measured concentration was reported to be > 1.22 mg/L.

RIFM, 2018d: The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. In this study, cultures of algae were exposed to WAFs individually prepared at loading rates of 0 (control), 1.0, 2.2, 4.6, 10, and 22 mg/L for 72 h. Nominal target concentrations were analytically verified at the start of the test, and after 24- and 72-h exposure. Time Weighted Average (TWA) concentrations were determined to be 0.16, 0.32, 0.25, 0.80, and 0.88 mg/L and the effect concentrations expressed as such. The 72-h ErC50 and 72-h EyC50 values based on TWA concentration were determined to be $>$ 0.88 mg/L, and 0.61 mg/L, respectively.

11.3.1.3. Other available data. The following additional data is available at this time:

The acute fish (*Danio rerio*) toxicity test was conducted according to the OECD 203 guideline (limit test), under semi-static conditions. The study was carried out using WAFs. The WAFs (for fresh media at $t = 0$ h, $t = 24$ h, $t = 48$ h, and $t = 72$ h) were prepared under closed conditions and by slow-stirring. This limit test was performed at the nominal loading rate of 0.50 mg/L. Based on the geometric means of measured

exposure concentrations, the 96-h LC50 value was determined to be higher than 0.23 mg/L (ECHA, 2018).

11.3.1.4. 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 Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.1	4.1
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	10–100	10–100
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined Regional Volume of Use for both CAS #s.

Based on available data, the RQ for this material is < 1. No additional assessment is necessary.

The RIFM PNEC is 0.23 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 volumes of use.

Literature Search and Risk Assessment Completed On: 06/28/21.

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 10/13/21.

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.2022.112859.

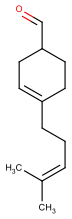
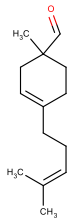
Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European 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, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization 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).

	Target Material	Read-across Material
Principal Name	Isohexenyl cyclohexenyl carboxaldehyde	1-Methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
CAS No.	37677-14-8	52475-86-2
Structure		
Similarity (Tanimoto Score)		1.00
Read-across Endpoint		• Repeated Dose Toxicity • Reproductive Toxicity
Molecular Formula	$C_{13}H_{20}O$	$C_{14}H_{22}O$
Molecular Weight (g/mol)	192.3	206.32
Melting Point (°C, EPI Suite)	27.71	47.47
Boiling Point (°C, EPI Suite)	278.05	285.50
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.783	0.349
Log Kow (KOWWIN v1.68 in EPI Suite)	4.73	5.19
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	4.348	1.512
Jmax (μg/cm^2/h, SAM)	21.3	10.6
Henry's Law (Pa·m3/mol, Bond Method, EPI Suite)	7.37E+001	9.79E+001
Repeated Dose Toxicity		
Repeated dose (HESS)	• Not categorized	• Not categorized
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH or NH2 group	• Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (moderate reliability)	• Toxicant (low reliability)
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 isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, 1-methyl-4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (CAS # 52475-86-2) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 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 isohexenyl cyclohexenyl carboxaldehyde (CAS # 37677-14-8) for the reproductive toxicity and repeated dose toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to a class of unsaturated alkyl-substituted cyclic aldehydes.
  - o The target material and the read-across analog share the same cyclohexene structure and unsaturated 4-methyl pentenyl substituent.
  - o The key difference between the target material and the read-across analog is that the read-across analog has a methyl group in the 1-position on the ring. This structural difference is toxicologically insignificant.
  - o Similarity between the target material and the read-across analog is indicated by the Tanimoto score. The difference between the structures that affect the Tanimoto score is toxicologically insignificant.
  - 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 OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The read-across analog and the target material have alerts of being toxicants with moderate reliability by the CAESAR model. The data described in the reproductive section confirm that the MOE is adequate at the current level of use. Therefore, the predictions are superseded by the data.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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