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Short Review

RIFM fragrance ingredient safety assessment, hexyl salicylate, CAS Registry Number 6259-76-3

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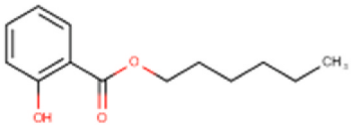

Name: Hexyl salicylate CAS Registry Number: 6259-76-3

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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., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017;

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Safford et al., 2015a; Safford 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; please note that the citation dates used for studies sourced from the ECHA website are the dates the dossiers were first published, not the dates that the studies were conducted
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

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

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

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

Hexyl salicylate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data from hexyl salicylate and read-across analog isoamyl salicylate (CAS # 87-20-7) show that hexyl salicylate is not expected to be genotoxic. Data from read-across analog amyl salicylate (CAS # 2050-08-0) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data provided hexyl salicylate a No Expected Sensitization Induction Level (NESIL) of 35000 μg/cm^2 for the skin sensitization endpoint. The photoirritation/photoallergenicity endpoints were evaluated based on data; hexyl salicylate is not photoirritating/photoallergenic. Data on hexyl salicylate provide a calculated MOE >100 for the local respiratory endpoint. The environmental endpoints were evaluated; hexyl 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 (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Genotoxicity: Not expected to be genotoxic. (RIFM, 2000d; RIFM, 2015a)	
Repeated Dose Toxicity: NOAEL = 281 mg/kg/day.	RIFM (2020a)
Reproductive Toxicity: NOAEL = 333 mg/kg/day.	RIFM (2020b)
Skin Sensitization: NESIL = 35000 μg/cm^2.	RIFM (2004a)
Photoirritation/ Photoallergenicity: Not photoirritating/photoallergenic.	(Forbes et al., 1977; RIFM, 2002; RIFM, 2003; RIFM, 2004b; RIFM, 1975b)
Local Respiratory Toxicity: NOAEC = 249 mg/m^3.	ECHA (2011)

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Critical Measured Value: 91% (OECD 301F)	RIFM (1995)
Bioaccumulation:	
Screening-level: 1012 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Critical Ecotoxicity Endpoint: *Daphnia magna* EC10: 0.045 mg/L	(Milligan et al., 2021)
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: *D. magna* EC10: 0.045 mg/L	(Milligan et al., 2021)
RIFM PNEC is: 4.5 μg/L	
• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe >1	

1. Identification

1. **Chemical Name:** Hexyl salicylate
2. **CAS Registry Number:** 6259-76-3
3. **Synonyms:** Benzoic acid, 2-hydroxy-, hexyl ester; Hexyl o-hydroxybenzoate; ﾋﾄﾞﾛｷｼ安息香酸ｱﾙｷﾙ(C = 1 ~ 22); Hexyl salicylate
4. **Molecular Formula:** $C_{13}H_{18}O_3$
5. **Molecular Weight:** 222.28 g/mol
6. **RIFM Number:** 630
7. **Stereochemistry:** No stereoisomer possible.

2. Physical data

1. **Boiling Point:** >200 °C (Fragrance Materials Association [FMA]), 327.79 °C (EPI Suite v4.11)
2. **Flash Point:** >200 °F; closed cup (FMA), 151 °C (Globally Harmonized System)
3. **Log K_{OW}:** 5.5 at 30 °C (RIFM, 1996), 5.06 (EPI Suite v4.11)
4. **Melting Point**: 99.68 °C (EPI Suite v4.11)
5. **Water Solubility:** 6.084 mg/L (EPI Suite v4.11)
6. **Specific Gravity:** 1.040 (FMA), 1.04 g/mL (RIFM, 1994)
7. **Vapor Pressure:** 0.0000118 mm Hg at 20 °C (EPI Suite v4.0), 2.44e-005 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm with peak absorbance at 305 nm and returning to baseline by 330 nm; molar absorption coefficients (3294, 3300, 3420 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are above the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Arctander, 1969: Colorless oily liquid. Very faint, sweet-herbaceous, and floral odor with dry-bark-like green undertones.

3. Volume of use (worldwide band)

1. >1000 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.2.6)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.74% (RIFM, 2018)
2. **Inhalation Exposure*:** 0.0022 mg/kg/day or 0.16 mg/day (RIFM, 2018)
3. **Total Systemic Exposure**:** 0.0037 mg/kg/day (RIFM, 2018)

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

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

5. Derivation of systemic absorption

1. **Dermal:** 7.8% SABS

ECHA REACH Dossier: Hexyl salicylate (ECHA, 2011): An *in vitro* human skin absorption study for hexyl salicylate (CAS # 6259-76-3) at concentrations of 0.1%, 20%, or 100% (in dipropylene glycol) was conducted following OECD TG 428 guidelines for 24 h. The test material was 14C-radiolabelled and applied to split-thickness abdominal or breast skin membranes (N = 8) obtained from 4 female donors under unoccluded conditions. Exposure to the test material occurred for 8 h and then was terminated by washing with a 3% soap solution (which removed 93.5%, 87.9%, and 97.6% of the applied activity at each dose, respectively). The skin membranes were tape-stripped at the termination of the study 24 h after exposure. The dermal absorption rate was 2.7%, 7.8%, and 0.8% for the 0.1%, 20%, and 100% solutions, respectively. The most conservative value of 7.8% was selected for this study. The overall recovery of hexyl salicylate in human skin was 93.5% ± 2.0%, 97.6% ± 0.9%, and 98.5% ± 1.9% for the 0.1%, 20%, and 100% solutions, respectively.

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 v4.5 (OECD, 2021b)
I	I	I

2. **Analogs Selected**:

a. **Genotoxicity:** Isoamyl salicylate (CAS # 87-20-7)
b. **Repeated Dose Toxicity:** Amyl salicylate (CAS # 2050-08-0)
c. **Reproductive Toxicity:** Amyl salicylate (CAS # 2050-08-0)
d. **Skin Sensitization:** None
e. **Photoirritation/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

Hexyl salicylate is reported to occur in the following foods by the VCF*:

Apple brandy (Calvados).

*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 08/02/23 (ECHA, 2011).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for hexyl salicylate 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)	1.5
2	Products applied to the axillae	0.80
3	Products applied to the face/body using fingertips	8.0
4	Products related to fine fragrances	15
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	3.8
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	3.8
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	3.8
5D	Baby cream, oil, talc	1.3
6	Products with oral and lip exposure	0.15
7	Products applied to the hair with some hand contact	6.2
8	Products with significant ano-genital exposure (tampon)	1.3
9	Products with body and hand exposure, primarily rinse-off (bar soap)	29
10A	Household care products with mostly hand contact (hand dishwashing detergent)	10
10B	Aerosol air freshener	37
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	1.3
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

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 hexyl salicylate, the basis was the subchronic reference dose of 2.81 mg/kg/day, a skin absorption value of 7.80%, and a skin sensitization NESIL of 35000 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 v3.2.10.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, hexyl salicylate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Hexyl salicylate was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) with and without metabolic activation, positive for genotoxicity with metabolic activation, and negative for genotoxicity without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures (Thakkar et al., 2022). While the BlueScreen assay on the target material showed positive results, data from additional assays on the target material and a read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of hexyl salicylate was assessed in an Ames study conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102 were treated with the test material in dimethyl sulfoxide (DMSO) in the presence of metabolic activation at concentrations up to 5000 μg/plate. There were no significant increases in the number of revertant colonies for any of the test conditions in any of the strains (RIFM, 2000d). Under the conditions of the study, hexyl salicylate was considered negative in the Ames test.

There are no studies assessing the clastogenic activity of hexyl salicylate; however, read-across can be made to isoamyl salicylate (CAS # 87-20-7; see Section VI).

The clastogenic activity of isoamyl 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 isoamyl salicylate in solvent, DMSO, at concentrations up to 200 μg/mL in the presence and absence of metabolic activation (S9) for 4 and 24 h. Isoamyl salicylate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2015a). Under the conditions of the study, isoamyl salicylate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to hexyl salicylate.

Based on the available data, isoamyl salicylate does not present a concern for genotoxic potential, and this can be extended to hexyl salicylate.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/01/22.

11.1.2. Repeated dose toxicity

The MOE for hexyl 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 on hexyl salicylate. Read-across material amyl salicylate (CAS # 2050-80-0; see Section VI) has sufficient data to support the repeated dose toxicity endpoint. In a GLP- and OECD 408-compliant study, 10 Wistar Han rats/sex/dose were administered amyl salicylate via diet at concentrations of 0, 750, 3750, and 7500 ppm (equivalent to doses of 0, 55, 281, and 569 mg/kg/day in males and 0, 67, 329, and 607 mg/kg/day in females, according to the study report) for 90 days. No mortality was observed throughout the study. No treatment-related adverse effects were observed in clinical signs, hematology, clinical chemistry, gross necropsy, organ weights, or histopathology. Reduced body weights and bodyweight gains, reflective of undernutrition, were observed in both sexes at the high dose. Based on reduced body weights and bodyweight gains observed in both sexes at 7500 ppm, the repeated dose toxicity NOAEC for this study was determined to be 3750 ppm (equivalent to 281 mg/kg/day in males and 329 mg/kg/day in females) (RIFM, 2020a).

In a GLP- and OECD 421-compliant study, 10 Wistar Han rats/sex/dose were administered amyl salicylate via diet at concentrations of 0, 500, 1500, and 5000 ppm (equivalent to doses of 0, 33, 100, and 333 mg/kg/day, according to the study report) for a minimum of 28 days. No mortality was observed throughout the study. No treatment-related adverse effects were observed in clinical signs, macroscopic examination, organ weights, or macroscopic examination. Reduced body weights and bodyweight gains were observed in females at 5000 ppm during premating but recovered during the remainder of the study period and, thus, were not considered adverse. Based on no treatment-related adverse effects up to the highest dose, the repeated dose toxicity NOAEC for this study was determined to be 5000 ppm (equivalent to 333 mg/kg/day) (RIFM, 2020b).

The most conservative NOAEL was derived from the OECD 408 study at 281 mg/kg/day.

Therefore, the hexyl salicylate MOE is equal to the amyl salicylate NOAEL (281 mg/kg/day) divided by the total systemic exposure (0.025 mg/kg/day) to hexyl salicylate, 281/0.0037, or 75946.

In addition, the total systemic exposure to hexyl salicylate (3.7 μg/kg/day) is below the TTC of a Cramer Class I material (30 μg/kg/day) for the repeated dose toxicity endpoint at the current level of use (Kroes et al., 2007).

11.1.2.1.1. Derivation of subchronic reference dose (RfD). s 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. (2020) and a subchronic RfD of 2.81 mg/kg/day.

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 subchronic RfD for hexyl salicylate was calculated by dividing the lowest NOAEL (from the Repeated Dose or Reproductive Toxicity sections) of 281 mg/kg/day by the uncertainty factor, 100 = 2.81 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/04/22.

11.1.3. Reproductive toxicity

The MOE for hexyl 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 hexyl salicylate. Read-across material amyl salicylate (CAS # 2050-08-0; see Section VI) has sufficient data to support the reproductive toxicity endpoint. In a GLP- and OECD 421-compliant study, 10 Wistar Han rats/sex/dose were administered amyl salicylate via diet at concentrations of 0, 500, 1500, and 5000 ppm (equivalent to doses of 0, 33, 100, and 333 mg/kg/day, according to the study report) for a minimum of 28 days. No treatment-related adverse effects were observed on mating and fertility indices, precoital time, number of implantations, estrous cycle, or histopathology of reproductive organs. No treatment-related adverse effects were observed on gestation, viability and lactation indices, gestation duration, parturition, maternal care, litter size, sex ratio, pup mortality, pup clinical signs, pup body weights, pup anogenital distance,

pup areola/nipple retention, T4 thyroid hormone levels, or macroscopic examination. Based on no treatment-related adverse effects up to the highest dose, the reproductive NOAEC for this study was determined to be 5000 ppm (equivalent to 333 mg/kg/day) (RIFM, 2020b).

Therefore, the amyl salicylate MOE for the developmental toxicity and fertility endpoints can be calculated by dividing the amyl salicylate NOAEL in mg/kg/day by the total systemic exposure to amyl salicylate, 333/0.0037, or 90000.

In addition, the total systemic exposure to hexyl salicylate (3.7 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: 03/04/22.

11.1.4. Skin sensitization

Based on the existing data, hexyl salicylate is considered a skin sensitizer with a defined NESIL of 35000 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, hexyl salicylate is considered a skin sensitizer (Table 1). The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). Hexyl salicylate was predicted not to be sensitizing based on OECD Guideline No. 497: Defined Approaches on Skin Sensitization (OECD, 2021a). Hexyl salicylate was negative in the direct peptide reactivity assay (DPRA) and KeratinoSens, inconclusive in the human cell line activation test (h-CLAT), and positive in the U-SENS test (RIFM, 2014; Urbisch et al., 2015; RIFM, 2015b; RIFM, 2015c; Piroird et al., 2015). In a murine local lymph node assay (LLNA), hexyl salicylate was found to be sensitizing with an EC3 value of 0.18% (45 μg/cm^2) (RIFM, 2006). In a guinea pig maximization test, hexyl salicylate did not lead to skin sensitization reactions (RIFM, 1981). In a human maximization test, no skin sensitization reactions were observed when tested at 2070 μg/cm^2 (RIFM, 1975a). Additionally, in a Confirmation of No Induction in Humans (CNIH) test with 35433 μg/cm^2 of hexyl salicylate in 3:1 diethyl phthalate:ethanol, no reactions indicative of sensitization were observed in any of the 103 volunteers (RIFM, 2004a).

Based on weight of evidence (WoE) from structural analysis, *in vitro* studies, animal studies, and human studies, hexyl salicylate is a sensitizer with a WoE NESIL of 35000 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. (2020) and a subchronic RfD of 2.81 mg/kg/day.

Table 1
Summary of existing data on hexyl salicylate.

WoE Skin Sensitization Potency Category[1]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm²	NOEL-HMT (induction) μg/cm²	LOEL[2] (induction) μg/cm²	WoE NESIL[3] μg/cm²	LLNA Weighted Mean EC3 Value μg/cm²	GPMT[4]	Buehler[4]
Very weak	35433	2070	NA	35000	45	Negative	NA
	In vitro Data[5]				*In silico* protein binding alerts (OECD Toolbox v4.5)		
	KE 1	KE 2	KE 3		Target Material	Autoxidation simulator	Metabolism simulator
	Negative	Negative	Inconclusive		No alert found	No alert found	No alert found

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; GPMT = Guinea Pig Maximization Test; HMT = Human Maximization Test; LOEL = lowest observed effect level; KE = Key Event; NA = Not Available.
[1]WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).
[2]Data derived from CNIH or HMT.
[3]WoE NESIL limited to 2 significant figures.
[4]Studies conducted according to the OECD TG 406 are included in the table.
[5]Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

Additional References: RIFM, 1968; Sharp (1978); RIFM, 2003; RIFM, 1967.

Literature Search and Risk Assessment Completed On: 03/29/22.

11.1.5. Photoirritation/photoallergenicity

Based on existing data, hexyl salicylate would not be expected to present a concern for photoirritation or photoallergenicity.

11.1.5.1. Risk assessment. The available UV/Vis spectra (OECD TG 101) for hexyl salicylate indicate significant absorbance between 290 and 700 nm, with peak absorbance at 305 nm and returning to baseline by 330 nm. The molar absorption coefficient for wavelengths between 290 and 700 nm is above the benchmark (1000 L mol^{-1} • cm^{-1}) of concern for photoirritating effects (Henry et al., 2009). Hexyl salicylate was not observed to result in photoirritating responses in the 3T3 Neutral Red Uptake (NRU) Photoirritation assay or in guinea pig, miniature swine assays, or human studies (Forbes et al., 1977; RIFM, 2002; RIFM, 2003; RIFM, 2004b; RIFM, 1975b). Based on existing data, hexyl salicylate would not be expected to present a concern for photoirritation or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate significant absorbance in the range of 290–700 nm, with a peak at 305 nm and a return to the baseline by 330 nm. The molar absorption coefficients (3294, 3300, and 3420 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are above the benchmark of concern for photoirritating effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/28/22.

11.1.6. Local Respiratory Toxicity

The MOE for hexyl salicylate is adequate for the respiratory endpoint at the current level of use.

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In a 28-day, repeat dose inhalation study conducted in Wistar rats (5/sex/dose), a NOAEC of 249 mg/m^3 was reported for hexyl salicylate (ECHA, 2011). Detailed clinical observations, body and organ weights, food consumption, hematology, clinical chemistry, gross pathology, as well as histopathology (adrenals, lungs, brain, spleen, heart, testes, kidneys, thymus, and liver) were all recorded. There were no treatment-related effects observed at any test concentration (0, 10.9, 52.3, or 249 mg/m^3). The NOAEC was determined to be 249 mg/m^3.

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

- (249 mg/m^3) × (1 m^3/1000 L) = 0.249 mg/L
- Minute ventilation of 0.17 L/min for a Sprague Dawley rat* × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 61.2 L/day
- (0.249 mg/L) × (61.2 L/d) = 15.2 mg/day
- (15.2 mg/day)/(0.0016 kg lung weight of rat**) = 9500 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.16 mg/day—this value was derived from the concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; Safford et al., 2015). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 0.25 mg/kg lung weight/day resulting in a MOE of 38000 (i.e., [9500 mg/kg lung weight/day]/[0.25 mg/kg lung weight/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to interspecies and intraspecies variation, the material exposure by inhalation at 0.16 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

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

Additional References: UGCM, 1997.

Literature Search and Risk Assessment Completed On: 03/23/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of hexyl 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, hexyl 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 hexyl 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, 2017a). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Based on the current VoU (2019), hexyl salicylate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies. ***Biodegradation***:

s: A 28-day biodegradation study was conducted according to the Council Directive 92/69 EEC Method C.4-D. Biodegradation of 60% was observed.

RIFM, 1995: The ready biodegradability of hexyl salicylate was evaluated by the manometric respirometry test according to the OECD 301F method. Biodegradation of 91% was observed after 28 days.

RIFM, 1994: The biodegradation of hexyl salicylate was evaluated according to the OECD 301B method. 10 mg/L of the test material was incubated for 28 days. Biodegradation of 89.9% was observed.

RIFM, 2000b: Hexyl salicylate was evaluated in a 28-day biodegradation study according to the ISO 14593 method. Biodegradation of 64% was observed.

Ecotoxicity:

RIFM, 1999: A 48-h acute toxicity test with *D. magna* was conducted with the test material. The geometric mean of EC0/EC100 at 48 h was 0.39 mg/L.

RIFM, 1983: A 24-h acute toxicity test with *D. magna* was conducted with the test material. The EC50 at 24 h was 1.5 mg/L.

RIFM, 2000c: An acute toxicity study was conducted for 96 h in freshwater fish (*Danio rerio*) according to the OECD 203 guidelines. The LC50 was reported to be between >100 mg/L.

Milligan et al., 2021: A *D. magna* reproduction test was conducted according to the OECD 211 method under flow-through conditions. *D. magna* was exposed to the test material at mean measured concentrations ranging from 0.01 to 0.140 mg/L for 21 days. The EC10 for reproduction, measured as the number of live young produced per reproductive day, was the most sensitive biological endpoint measured in the study and was reported to be 0.045 mg/L, based on time-weighted mean measured test concentrations.

RIFM, 2022: An early life-stage toxicity test with the fathead minnow was conducted according to the OECD 210 method. Fathead minnow embryos were exposed to a geometric series of 5 test concentrations, a negative (dilution water) control, and a solvent control under flow-through conditions. The exposure period included a 5-day embryo hatching period and a 28-day post-hatch juvenile growth period. In terms of overall survival, the EC10 was reported to be 0.143 mg/L based on time-weighted mean measured test concentrations.

11.2.1.3. Other available data. Hexyl salicylate has been registered under REACH, and the following additional data are available (ECHA, 2011):

A 48-h *D. magna* acute study was conducted according to the OECD 202 guidelines with a reported EC50 of 0.357 mg/L.

An algae growth inhibition test was conducted according to the OECD 201 method. Based on initial measured concentrations, the 72-h EyC50 was calculated to be 0.28 mg/L, the 72-h ErC50 was 0.61 mg/L, and NOEC was 0.15 mg/L.

11.2.2. 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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>0.268</u>			1000000	0.000268	
ECOSAR v2.0 Acute Endpoints **(Tier 2)**	0.486	0.728	0.191			Esters
ECOSAR v2.0 Acute Endpoints **(Tier 2)**	<u>0.183</u>	0.215	0.734	10000	0.0183	Phenols
ECOSAR v2.0 Acute Endpoints **(Tier 2)**	0.327	0.246	0.586			Neutral Organics
Tier 3: Measured Data, including read-across and REACH data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	>100		0.143			
Daphnia		0.357	<u>0.045</u>	10	4.5	
Algae		0.28	0.15			

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	5.5	5.5
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional VoU Tonnage Band	>1000	100–1000
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 4.5 μg/L. The revised PEC/PNECs for EU and NA are >1.

Literature Search and Risk Assessment Completed On: 03/08/23.

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
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine Technical Bulletin:** https://www.nlm.nih.gov/pubs/techbull/nd19/nd19_toxnet_new_locations.html
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA ChemView:** https://chemview.epa.gov/chemview/
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://pubchem.ncbi.nlm.nih.gov/source/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 08/02/23.

Conclusion

- Isoamyl salicylate (CAS # 87-20-7) was used as a read-across analog for the target material, hexyl salicylate (CAS # 6259-76-3), for the genotoxicity endpoint.
  - o The target material and the read-across analog belong to the class of salicylate esters.
  - o The key difference between the target material and read-across analog is that the target material has a hexyl alcohol fragment, while the read-across analog has an isopentyl alcohol fragment. The differences between structures do not essentially change the physical–chemical properties nor raise any additional structural alerts, and, therefore, the toxicity profiles are expected to be similar.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.5, 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 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.
- Amyl salicylate (CAS # 2050-08-0) was used as a read-across analog for the target material, hexyl salicylate (CAS # 6259-76-3), for the repeated dose toxicity and reproductive toxicity endpoints.
  - o The target material and the read-across analog belong to the class of salicylate esters.
  - o The key difference between the target material and read-across analog is that the target material has a hexyl alcohol fragment, while the read-across analog has a pentyl alcohol fragment. The differences between structures do not essentially change the physical–chemical properties nor raise any additional structural alerts, and, therefore, the toxicity profiles are expected to be similar.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.5, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o Both the target material and read-across analog have a strong binder alert (Estrogen Receptor [ER] Binding alert). The data described in the developmental and reproductive toxicity and repeated dose toxicity sections confirm that the MOE is adequate under the current usage. Therefore, the alert is 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.

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.2023.114217.

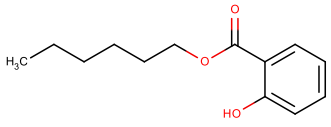
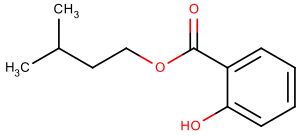
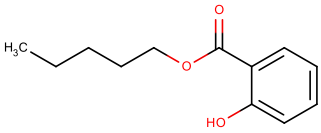
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). 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, 2017b).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (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 v4.5 (OECD, 2021b).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.5 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material
Principal Name	Hexyl salicylate	Isoamyl salicylate	Amyl salicylate
CAS No.	6259-76-3	87-20-7	2050-08-0
Structure			
Similarity (Tanimoto Score)		0.89	0.97
Endpoint		• Genotoxicity	• Repeated dose toxicity • Reproductive toxicity
Molecular Formula	$C_{13}H_{18}O_3$	$C_{12}H_{16}O_3$	$C_{12}H_{16}O_3$
Molecular Weight (g/mol)	222.28	208.26	208.26
Melting Point (°C, EPI Suite)	99.68	82.45	90.74
Boiling Point (°C, EPI Suite)	327.79	278.00	270.00
Vapor Pressure (Pa at 25 °C, EPI Suite)	0.00	0.09	0.11
Water Solubility (mg/L, at 25 °C, WSKOW v1.42 in EPI Suite)	6.08	145.00	18.94
Log K_{OW}	5.06	4.49	4.57
J_{max} (μg/cm^2/h, SAM)	0.86	17.91	2.44
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.89	1.43	1.43
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.5)	No alert found	No alert found	
DNA Binding (OECD QSAR Toolbox v4.5)	No alert found	No alert found	
Carcinogenicity (ISS)	No alert found	No alert found	
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found	
***In Vitro* Mutagenicity (Ames, ISS)**	No alert found	No alert found	
***In Vivo* Mutagenicity (Micronucleus, ISS)**	No skin sensitization reactivity domain alerts were identified	No skin sensitization reactivity domain alerts were identified	
Oncologic Classification	Phenol-type Compounds	Phenol-type Compounds	

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
Repeated Dose Toxicity			
Repeated Dose (HESS)	Not categorized		Not categorized
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.5)	Strong binder, OH group		Strong binder, OH group
Developmental Toxicity (CAESAR v2.1.6)	Non-toxicant (good reliability)		Non-toxicant (moderate reliability)
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.5)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on hexyl salicylate (CAS # 6259-76-3). Hence, *in silico* evaluation was conducted to determine read-across materials. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, isoamyl salicylate (CAS # 87-20-7) and amyl salicylate (CAS # 2050-08-0) were identified as read-across analogs with sufficient data for toxicological evaluation.

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