Food and Chemical Toxicology 197 (2025) 115141

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short Review

RIFM fragrance ingredient safety assessment, vanillin isobutyrate, CAS Registry Number 20665-85-4

A.M. Api [a], A. Bartlett [a], D. Belsito [b], D. Botelho [a], M. Bruze [c], A. Bryant-Friedrich [d], G.A. Burton Jr. [e], M.A. Cancellieri [a], H. Chon [a], M.L. Dagli [f], W. Dekant [g], C. Deodhar [a], K. Farrell [a], A.D. Fryer [h], L. Jones [a], K. Joshi [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], H. Moustakas [a], J. Muldoon [a], T.M. Penning [i], G. Ritacco [a], N. Sadekar [a], I. Schember [a], T.W. Schultz [j], F. Siddiqi [a], I.G. Sipes [k], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [l]

[a] *Research Institute for Fragrance Materials, Inc., 1200 MacArthur Boulevard, Suite 306, Mahwah, NJ, 07430, USA*
[b] *Member Expert Panel for Fragrance Safety, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel for Fragrance Safety, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member Expert Panel for Fragrance Safety, Pharmaceutical Sciences, Wayne State University, 42 W. Warren Ave., Detroit, MI, 48202, USA*
[e] *Member Expert Panel for Fragrance Safety, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[f] *Member Expert Panel for Fragrance Safety, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member Expert Panel for Fragrance Safety, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member of Expert Panel for Fragrance Safety, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[j] *Member Expert Panel for Fragrance Safety, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996- 4500, USA*
[k] *Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[l] *Member Expert Panel for Fragrance Safety, The Journal of Dermatological Science (JDS), Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

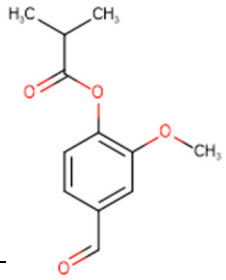
ARTICLE INFO

Handling Editor: Dr. Bryan Delaney

* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

https://doi.org/10.1016/j.fct.2024.115141
Received 18 November 2024; Accepted 22 November 2024
Available online 27 November 2024

Version: 111524. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all Research Institute for Fragrance Materials (RIFM) Fragrance Ingredient Safety Assessments is here: http://fragrancematerialsafetyresource.elsevier.com.



Name: Vanillin isobutyrate
CAS Registry Number: 20665-85-4

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
CAESAR - Computer-Assisted Evaluation of industrial chemical Substances According to Regulations
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; Safford et al., 2015, 2017, 2024) 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
HESS - Hazard Evaluation Support System; a repeated dose profiler that is used to identify the toxicological profiler of chemicals
IFRA - The International Fragrance Association
IRB - Institutional Review Board
ISS - Istituto Superiore di Sanità (Italian National Institute of Health)
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
OASIS - OASIS Laboratory of Mathematical Chemistry (LMC)
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
Toxtree - an *in silico* tool that can estimate toxic hazard by applying a decision tree approach
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food

(*continued on next column*)

(*continued*)

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 material was not evaluated for photoallergy due to a lack of suitable data and validated *in vitro* tests.

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. This material was not evaluated for photoallergy due to a lack of suitable data and validated *in vitro* tests.

Vanillin isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that vanillin isobutyrate is not genotoxic. Data on vanillin isobutyrate provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog ethyl vanillin (CAS # 121-32-4) show that there are no safety concerns for vanillin isobutyrate for skin sensitization under the current declared levels of use. Vanillin isobutyrate is expected to present a concern for photoirritation with a No Observed Effect Level (NOEL) of 3%. Vanillin isobutyrate was not evaluated for photoallergenicity due to a lack of suitable data and validated *in vitro* tests. To address this data gap, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of vanillin isobutyrate. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to vanillin isobutyrate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; vanillin isobutyrate 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, 1986; RIFM, 2015c)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.	RIFM (2016d)
Reproductive Toxicity: NOAEL = 1000 mg/kg/day.	RIFM (2016d)
Skin Sensitization: No concern for skin sensitization.	(Basketter et al., 2001; Basketter et al., 2002; Basketter et al., 2003; Roberts et al., 2007; RIFM, 1997; RIFM, 2011)
Photoirritation/ Photoallergenicity: Photoirritating. NOEL for photoirritation = 3%; Maximum Acceptable Concentration = 0.6%; not evaluated for photoallergy.	(RIFM, 2015a; RIFM, 2016b; RIFM, 2016a)

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

Environmental Safety Assessment

Hazard Assessment:	
Persistence: Critical Measured Value: 94% (OECD 301F)	RIFM (2012)
Bioaccumulation: Screening-level: 15.22 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 299.4 mg/L	(Salvito et al., 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

(*continued on next page*)

(*continued*)

Screening-level: PEC/PNEC (North America and Europe) < 1	(Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 299.4 mg/L	(Salvito et al., 2002)
RIFM PNEC is: 0.2994 μg/L	
• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe <1; cleared at the screening-level	

1. Identification

1. **Chemical Name:** Vanillin isobutyrate
2. **CAS Registry Number:** 20665-85-4
3. **Synonyms:** m- Anisaldehyde, 4-hydroxy, 2-methyl propionate; Benzaldehyde, 4-hydroxy, 3-methoxy, 2-methylpropanoate; 4-Formyl-2-methoxyphenyl 2-methylpropanoate; 4-Formyl-2-methoxyphenyl isobutyrate; Isobutyric acid, ester with vanillin; 3-Methoxy-4-isobutyrylbenzaldehyde; Propanoic acid, 2-methyl, 4-formyl-2-methoxyphenyl ester; Isobutavan; イソ酪酸バニリン; (4-Formyl-2-methoxyphenyl) 2-methylpropanoate; 4-Isobutyroyloxy-3-methoxybenzaldehyde; Vanillin isobutyrate
4. **Molecular Formula:** $C_{12}H_{14}O_4$
5. **Molecular Weight:** 222.24 g/mol
6. **RIFM Number:** 5094
7. **Stereochemistry:** No stereoisomer possible.

2. Physical data

1. **Boiling Point:** 566 K (293 °C) (RIFM, 2013b), 314.04 °C (EPI Suite v4.11)
2. **Flash Point:** >93 °C (Globally Harmonized System [GHS]), 168 °C at 1013 hPa (not classified as a flammable liquid) (RIFM, 2014d)
3. **Log K_{OW}:** 2.0 (RIFM, 2013a), 2.3 (EPI Suite v4.11)
4. **Melting Point**: 82.28 °C (EPI Suite v4.11), 29 °C (302 K) (RIFM, 2016e)
5. **Water Solubility:** 366.1 mg/L at 25 °C (EPI Suite v4.11), 573 ± 22.8 mg/L at 20 °C at pH 4.2 (RIFM, 2014e)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.000262 mm Hg at 25 °C (EPI Suite v4.11), 0.017, 0.031, and 0.49 Pa at 20, 25, and 50 °C, respectively (RIFM, 2014c)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm, with distinct peaks at 304 nm (under neutral and acidic conditions) and 349 nm (under basic conditions) and returning to the baseline by 390 nm. Maximum molar absorption coefficients within this range (208, 1018, and 7903 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:** Colorless liquid with a heavy, sweet (vanillin, nutmeg) odor

3. Volume of use (worldwide band)

1. 10–100 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.4.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.27% (RIFM, 2023)
2. **Inhalation Exposure*:** 0.0014 mg/kg/day or 0.098 mg/day (RIFM, 2023)
3. **Total Systemic Exposure**:** 0.0097 mg/kg/day (RIFM, 2023)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017, 2024).

**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, 2017; Safford et al., 2015, 2017, 2024).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.5
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** Ethyl vanillin (CAS # 121-32-4)
   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

Vanillin isobutyrate 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 11/15/24.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for vanillin isobutyrate 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)	0.60
2	Products applied to the axillae	0.60
3	Products applied to the face/body using fingertips	0.60
4	Products related to fine fragrances	0.60

(*continued on next page*)

(*continued*)

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.60
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.60
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.60
5D	Baby cream, oil, talc	0.20
6	Products with oral and lip exposure	0.27
7A	Rinse-off products applied to the hair with some hand contact	3.0
7B	Leave-on products applied to the hair with some hand contact	0.60
8	Products with significant ano-genital exposure (tampon)	0.20
9	Products with body and hand exposure, primarily rinse-off (bar soap)	3.0
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.81
10B	Aerosol air freshener	0.60
11A	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate without UV exposure	0.20
11B	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate with potential UV exposure	0.60
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 vanillin isobutyrate, the basis was the subchronic reference dose of 3.33 mg/kg/day, a predicted skin absorption value of 80%, and a photoirritation NOEL of 3% (Maximum Safe-Use Level: 0.6%).

As a conservative approach, we assumed that 100% of the material exposed via the skin is bioavailable (see Section V), thereby deriving the most stringent MOE. Since the MOE is > 100 (see the repeated dose and reproductive toxicity sections), we then refined the exposure to 80% using an *in silico* Skin Absorption Model (SAM) to determine the Maximum Allowable Concentrations for each category listed in Section X.

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

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.4.3.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, vanillin isobutyrate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of vanillin isobutyrate has been evaluated in a bacterial reverse mutation assay conducted equivalent to OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with vanillin isobutyrate 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, 1986). Under the conditions of the study, vanillin isobutyrate was not mutagenic in the Ames test.

A mammalian cell gene mutation assay (mouse lymphoma assay) was conducted according to OECD TG 490 and GLP guidelines. Mouse lymphoma cells were treated with vanillin isobutyrate in DMSO at concentrations up to 1600 μg/mL for 3 h with and without metabolic activation and at concentrations up to 500 μg/mL for 24 h without metabolic activation. There were statistically significant increases in the frequency of mutant colonies observed in the 3-h treatment condition in the absence of metabolic activation. However, these increases were observed at a highly toxic dose with a 10% survival rate and were not dose-dependent and, hence, cannot be considered a biologically relevant outcome. There were no statistically significant increases observed in the 3-h treatment condition in the presence of metabolic activation or the 24-h treatment condition in the absence of metabolic activation (RIFM, 2016c). Under the conditions of the study, the mutagenicity of vanillin isobutyrate was concluded to be equivocal based on biologically non-relevant increases at a toxic dose in a 3-h treatment (-S9) test condition only. Considering this is only observed at a highly toxic dose without dose-dependent increases at any other dose level, the overall outcome of the study can be concluded to be negative. As an additional weight of evidence (WoE), the hydrolysis products vanillin and isobutyric acid are non-mutagenic and non-carcinogenic based on the available data (ECHA, 2016).

The clastogenic activity of vanillin isobutyrate 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 vanillin isobutyrate in water at concentrations up to 1600 μg/mL in the dose range finding study; in the main study, micronuclei analysis was conducted at concentrations up to 975 μg/mL in the presence and absence of metabolic activation. Vanillin isobutyrate did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2015c). Under the conditions of the study, vanillin isobutyrate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, vanillin isobutyrate does not present a concern for genotoxic potential.

Additional References: RIFM, 1983a.

Literature Search and Risk Assessment Completed On: 07/19/24.

11.1.2. Repeated dose toxicity

The MOE for vanillin isobutyrate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on vanillin isobutyrate. In a GLP- and OECD 422-compliant study, 10 CRL:WI(Han) rats/sex/dose were administered vanillin isobutyrate via diet at doses of 0, 100, 300, and 1000 mg/kg/day. Males were treated for 2 weeks pre-mating, during pairing, and 2 weeks further until necropsy; females were treated for 2 weeks pre-mating, during pairing, until day 4 postpartum. No mortality occurred throughout the study period. There were no treatment-related adverse effects in clinical signs, body weights, bodyweight gains, locomotor activity, hematology, clinical chemistry, organ weights, gross pathology, or microscopic pathology. Based on no treatment-related adverse effects up to the highest dose, the repeated dose toxicity NOAEL for this study was considered to be 1000 mg/kg/day (RIFM, 2016d).

A default safety factor of 3 was used when deriving a NOAEL from OECD 422 studies (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

The derived NOAEL for the repeated dose toxicity data is 1000/3 or 333 mg/kg/day.

Therefore, the vanillin isobutyrate MOE for the repeated dose toxicity endpoint can be calculated by dividing the vanillin isobutyrate

NOAEL in mg/kg/day by the total systemic exposure to vanillin isobutyrate, 333/0.0097 or 34330.

Additionally, the total systemic exposure to vanillin isobutyrate (9.7 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.

11.1.2.1.1. Derivation of subchronic reference dose (RfD). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (2020) and a subchronic RfD of 3.33 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 vanillin isobutyrate was calculated by dividing the lowest NOAEL (from the Repeated Dose or Reproductive Toxicity sections) of 333 mg/kg/day by the uncertainty factor, 100 = 3.33 mg/kg/day.

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

Table 1
Summary of existing data on ethyl vanillin as a read-across for vanillin isobutyrate.

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]
No evidence of sensitization[7]	8858	1380	NA	NA	Negative up to 12500 (50%)	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	Positive	Negative (U-SENS) Positive (h-CLAT)		No alert found	No alert found	Schiff base formation

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; EC3 = concentration of test chemical required to induce a 3-fold increase in lymph node cell proliferation; GPMT = Guinea Pig Maximization Test; 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.

[6]Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

[7]Determined based on Criteria for the RIFM safety evaluation process for fragrance ingredients (Api et al., 2015).

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/18/24.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on vanillin isobutyrate. In a GLP- and OECD 422-compliant study, 10 CRL:WI(Han) rats/sex/dose were administered vanillin isobutyrate via diet at doses of 0, 100, 300, and 1000 mg/kg/day. Males were treated for 2 weeks pre-mating, during pairing, and 2 weeks further until necropsy; females were treated for 2 weeks pre-mating, during pairing, until day 4 postpartum. No mortality occurred throughout the study period. No treatment-related adverse effects were observed in mating, fertility, fecundity indices, gestation length, number of implantations, number of live births, pup survival, or pup observations. Based on no treatment-related adverse effects seen up to the highest dose, the reproductive toxicity NOAEL for this study was considered to be 1000 mg/kg/day (RIFM, 2016d).

Therefore, the vanillin isobutyrate MOE for the reproductive toxicity endpoint can be calculated by dividing the vanillin isobutyrate NOAEL in mg/kg/day by the total systemic exposure to vanillin isobutyrate, 1000/0.0097, or 103093.

In addition, the total systemic exposure to vanillin isobutyrate (9.7 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: 09/18/24.

11.1.4. Skin sensitization

Based on the existing data on read-across material ethyl vanillin (CAS # 121-32-4), vanillin isobutyrate presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for vanillin isobutyrate. Therefore, read-across material ethyl vanillin (CAS # 121-32-4; see Section VI) was used for the risk assessment of vanillin isobutyrate. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, vanillin isobutyrate is not considered a skin sensitizer. The chemical structure of these materials indicates that they would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). Read-across material ethyl vanillin was predicted to be negative in a direct peptide reactivity assay (DPRA), positive in KeratinoSens, negative in the human cell line activation test (h-CLAT), and both positive and negative in the U-SENS test (Natsch, 2013; Piroird et al., 2015; Urbisch, 2015). In a murine local lymph node assay (LLNA), read-across material ethyl vanillin was not found to be sensitizing when tested up to 50% (12500 μg/cm^2) (Basketter et al., 2001; Basketter et al., 2002; Basketter et al., 2003; Roberts et al., 2007). In guinea pig maximization tests, vanillin isobutyrate and read-across material ethyl vanillin did not lead to skin sensitization reactions (RIFM, 1983b; RIFM, 1997). In a human maximization test, no skin sensitization reactions were observed when the read-across material was tested at 1380 μg/cm^2 (RIFM, 1970). In a Confirmation of No Induction in Humans test (CNIH) with 590 μg/cm^2 vanillin isobutyrate in 1:3 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 109 volunteers (RIFM, 2009). Additionally, in a CNIH with 8858 μg/cm^2 of read-across material ethyl vanillin in 1:3 EtOH:DEP, no reactions indicative of sensitization were observed in any of the 108 volunteers (RIFM, 2011).

Based on WoE from structural analysis, *in vitro* studies, animal studies, and human studies on the read-across material as well as the target material, vanillin isobutyrate does not present a concern for skin sensitization.

Additional References: Klecak (1985).

Literature Search and Risk Assessment Completed On: 07/25/24.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra and the available study data, vanillin isobutyrate has photoirritating potential. Vanillin isobutyrate was not evaluated for photoallergy due to a lack of suitable data and validated *in vitro* tests. To address this data gap, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of vanillin isobutyrate.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate significant absorption between 290 and 700 nm. The corresponding molar absorption coefficients are above the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). In an *in vitro* 3T3 Neutral Red Uptake photoirritation test (OECD TG 432), vanillin isobutyrate was found to have photoirritating potential (RIFM, 2015a). In a reconstructed human epidermis photoirritation test (OECD TG 498), concentrations of 0.3%, 1%, and 3% vanillin isobutyrate in PEG were not found to be photoirritating (RIFM, 2016b). In a human photoirritation test, concentrations of 0.3%, 1%, and 3% vanillin isobutyrate in 1:3 EtOH:DEP did not result in photoirritation. The NOEL for photoirritation in human volunteers was 3% (RIFM, 2016a). Considering a safety factor for photoirritation of 5, the maximum acceptable concentration for vanillin isobutyrate based on photoirritation alone is 0.6%. Maximum acceptable concentrations across all finished product categories and all endpoints may be found in Section X. Vanillin isobutyrate was not evaluated for photoallergy due to a lack of suitable data and validated *in vitro* tests. To address this data gap, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of vanillin isobutyrate.

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, at 304 nm (under neutral and acidic conditions) and 349 nm (under basic conditions) and returning to baseline by 390 nm. Maximum molar absorption coefficients within this range (208, 1018, and 7903 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are above the benchmark of concern for photoirritant or photoallergenic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/27/24.

11.1.6. Local Respiratory Toxicity

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

11.1.6.1. Risk assessment. There are limited inhalation data available on vanillin isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.098 mg/day. This exposure is 14.3 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: RIFM, 2018.

Literature Search and Risk Assessment Completed On: 07/18/24.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of vanillin isobutyrate 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, vanillin isobutyrate was identified as a fragrance material with no 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 vanillin isobutyrate 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 (IFRA, 2019), vanillin isobutyrate does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.1.1.1. Biodegradation. **RIFM, 2012**: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F method. Vanillin isobutyrate undergoes 94% biodegradation after 28 days.

11.2.1.1.2. Ecotoxicity. **RIFM, 2014a:** An algae growth inhibition test was conducted according to the OECD 201 method. Based on the geometric mean of each test concentration, the 72-h EC50 for growth rate (ErC50) was determined to be > 40.0 mg/L (48.5 mg/L extrapolated value). The 72-h EC50 value for yield (EyC50) was 39.6 mg/L (nominal) and 14.9 mg/L (actual).

RIFM, 2014b: A *Daphnia magna* acute immobilization study was conducted according to the OECD 202 method under semi-static conditions. The 48-h EC50 was reported to be 36.2 mg/L.

RIFM, 2015b: A fish (rainbow trout) acute toxicity test was conducted according to the OECD 203 method. Under the conditions of the study, the 96-h LC50 was reported to be 5.97 mg/L based on measured concentrations.

11.2.1.1.3. Other available data. Vanillin isobutyrate has been registered for REACH with no additional data at this time.

11.2.1.2. Risk assessment refinement. Since vanillin isobutyrate has passed the screening criteria, measured data are included for completeness only and have 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 underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.0	2.0
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	10–100	10–100
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.2994 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 07/08/24.

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 11/15/24.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	299.4			1000000	0.2994	

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.2024.115141.

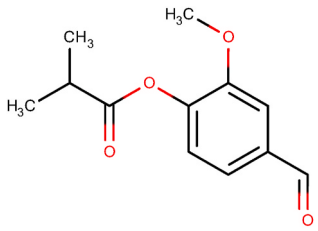
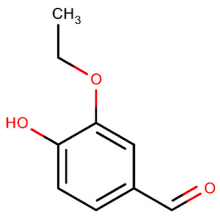
Appendix

Read-across Justification:

Methods

The read-across analog was 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 Chemicals 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, 2021).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- 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, 2021).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- 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
Principal Name	Vanillin isobutyrate	Ethyl vanillin
CAS No.	20665-85-4	121-32-4
Structure		
Similarity (Tanimoto Score)		0.65
Endpoint		Skin sensitization
Molecular Formula	$C_{12}H_{14}O_4$	$C_9H_{10}O_3$

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Molecular Weight (g/mol)	222.24	166.18
Melting Point (°C, EPI Suite)	82.28	77.50
Boiling Point (°C, EPI Suite)	314.04	294.00
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.03	0.00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	366.10	2820.00
Log K_{OW}	2.30	1.58
J_{max} (μg/cm^2/h, SAM)	2.07	20.72
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	0.00	0.00
Skin Sensitization		
Protein Binding (OASIS v1.1)	No alert found	No alert found
Protein Binding (OECD)	Acylation|Acylation ≫ Direct Acylation Involving a Leaving group|Acylation ≫ Direct Acylation Involving a Leaving group ≫ Acetates	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	Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Schiff base formation identified.	Alert for Schiff base formation identified.
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.5)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on 4-acetoxy-3-ethoxybenzaldehyde (CAS # 72207-94-4). 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, ethyl vanillin (CAS # 121-32-4) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusion

- Ethyl vanillin (CAS # 121-32-4) was used as a read-across analog for the target material, 4-acetoxy-3-ethoxybenzaldehyde (CAS # 72207-94-4), for the skin sensitization endpoint.
  - o The target material and read-across analog belong to the class of aromatic aldehydes. The key difference between the target material and the read-across analog is that the read-across analog has a para-hydroxyl group and an ethoxy substituent, whereas the target material has a *para*-isobutyrate ester and a methoxy substituent. This structural difference is toxicologically insignificant.
  - 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 an alert for Schiff base formation (Skin Sensitization Reactivity Domains [Toxtree v2.6.13]). This alert is due to the presence of an aldehyde in both substances. In addition, the target material has an acylation alert that arises due to the presence of an acetate-type moiety in the target material. However, the data described in the skin sensitization section show that the read-across analog does not pose a concern for skin sensitization. Therefore, based on structural similarity and data for the read-across analog, 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.

References

Api, A.M., Basketter, D., Bridges, J., Cadby, P., et al., 2020. Updating exposure assessment for skin sensitization quantitative risk assessment for fragrance materials. Regul. Toxicol. Pharmacol. 2020, 118, 104805.

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Basketter, D.A., Gilmour, N., Dearman, R.J., Kimber, I., Ryan, C.A., Gerberick, F., 2003. Classification of skin sensitisation potency using the local lymph node assay. Toxicologist 72 (S-1), 101.

Basketter, D.A., Wright, Z., Gilmour, N.J., Ryan, C.A., Gerberick, G.F., Robinson, M.K., Dearman, R.J., Kimber, I., 2002. Prediction of human sensitization potency using Local Lymph Node Assay EC3 values. Toxicologist 66 (1-S), 240.

Basketter, D.A., Wright, Z.M., Warbrick, E.V., Dearman, R.J., Kimber, I., Ryan, C.A., Gerberick, G.F., White, I.R., 2001. Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis 45 (2), 89–94.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. Chem. Cent. J. 4 (Suppl. 1), S4.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S. H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

Cottrez, F., Boitel, E., Ourlin, J.C., Peiffer, J.L., et al., 2016. A 3D reconstituted epidermis based model for quantifying chemical sensitization potency: reproducibility and predictivity results from an inter-laboratory study. Toxicol. Vitro 32, 248–260.
Date, M.S., O'Brien, D., Botelho, D.J., Schultz, T.W., et al., 2020. Clustering a chemical inventory for safety assessment of fragrance ingredients: identifying read-across analogs to address data gaps. Chem. Res. Toxicol. 33 (7), 1709–1718.
ECHA, 2012. Guidance on information requirements and chemical safety assessment: chapter R.8: characterisation of dose [concentration]-response for human health. Retrieved from. https://echa.europa.eu/en/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.
ECHA, 2016. 4-Formyl-2-methoxyphenyl isobutyrate registration dossier. Retrieved from. https://chem.echa.europa.eu/100.039.945/dossier-view/b67cc80f-c5a1-4d67-b43b-7f9e86adfb45/75b7082e-e7a3-422c-9967-dfd6da3fb3e8_75b7082e-e7a3-422c-9967-dfd6da3fb3e8?searchText=20665-85-4.
ECHA, 2017a. Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT Assessment. Retrieved from. https://echa.europa.eu/en/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.
ECHA, 2017b. Read-across assessment framework (RAAF). Retrieved from. https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a.
Forreryd, A., Zeller, K.S., Lindberg, T., Johansson, H., Linstedt, M., 2016. From genome-wide arrays to tailor-made biomarker readout - progress towards routine analysis of skin sensitizing chemicals with GARD. Toxicol. Vitro 37, 178–188.
Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.
IFRA (International Fragrance Association), 2019. Volume of Use Survey. January-December 2019.
Klecak, G., 1985. The freund's complete adjuvant test and the open epicutaneous test. Curr. Probl. Dermatol. 14, 152–171.
Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.
Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.
Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A., Basketter, D., 2021. Fragrance skin sensitization evaluation and human testing: 30-year experience. Dermatitis 32 (5), 339–352.
Natsch, A., Ryan, C.A., Foertsch, L., Emter, R., Jaworska, J., Gerberick, F., Kern, P., 2013. A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. J. Appl. Toxicol. 33 (11), 1337–1352.
OECD, 2015. Guidance document on the reporting of integrated Approaches to testing and assessment (IATA). ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.
OECD, 2021. The OECD QSAR Toolbox, v3.2–4.5. Retrieved from. http://www.qsartoolbox.org/.
Piroird, C., Ovigne, J.-M., Rousset, F., Martinozzi-Teissier, S., Gomes, C., Cotovio, J., Alepee, N., 2015. The Myeloid U937 Skin Sensitization Test (U-SENS) addresses the activation of dendritic cell event in the adverse outcome pathway for skin sensitization. Toxicol. Vitro 29 (5), 901–916.
RIFM (Research Institute for Fragrance Materials, Inc.), 1970. The Contact Sensitizing Potential of Fragrance Materials in Humans. Report to RIFM. RIFM Report Number 1760. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1983a. Examination of Vanillin Isobutyrate (Isobutavan) for Mutagenic Activity in the Ames Test. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 52856.
RIFM (Research Institute for Fragrance Materials, Inc.), 1983b. Sensitization Test with Vanillin Isobutyrate (Isobutavan) in guinea Pigs. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 52857.
RIFM (Research Institute for Fragrance Materials, Inc.), 1986. Vanillin Isobutyrate: Reverse Mutation Test "Ames Test" with S. typhimurium and E. coli. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Takasago International Corporation. RIFM report number 41271.
RIFM (Research Institute for Fragrance Materials, Inc.), 1997. Ethyl Vanillin: Magnusson and Kligman Maximisation Test in the guinea Pig. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Symrise. RIFM report number 53662.
RIFM (Research Institute for Fragrance Materials, Inc.), 2009. Repeated Insult Patch Test with Vanillin Isobutyrate. RIFM Report Number 58221. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2011. Repeated Insult Patch Test with Ethyl Vanillin. RIFM Report Number 62177. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2012. Ready Biodegradability of Vanillin Isobutyrate (Isobutavan). Unpublished Report from Givaudan. RIFM Report Number 63211. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013a. Partition Coefficient N-Octanol/water of Vanillin Isobutyrate (Isobutavan). Unpublished Report from Givaudan. RIFM Report Number 65201. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013b. The Boiling Point of Vanillin Isobutyrate (Isobutavan). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 66699.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014a. Vanillin Isobutyrate (Isobutavan): Toxicity to the Single Cell Green Alga Pseudokirchneriella Subcapitata. Unpublished Report from Givaudan. RIFM Report Number 71725. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014b. Vanillin Isobutyrate (Isobutavan): Toxicity to the Water Flea Daphnia Magna Straus Using the Semi-static Acute Immobilization Test. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71727.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014c. Vanillin Isobutyrate (Isobutavan): Determination of Vapour Pressure. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71735.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014d. Vanillin Isobutyrate (Isobutavan): Determination of Flash Point. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71736.
RIFM (Research Institute for Fragrance Materials, Inc.), 2014e. Vanillin Isobutyrate (Isobutavan): Determination of Water Solubility. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71740.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015a. Vanillin Isobutyrate: Neutral Red Uptake Phototoxicity Assay in BALB/c 3T3 Mouse Fibroblasts. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 69591.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015b. Vanillin Isobutyrate (Isobutavan): Toxicity to the Rainbow Trout Oncorhynchus mykiss Using the Semi-static Acute Toxicity Test. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71728.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015c. Vanillin Isobutyrate (Isobutavan): in Vitro Micronucleus Assay in Cultured Peripheral Human Lymphocytes (HPBL). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71732.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016a. Modified Phototoxicity Test with Vanillin Isobutyrate. RIFM Report Number 69336. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016b. Vanillin Isobutyrate: Phototoxicity Assay Using the EpiDerm™ Skin Model: Multi-Dose Assay. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 69758.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016c. Vanillin Isobutyrate (Isobutavan): Evaluation of the Mutagenic Activity in an in Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71731.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016d. Vanillin Isobutyrate (Isobutavan): Oral (Dietary) Combined Repeated Dose Toxicity Study with Reproduction/developmental Toxicity Screening Test in the Rat. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71733.
RIFM (Research Institute for Fragrance Materials, Inc.), 2016e. Vanillin Isobutyrate (Isobutavan): Determination of Melting Point. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 71737.
RIFM (Research Institute for Fragrance Materials, Inc.), 2018. Vanillin Isobutyrate (Isobutavan): Acute Inhalation Toxicity (Acute Toxic Class Method) in Rats. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Givaudan. RIFM report number 77239.
RIFM (Research Institute for Fragrance Materials, Inc.), 2023. Exposure Survey 41. August 2023.
Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C. A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.
Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2024. Corrigendum to "Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products". Regul. Toxicol. Pharmacol. 72 (3), 105545, 673-68]. Regul Toxicol Pharmacol.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.
Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74, 164–176.
Urbisch, D., Mehling, A., Guth, K., Ramirez, T., Honarvar, N., et al., 2015. Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul. Toxicol. Pharmacol. 71 (2), 337–351.
US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v2.0. United States Environmental Protection Agency, Washington, DC, USA.