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RIFM fragrance ingredient safety assessment, 3-methyl-2-butenyl salicylate, CAS Registry Number 68555-58-8

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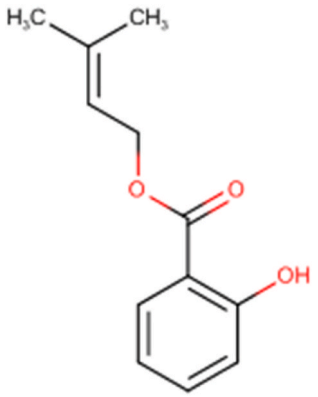

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015a; 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.

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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.

3-Methyl-2-butenyl salicylate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data and read-across to 1,3-dimethyl-3-butenyl salicylate (CAS # 80118-10-1) show that 3-methyl-2-butenyl salicylate is not expected to be genotoxic. Data on read-across material *cis*-3-hexenyl salicylate (CAS # 65405-77-8) provided a calculated Margin of Exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints and provided 3-methyl-2-butenyl salicylate a No Expected Sensitization Induction Level (NESIL) of 14000 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 3-methyl-2-butenyl salicylate 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; the exposure to 3-methyl-2-butenyl salicylate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 3-methyl-2-butenyl salicylate 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 expected to be genotoxic. (RIFM, 2018c; RIFM, 2020c)
Repeated Dose Toxicity: NOAEL = 40 mg/kg/day. (JECDB, 2013)
Reproductive Toxicity: Developmental toxicity NOAEL: 120 mg/kg/day. Fertility NOAEL: 120 mg/kg/day. JECDB (2013)
Skin Sensitization: NESIL = 14000 μg/cm^2. RIFM (2013)
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: 86% (OECD 301F) RIFM (2010)
Bioaccumulation:
Screening-level: 376.9 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: 96-h Algae EC50: 0.451 mg/L (ECOSAR; US EPA, 2012b)
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 Algae EC50: 0.451 mg/L (ECOSAR; US EPA, 2012b
RIFM PNEC is: 0.0451 μg/L
- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

1. **Chemical Name:** 3-Methyl-2-butenyl salicylate
2. **CAS Registry Number:** 68555-58-8

3. **Synonyms:** Benzoic acid, 2-hydroxy-, 3-methyl-2-butenyl ester; Prenyl salicylate; 3-Methylbut-2-en-1-yl salicylate; 3-Methyl-2-butenyl salicylate
4. **Molecular Formula:** $C_{12}H_{14}O_3$
5. **Molecular Weight:** 206.24
6. **RIFM Number:** 31
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 125 °C at 2 mm Hg (Fragrance Materials Association [FMA]), 314.96 °C (EPI Suite), 215.7 °C at 1013 hPa (RIFM, 2018a)
2. **Flash Point:** >200 °F; CC (FMA), >93 °C (Globally Harmonized System), 142.5 °C at 1013 hPa (average corrected and rounded down to nearest multiple of 0.5 °C) (RIFM, 2018b)
3. **Log K_{OW}:** 4.41 (EPI Suite), 4.49 at 25 °C (RIFM, 2018d)
4. **Melting Point**: 84.59 °C (EPI Suite)
5. **Water Solubility:** 26.51 mg/L (EPI Suite)
6. **Specific Gravity:** 1.09 (FMA)
7. **Vapor Pressure:** 7.4e-005 mm Hg at 25 °C (EPI Suite), 0.0000367 mm Hg at 20 °C (EPI Suite v4.0)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm. Molar absorption coefficients (60.9 L mol^{-1} • cm^{-1}, 86.0 L mol^{-1} • cm^{-1}, and 409.7 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions respectively) are below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** A colorless liquid

3. Volume of use (worldwide band)

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

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v2.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.1% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00016 mg/kg/day or 0.012 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0023 mg/kg/day (RIFM, 2019)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I

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

2. Analogs Selected:
   a. **Genotoxicity:** 1,3-Dimethyl-3-butenyl salicylate (CAS # 80118-10-1)
   b. **Repeated Dose Toxicity:** *cis*-3-Hexenyl salicylate (CAS # 65405-77-8)
   c. **Reproductive Toxicity:** *cis*-3-Hexenyl salicylate (CAS # 65405-77-8)
   d. **Skin Sensitization:** *cis*-3-Hexenyl salicylate (CAS # 65405-77-8)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

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

8. Natural occurrence

3-Methyl-2-butenyl salicylate 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

Available; accessed 10/06/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 3-methyl-2-butenyl 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)	0.0024
2	Products applied to the axillae	0.32
3	Products applied to the face/body using fingertips	0.029
4	Products related to fine fragrances	5.2
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	1.5
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	1.4
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0024
5D	Baby cream, oil, talc	0.00080
6	Products with oral and lip exposure	0.0024
7	Products applied to the hair with some hand contact	0.092
8	Products with significant ano-genital exposure (tampon)	0.00080
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.48
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.067
10B	Aerosol air freshener	2.4
11	Products with intended skin contact but minimal transfer of fragrance to	0.00080

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
	skin from inert substrate (feminine hygiene pad)	
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	25

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 3-methyl-2-butenyl, the basis was the reference dose of 0.40 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 14000 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 v3.0.5.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 3-methyl-2-butenyl salicylate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 3-methyl-2-butenyl salicylate 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 pre-incubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 3-methyl-2-butenyl salicylate in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2018c). Under the conditions of the study, 3-methyl-2-butenyl salicylate was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of 3-methyl-2-butenyl salicylate; however, read-across can be made to 1,3-dimethyl-3-butenyl salicylate (CAS # 80118-10-1; see Section VI).

The clastogenic activity of 1,3-dimethyl-3-butenyl salicylate 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 1,3-dimethyl-3-butenyl salicylate in dimethyl sulfoxide (DMSO) at concentrations up to 2000 μg/mL in a dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 200 μg/mL in the presence and absence of metabolic activation. 1,3-Dimethyl-3-butenyl salicylate did not induce binucleated cells with micronuclei when tested up to the cytotoxic level concentration in either the presence or absence of an S9 activation system (RIFM, 2020c). Under the conditions of the study, 1, 3-dimethyl-3-butenyl salicylate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 3-methyl-2--butenyl salicylate.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/28/21.

11.1.2. Repeated dose toxicity

The MOE for 3-methyl-2-butenyl salicylate 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 for 3-methyl-2-butenyl salicylate. Read-across material *cis*-3-hexenyl salicylate (CAS # 65405-77-8; see Section VI) has sufficient repeated dose toxicity data.

In an OECD TG 422 study, 12 Sprague Dawley SPF rats/sex/dose were administered *cis*-3-hexenyl salicylate via gavage at doses of 0 (control group: corn oil), 40, 120, and 360 mg/kg. An additional 10 females/dose were treated with 0 mg/kg/day and 360 mg/kg/day as non-mating groups. Males were treated for 14 days before mating and throughout the mating period until the day before euthanasia (42 days). Females in the mating group were treated for 14 days before mating and throughout the mating and gestation periods until 4 days before nursing (41–49 days). Females in the non-mating group were treated for 42 days. Additionally, 5 males in the mating group and 5 females in the non-mating group at 0 and 360 mg/kg were maintained for a recovery period of 14 days after dosing. No treatment-related effects were observed in any parameter at 40 or 120 mg/kg/day. At 360 mg/kg, a total of 3 females from the mating group died on day 23 of gestation and day 1 of lactation. No treatment-related effects were observed in general clinical observations, functional testing, grip strength tests, or locomotor activity tests. Decreased body weight and bodyweight gain during gestation were observed, as well as decreased bodyweight gain and food intake consumption during lactation. Increased water consumption and urine output and decreased urinary osmotic pressure were observed in males. Decreased red blood cell count and increased mean cell volume, mean corpuscular hemoglobin volume, and reticulocyte count were observed in males; these hematological changes were the only effects to persist through the recovery period. Prolonged activated partial thromboplastin time and prolonged prothrombin time were observed in males. Decreased platelet count was observed in mating group females. Increased levels of AST, A/G ratio, and inorganic phosphorus were observed in both sexes. Additionally, increased levels of total bile acids, phospholipids, albumin, and creatinine were observed in males. Increased levels of creatinine were found in mating group females. Increased levels of ALT and triglyceride as well as decreased levels of glucose, potassium, and chlorine were observed in non-mating group females. Increased liver weight in non-mating group females and decreased pituitary weight in mating group females were observed. Increased frequency of glandular stomach dark red coloring, glandular stomach erosion, and femur trabecular bone were observed in both sexes (JECDB, 2013). Based on body weight and food consumption in females as well as hematological and clinical chemistry changes in both sexes at 360 mg/kg/day, the NOAEL for this study was considered to be 120 mg/kg/day.

A default safety factor of 3 was used when deriving a NOAEL from the 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 120/3 or 40 mg/kg/day.

Therefore, the 3-methyl-2-butenyl salicylate MOE for the repeated dose toxicity endpoint can be calculated by dividing the *cis*-3-hexenyl salicylate NOAEL in mg/kg/day by the total systemic exposure to 3-Methyl-2-butenyl salicylate, 40/0.0023 or 17391.

In addition, the total systemic exposure to 3-methyl-2-butenyl salicylate (2.3 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, 2020b) and a reference dose (RfD) of 0.40 mg/kg/day.

11.1.2.1.1. Derivation of RfD. The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The RfD for 3-methyl-2-butenyl salicylate was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive

Toxicity sections) of 40 mg/kg/day by the uncertainty factor, 100 = 0.40 mg/kg/day.

*The Expert Panel for Fragrances Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/29/21.

11.1.3. Reproductive toxicity

The MOE for 3-methyl-2-butenyl salicylate 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 3-methyl-2-butenyl salicylate. Read-across material (Z)-3-hexenyl salicylate (CAS # 65405-77-8; see Section VI) has sufficient reproductive toxicity data.

There are sufficient reproductive toxicity data on (Z)-3-hexenyl salicylate. An OECD 422/GLP combined repeated dose toxicity study with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were exposed to the test material 4-methylpent-3-en-2-one at doses of 40, 120, or 360 in corn oil via oral gavage. Rats were treated for 14 days pre-mating, during mating, and for females in gestation periods until 4 days before nursing (41–49 days). No treatment-related effects were observed in the estrous cycle, the number of days required to mate, copulation rate, insemination rate, and conception rate, the sex ratio at 0 and 4 days after birth, or macroscopic findings at 4 days after birth. During the lactation period, poor lactation was observed in mid- and high-dose groups, and 3 dams along with all suckling rats died during the lactation period only in the 360 mg/kg group. In the high-dose group, a tendency toward low birth rates and a prolonged gestation period, low implantation count, a tendency toward low delivery rates, and a tendency toward a high number of stillbirths and low number in litter were observed. In addition, vestigial tails, holorachischisis, and exencephaly were also observed. Low body weights in males and females at 0 and 4 days after birth and low body weight increase rates during that period were observed, and both a tendency toward low birth rates and a low survival rate 4 days after birth were also shown. In the 40 mg/kg and 120 mg/kg groups, no effects resulting from the administration of the test material were observed. Hence, the NOAEL for fertility was considered to be 120 mg/kg/day, based on the tendency toward low birth rates, prolonged gestation period, and low implantation count at 360 mg/kg/day. The NOAEL for developmental toxicity was considered to be 120 mg/kg/day based on a high number of stillbirths and low number in litter, vestigial tails, holorachischisis, exencephaly, and low survival rates observed at 360 mg/kg/day (JECDB, 2013).

Therefore, the 3-methyl-2-butenyl salicylate MOE for the developmental toxicity endpoint can be calculated by dividing the (Z)-3-hexenyl salicylate NOAEL in mg/kg/day by the total systemic exposure to 3-methyl-2-butenyl salicylate, 120/0.0023 or 52174.

The 3-methyl-2-butenyl salicylate MOE for the fertility endpoint can be calculated by dividing the (Z)-3-hexenyl salicylate NOAEL in mg/kg/day by the total systemic exposure to 3-methyl-2-butenyl salicylate, 120/0.0023 or 52174.

In addition, the total systemic exposure to 3-methyl-2-butenyl salicylate (2.3 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: 05/07/21.

11.1.4. Skin sensitization

Based on the existing data and read-across material *cis*-3-hexenyl salicylate (CAS # 65405-77-8), 3-methyl-2-butenyl salicylate is considered a skin sensitizer with a defined NESIL of 14000 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for 3-methyl-2-butenyl salicylate. Based on the existing data and read-across material *cis*-3-hexenyl salicylate (CAS # 65405-77-8; see Section VI), 3-methyl-2-butenyl salicylate is considered a skin sensitizer. The chemical structure of these materials indicate that they would not be expected to react directly with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). 3-Methyl-2-butenyl salicylate was found to be negative in an *in vitro* direct peptide reactivity assay and KeratinoSens test (ECHA, 2019). However, in a murine local lymph node assay (LLNA), read-across material *cis*-3-hexenyl salicylate was found to be sensitizing with an EC3 value of 3.6% (902 μg/cm^2) (RIFM, 1999a). In a guinea pig maximization test, the read-across material *cis*-3-hexenyl salicylate did not present reactions indicative of sensitization at 100% (ECHA, 2013; RIFM, 1999b). In 2 human maximization tests (HMTs), no skin sensitization reactions were observed with 20% (13800 μg/cm^2) 3-methyl-2-butenyl salicylate and with 3% (2070 μg/cm^2) read-across material *cis*-3-hexenyl salicylate (RIFM, 1978; RIFM, 1975). Additionally, in 2 Confirmation of No Induction in Humans tests (CNIHs) with 26% or 14325 μg/cm^2 and 15% or 8264 μg/cm^2 read-across material *cis*-3-hexenyl salicylate in 1:3 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 103 and 110 volunteers, respectively (RIFM, 2013; RIFM, 2012).

Based on weight of evidence (WoE) from structural analysis, human studies, and data on read-across material *cis*-3-hexenyl salicylate, 3-methyl-2-butenyl salicylate is a moderate sensitizer with a WoE NESIL of 14000 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, 2020b) and a reference dose of 0.40 mg/kg/day.

Additional References: RIFM, 2000; Klecak (1985); RIFM, 1981.

Literature Search and Risk Assessment Completed On: 05/06/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 3-methyl-2-butenyl salicylate would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 3-methyl-2-butenyl salicylate in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry

Table 1
Data summary for *cis*-3-hexenyl salicylate as read-across for 3-methyl-2-butenyl salicylate.

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[c] μg/cm^2
902.5	Moderate	14325	13800	NA	14000

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.

[c] WoE NESIL limited to 2 significant figures.

et al., 2009). Based on the lack of absorbance, 3-methyl-2-butenyl salicylate does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm, peaking at about 300 nm. The molar absorption coefficients under neutral, acidic, and basic conditions (60.9, 86.0, 409.7 L mol^{-1} • cm^{-1}, respectively) are well 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: 04/29/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 3-methyl-2-butenyl salicylate is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are insufficient inhalation data available on 3-methyl-2-butenyl salicylate. Based on the Creme RIFM Model, the inhalation exposure is 0.012 mg/day. This exposure is 116.7 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: Belsito et al., 2007.

Literature Search and Risk Assessment Completed On: 05/04/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 3-methyl-2-butenyl salicylate 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, 3-methyl-2-butenyl salicylate 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 3-methyl-2-butenyl salicylate 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), 3-methyl-2-butenyl salicylate 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, 2010: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guideline. Biodegradation of 86% was observed after 28 days.

Ecotoxicity: No data available.

11.2.2.1.2. Other available data. 3-Methyl-2-butenyl salicylate has been registered for REACH with the following additional data available at this time (ECHA, 2019):

The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guideline. Biodegradation of 80% was observed after 28 days.

The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions. The 48-h EC50 value based on mean measured concentrations was reported to be 3.96 mg/L.

The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC50 values based on mean measured concentrations for growth rate and yield were reported to be 0.745 mg/L and 0.69 mg/L, respectively.

11.2.3. Risk assessment refinement

Since 3-Methyl-2-butenyl salicylate has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are highlighted.

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	4.49	4.49
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.0451 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: 05/06/21.

12. Literature Search*

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1.90			1000000	0.00190	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.080	1.735	0.504			Esters
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.528	0.465	1.693			Phenols
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.595	2.062	0.451	10000	0.0451	Vinyl/Allyl Esters
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.162	0.824	1.540			Neutral Organics

- **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 10/06/21.

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.2021.112735.

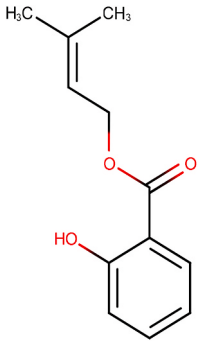
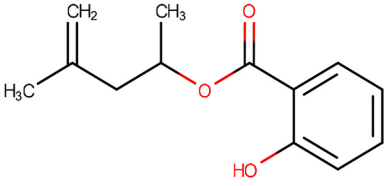
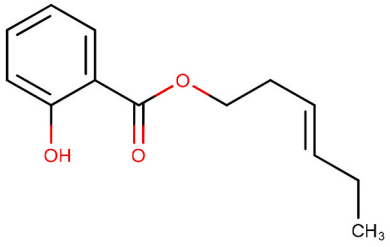
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (RIFM, 2020a). These criteria are in compliance with 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, 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	Read-across Material
Principal Name	3-Methyl-2-butenyl salicylate	1,3-Dimethyl-3-butenyl salicylate	*cis*-3-Hexenyl salicylate
CAS No.	68555-58-8	80118-10-1	65405-77-8
Structure			
Similarity (Tanimoto Score)		0.62	0.69
Endpoint		• Genotoxicity	• Skin sensitization • Repeated dose toxicity • Reproductive toxicity
Molecular Formula	$C_{12}H_{14}O_3$	$C_{13}H_{16}O_3$	$C_{13}H_{16}O_3$
Molecular Weight	206.241	220.268	220.268
Melting Point (°C, EPI Suite)	84.59	84.53	99.52
Boiling Point (°C, EPI Suite)	314.96	315.16	331.71
Vapor Pressure (Pa @ 25°C, EPI Suite)	9.87E-03	9.76E-03	2.60E-03
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	2.65E+01	8.44E+00	9.52E+00
Log KOW	4.41	4.91	4.84
J_{max} (μg/cm^2/h, SAM)	3.20	1.14	1.25
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.49E+00	1.67E+00	1.67E+00
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	No alert found	
DNA Binding (OECD QSAR Toolbox v4.2)	No alert found	No alert found	
Carcinogenicity (ISS)	No alert found No alert found	No alert found No alert found	

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
DNA Binding (Ames, MN, CA, OASIS v1.1)			
***In Vitro* Mutagenicity (Ames, ISS)**	No alert found	No alert found	
***In Vivo* Mutagenicity (Micronucleus, ISS)**	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	
Oncologic Classification	Phenol-type Compounds	Phenol-type Compounds	
Repeated Dose Toxicity			
Repeated Dose (HESS)	Mefenamic Acid (Hepatotoxicity) Alert|Menadione (Hepatotoxicity) Alert		Not categorized
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	Strong binder, OH group		Strong binder, OH group
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (moderate reliability)		Non-toxicant (moderate reliability)
Skin Sensitization			
Protein Binding (OASIS v1.1)	SN2|SN2 ≫ SN2 Reaction at a sp3 carbon atom|SN2 ≫ SN2 Reaction at a sp3 carbon atom ≫ Activated alkyl esters and thioesters		No alert found
Protein Binding (OECD)	SN2|SN2 ≫ SN2 reaction at sp3 carbon atom|SN2 ≫ SN2 reaction at sp3 carbon atom ≫ Allyl acetates and related chemicals		No alert found
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)	SN2|SN2 ≫ SN2 Reaction at a sp3 carbon atom|SN2 ≫ SN2 Reaction at a sp3 carbon atom ≫ Activated alkyl esters and thioesters		No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Acyl Transfer agent identified.		No skin sensitization reactivity domain alerts 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 3-methyl-2-butenyl salicylate (CAS # 68555-58-8). 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, materials 1,3-dimethyl-3-butenyl salicylate (CAS # 80118-10-1) and *cis*-3-hexenyl salicylate (CAS # 65405-77-8) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 1,3-Dimethyl-3-butenyl salicylate (CAS # 80118-10-1) was used as a read-across analog for the target material 3-methyl-2-butenyl salicylate (CAS # 68555-58-8) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the class of esters of salicylic acid.
  - o The target material and the read-across analog share a salicylic acid portion.
  - o The key difference between the target material and the read-across analog is that the target material has a vinylene unsaturation on the alcohol portion while the read-across analog has a vinyl unsaturation on the alcohol portion. With this structural difference, the read-across analog is predicted to be more reactive for genotoxicity.
  - o Similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o 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 target material and the read-across analog have an alert for micronucleus assay H-acceptor-path3-H-acceptor by *in vivo* mutagenicity by the ISS model. They are also classified as phenol-type compounds by the oncologic classifier of the OECD QSAR Toolbox. These alerts are due to the fact that the substances are esters of salicylic acids. The OHCCC═O substructure is responsible for having the H-acceptor alert as 2 H-bond acceptors are separated by 2 carbon atoms. The phenolic classification of the substances is due to the fact that salicylic acid possesses a phenolic substructure. The data on the read-across analog confirm that the material does not pose a concern for genetic toxicity. Therefore, based on the structural similarity between the target material and the read-across analog as well as the data for the read-across analog, the *in silico* alerts are superseded by the data.

- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

- *cis*-3-Hexenyl salicylate (CAS # 65405-77-8) was used as a read-across analog for the target material 3-methyl-2-butenyl salicylate (CAS # 68555-58-8) for the skin sensitization, reproductive toxicity, and repeated dose toxicity endpoints.
  - The target material and the read-across analog are structurally similar and belong to the class of esters of salicylic acid.
  - The target material and the read-across analog share a salicylic acid portion.
  - The key difference between the target material and the read-across analog is that the target material has a branched vinylene unsaturation on the alcohol portion while the read-across analog has a straight chain vinylene unsaturation on the alcohol portion. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - 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.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - The target material has a mefenamic acid (hepatotoxicity) alert and a menadione (hepatotoxicity) alert by HSESS categorization for repeated dose toxicity. The read-across analog does not have that alert.
  - The target material is categorized as toxic for the developmental toxicity endpoint by the CAESAR model. The read-across analog is not categorized as such. Both substances are predicted to be strong estrogen receptors binders. The data for the read-across analog confirm that the MOE for the material is adequate at the current level of use. Therefore, based on the structural similarity between the target and the read-across analog as well as the data for the read-across analog, the *in silico* alert is superseded by the data.
  - The target material is predicted to undergo SN2 reaction forming adducts with proteins. The data on the read-across analog confirm that the material is a skin sensitizer. The alert is consistent with the data.
  - The target has an alert for weak skin sensitizer. The alert is due to the presence of the 2–3 position vinylene unsaturation on the alcohol portion of the ester functionality. This substructure is present in the training set in the profile within OECD QSAR Toolbox v4.2. But the target has a 3-methyl substitution on the 2–3 unsaturation, which renders the target material non-reactive. Therefore, the target material is considered out of the structural domain from the training set. The presence of negative DPRA data on the target supersedes the alert. Also, *in vivo* data (LLNA and guinea pig data; please see skin sensitization section for detailed explanation on the data) on the read-across confirm that the read-across analog is more reactive compared to the target.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - 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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