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Short Review

RIFM fragrance ingredient safety assessment, 2-methyl-4-phenyl-2-butyl isobutyrate, CAS registry number 10031-71-7

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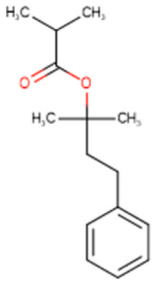


Name: 2-Methyl-4-phenyl-2-butyl isobutyrate
CAS Registry Number: 10031-71-7

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; B. Safford et al., 2015; B. Safford et al., 2024; B. Safford et al., 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency; 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
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative

(*continued on next column*)

(*continued*)

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.

2-Methyl-4-phenyl-2-butyl isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Target data and data from read-across analog 1,1-dimethyl-2-phenylethyl acetate (CAS # 151-05-3) show that 2-methyl-4-phenyl-2-butyl isobutyrate is not expected to be genotoxic. Data on read-across analog 1,1-dimethyl-2-phenylethyl acetate (CAS # 151-05-3) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog benzyl acetate (CAS # 140-11-4) show that there are no safety concerns for 2-methyl-4-phenyl-2-butyl isobutyrate for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2-methyl-4-phenyl-2-butyl isobutyrate is not expected to be photoirritating/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to 2-methyl-4-phenyl-2-butyl isobutyrate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 2-methyl-4-phenyl-2-butyl 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 (VoU) 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, 2001; RIFM, 2014b)
Repeated Dose Toxicity: NOAEL = 360 mg/kg/day.	ECHA (2017c)
Reproductive Toxicity: Developmental toxicity and Fertility NOAEL = 1000 mg/kg/day.	ECHA (2017c)
Skin Sensitization: Not a concern for skin sensitization.	(RIFM, 1985b; RIFM, 1986; RIFM, 1987; RIFM, 1988a)
Photoirritation/Photoallergenicity: Not expected to be a photoirritant/photoallergen.	(UV/Vis Spectra; RIFM Database)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence:	
Screening-level: 2.56 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 728 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 1.069 mg/L	(Salvito et al., 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) < 1	(Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 1.069 mg/L	(Salvito et al., 2002)

RIFM PNEC is: 0.001069 µg/L

- **Revised PEC/PNECs (IFRA 2019 VoU):** North America and Europe (not reported): Not applicable; cleared at the screening-level

1. Identification

1. **Chemical Name:** 2-Methyl-4-phenyl-2-butyl isobutyrate
2. **CAS Registry Number:** 10031-71-7
3. **Synonyms:** Dimethyl phenethyl carbinyl isobutyrate; 1,1-Dimethyl-3-phenylpropyl isobutyrate; D.M.P.E.C. isobutyrate; D.M.P.E.C. 2-methylpropanoate; 2-Methyl-4-phenyl-2-butyl 2-methylpropanoate; Phenylethyl dimethyl carbinyl isobutyrate; Propanoic acid, 2-methyl-, 1,1-dimethyl-3-phenylpropyl ester; 1,1-Dimethyl-3-phenylpropyl 2-methylpropanoate; Isobutyric acid, 1,1-dimethyl-3-phenylpropyl ester; 2-Methyl-4-phenyl-2-butyl isobutyrate
4. **Molecular Formula:** $C_{15}H_{22}O_2$
5. **Molecular Weight:** 234.33 g/mol
6. **RIFM Number:** 5048
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 291.23 °C (EPI Suite v4.11)
2. **Flash Point:** >200 °F; closed cup (Fragrance Materials Association [FMA])
3. **Log K_{OW}:** 4.84 (EPI Suite v4.11)
4. **Melting Point**: 49.1 °C (EPI Suite v4.11)
5. **Water Solubility:** 2.109 mg/L at 25 °C (EPI Suite v4.11)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.003 mm Hg at 20 °C (FMA), 0.00187 mm Hg (EPI Suite v4.11)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. <0.1 metric ton per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.00015% (RIFM, 2020)
2. **Inhalation Exposure*:** <0.00001 mg/kg/day or 0.0000008 mg/day (RIFM, 2020)
3. **Total Systemic Exposure**:** 0.0000029 mg/kg/day (RIFM, 2020)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low* (Expert Judgment)

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

*See the Appendix below for details.

2. **Analogs Selected**:
   a. **Genotoxicity:** 1,1-Dimethyl-2-phenylethyl acetate (CAS # 151-05-3)
   b. **Repeated Dose Toxicity:** 1,1-Dimethyl-2-phenylethyl acetate (CAS # 151-05-3)
   c. **Reproductive Toxicity:** 1,1-Dimethyl-2-phenylethyl acetate (CAS # 151-05-3)
   d. **Skin Sensitization:** Benzyl acetate (CAS # 140-11-4); Weight of Evidence (WoE) material: 2-Phenoxyethyl isobutyrate (CAS # 103-60-6)
   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 for inclusion in this safety assessment.
Additional References: None.

8. Natural occurrence

2-Methyl-4-phenyl-2-butyl 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

2-Methyl-4-phenyl-2-butyl isobutyrate has not been pre-registered; no dossier available as of 04/19/24.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 2-methyl-4-phenyl-2-butyl isobutyrate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 2-Methyl-4-phenyl-2-butyl isobutyrate 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, 2014a). These positive results were observed at cytotoxic concentrations that were within the acceptable range for the BlueScreen assay (positive: <80% relative cell density). BlueScreen is a human cell-based assay for

measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures (Thakkar et al., 2022). Additional assays on an appropriate read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no studies assessing the mutagenic activity of 2-methyl-4-phenyl-2-butyl isobutyrate; however, read-across can be made to 1,1-dimethyl-2-phenylethyl acetate (CAS # 151-05-3; see Section VI).

The mutagenic activity of 1,1-dimethyl-2-phenylethyl acetate has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with 1,1-dimethyl-2-phenylethyl acetate 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, 2001). Under the conditions of the study, 1,1-dimethyl-2-phenylethyl acetate was not mutagenic in the Ames test, and this can be extended to 2-methyl-4-phenyl-2-butyl isobutyrate.

The clastogenic activity of 2-methyl-4-phenyl-2-butyl 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 2-methyl-4-phenyl-2-butyl isobutyrate in DMSO at concentrations up to 2343 μg/mL in the dose range finding (DRF) study; in the main study, micronuclei analysis was conducted at concentrations up to 280 μg/mL in the presence and absence of metabolic activation. 2-Methyl-4-phenyl-2-butyl isobutyrate did induce binucleated cells with micronuclei at 57.6 μg/mL in the 24-h treatment in the absence of an S9 activation system (RIFM, 2014b). However, the MNBN frequencies at these concentrations were within the vehicle historical control ranges. Therefore, the statistically significant increases at these concentrations were considered biologically non-relevant and not indicative of clastogenic effects. Under the conditions of the study, 2-methyl-4-phenyl-2-butyl isobutyrate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 1,1-dimethyl-2-phenylethyl acetate does not present a concern for genotoxic potential, and this can be extended to 2-methyl-4-phenyl-2-butyl isobutyrate.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/04/23.

11.1.2. Repeated dose toxicity

The MOE for 2-methyl-4-phenyl-2-butyl isobutyrate 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 2-methyl-4-phenyl-2-butyl isobutyrate. There are sufficient data on read-across material 1,1-dimethyl-2-phenylethyl acetate (CAS # 151-05-3, see Section VI) that can be used to support the repeated dose toxicity endpoint. An OECD 408- and GLP-compliant (90-day oral toxicity study) test was conducted in Sprague Dawley rats. Groups of 10 rats/sex/dose were administered test material 1,1-dimethyl-2-phenylethyl acetate via oral gavage in 0.1% Tween 80 at doses of 0, 90, 180, or 360 mg/kg/day. In addition, there was also a high-dose recovery group of 5 males and females. No statistically significant changes in mean body weight and mean bodyweight gain were observed during the treatment period in males and females. A transient clinical sign of diarrhea was observed in all the treated groups. No adverse effects were seen in hematological findings, clinical biochemistry, and urinalysis. Furthermore, no treatment-related adverse effects were observed with respect to organ weights, gross pathology, and histopathology. Thus, based on overall observations, the NOAEL was considered to be 360 mg/kg/day, the highest dose tested (ECHA, 2017c).

Another OECD 422- and GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening test was also conducted in Wistar Han rats. Groups of 10 male and 13 female rats/dose were administered test material 1,1-dimethyl-2-phenylethyl acetate at doses of 250, 500, and 1000 mg/kg/day via oral gavage in corn oil. Males were treated for 14 days pre-mating, during mating, and post-mating till 28 days, and females were treated for 14 days pre-mating, during mating, and at least 13 days after delivery. During the study, no animal mortality was reported. No treatment-related adverse effects were reported for urinalysis, hematology, blood chemistry, and sensory function. A slight decrease in bodyweight gain during the pre-mating period (both males and females) and mating period (only males) was observed at the highest dose. Reddening and abscesses in the lungs and enlargement of mediastinal lymph nodes were also observed. However, as these findings were not dose-dependent and were also present in animals from the control group, they were considered to be unrelated to treatment. No adverse effects were seen in hematological findings, clinical biochemistry, and urinalysis. Furthermore, no treatment-related adverse effects were observed with respect to organ weights, gross pathology, and histopathology. Thus, the NOAEL was considered to be 500 mg/kg/day, based on the decrease in bodyweight gains seen at the highest dose (ECHA, 2017c).

Considering both OECD 408 and OECD 422 studies, a more robust NOAEL of 360 mg/kg/day from the OECD 408 (90-day) study was considered for the safety assessment.

Therefore, the 2-methyl-4-phenyl-2-butyl isobutyrate MOE for the repeated dose toxicity endpoint can be calculated by dividing the 1,1-dimethyl-2-phenylethyl acetate NOAEL in mg/kg/day by the total systemic exposure for 2-methyl-4-phenyl-2-butyl isobutyrate, 360/0.0000029 or 124137931.

In addition, the total systemic exposure to 2-methyl-4-phenyl-2-butyl isobutyrate (0.0029 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/17/23.

11.1.3. Reproductive toxicity

The MOE for 2-methyl-4-phenyl-2-butyl isobutyrate 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 2-methyl-4-phenyl-2-butyl isobutyrate. There are sufficient data on read-across material 1,1-dimethyl-2-phenylethyl acetate (CAS# 151-05-3, see Section VI) that can be used to support the reproductive toxicity endpoint.

An OECD 422- and GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening test was conducted in Wistar Han rats. Groups of 10 male and 13 female rats/dose were administered test material 1,1-dimethyl-2-phenylethyl acetate at doses of 250, 500, and 1000 mg/kg/day via oral gavage in corn oil. Males were treated for 14 days pre-mating, during mating, and post-mating till 28 days, and females were treated for 14 days pre-mating, during mating, and at least 13 days after delivery. During the study, no animal mortality was reported. There were no treatment-related effects on any mating and fertility parameters in the P-generation males and females at any dose. No treatment-related effects were seen in estrous cycling or litter parameters in the P-generation females at any dose. In the F1 generation pups, there were no treatment-related clinical observations or effects on anogenital distance, nipple retention (males), or mean pup body weights at any dose. In addition, there were no treatment-related macroscopic or microscopic observations in the F1 generation pups at any dose. Based on no effects seen up to the highest dose, the NOAEL for this study was determined to be 1000 mg/kg/day (ECHA, 2017c).

In another OECD 414- and GLP-compliant prenatal developmental toxicity study, 25 female Sprague Dawley rats/group were administered dose levels of 0 and 1000 mg/kg/day in 0.1% Tween 80 via oral gavage from gestation days (GDs) 5–19. No mortality was observed. No treatment-related changes in body weights, bodyweight gain, and feed consumption were observed in females. No gross lesions were observed in dams during necropsy in any of the doses tested. No treatment-related or toxicologically relevant effects were seen in fetuses with respect to external, visceral, and skeletal examinations. Thus, the NOAEL for developmental toxicity was considered to be 1000 mg/kg/day, based on the absence of treatment-related adverse effects on the development of pups up to the highest dose tested (ECHA, 2017c).

Therefore, the 2-methyl-4-phenyl-2-butyl isobutyrate MOE for the developmental toxicity and fertility endpoint can be calculated by dividing the 1,1-dimethyl-2-phenylethyl acetate NOAEL in mg/kg/day by the total systemic exposure for 2-methyl-4-phenyl-2-butyl isobutyrate, 1000/0.0000029 or 344827586.

In addition, the total systemic exposure to 2-methyl-4-phenyl-2-butyl isobutyrate (0.0029 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/17/23.

11.1.4. Skin sensitization

Based on the existing data on the read-across material benzyl acetate and WoE material 2-phenoxyethyl isobutyrate, 2-methyl-4-phenyl-2-butyl isobutyrate presents no concern for skin sensitization.

11.1.4.1. Risk assessment. No skin sensitization studies are available for 2-methyl-4-phenyl-2-butyl isobutyrate. Therefore, benzyl acetate (CAS # 140-11-4; see Section VI) was used for the risk assessment of 2-methyl-4-phenyl-2-butyl isobutyrate. The data on the read-across material are summarized in Table 1. Additionally, 2-phenoxyethyl isobutyrate (CAS # 103-60-6; see Section VI) was used as WoE. Based on the existing data on the read-across and WoE materials, 2-methyl-4-phenyl-2-butyl isobutyrate is not considered a skin sensitizer. 2-Methyl-4-phenyl-2-butyl isobutyrate and WoE material 2-phenoxyethyl isobutyrate are predicted *in silico* to be non-reactive with skin proteins directly, while read-across material benzyl acetate is predicted *in silico* to be reactive with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). In a murine local lymph node assay (LLNA), WoE material 2-phenoxyethyl isobutyrate was found to be non-sensitizing when tested up to 100% (25000 μg/cm^2) (RIFM, 2002). In a guinea pig maximization test, read-across material benzyl acetate did not lead to skin sensitization reactions (RIFM, 1985b). In a guinea pig Buehler test, read-across material benzyl acetate did not present reactions indicative of sensitization (RIFM, 1986). In a human maximization test, no skin

Table 1
Summary of existing data on benzyl acetate as a read-across for 2-methyl-4-phenyl-2-butyl isobutyrate.

WoE Skin Sensitization Potency Category[1]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL (induction) μg/cm^2	WoE NESIL μg/cm^2	LLNA Weighted Mean EC3 Value μg/cm^2	GPMT[3]	Buehler[3]
No evidence of sensitization[4]	9449	5520	N/A	N/A	N/A	Negative	Negative
	In vitro Data				*In silico* protein binding alerts (OECD Toolbox v4.5)		
	KE 1	KE 2	KE 3		Target Material	Autoxidation simulator	Metabolism simulator
	N/A	N/A	N/A		SN2	No alert found	No alert found

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans; HMT = Human Maximization Test; LOEL = lowest observed effect level; GPMT = Guinea Pig Maximization Test; KE = Key Event; N/A = Not Available.
1WoE 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).
2Data derived from CNIH or HMT.
3Studies conducted according to the OECD TG 406 are included in the table.
4Determined based on Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients (Api et al., 2015).

sensitization reactions were observed when read-across material benzyl acetate was tested at 5520 μg/cm^2 (Greif, 1967). In a human maximization test, no skin sensitization reactions were observed when WoE material 2-phenoxyethyl isobutyrate was tested at 2760 μg/cm^2 (RIFM, 1973). In a Confirmation of No Induction in Humans test (CNIH) with 387 μg/cm^2 of WoE material 2-phenoxyethyl isobutyrate in ethanol, no reactions indicative of sensitization were observed in any of the 38 volunteers (RIFM, 1965). In 3 separate CNIH tests with 14496 μg/cm^2, 2325 μg/cm^2, and 5814 μg/cm^2 of read-across material benzyl acetate in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 35, 42, and 35 volunteers, respectively (RIFM, 1975e; RIFM, 1975a; RIFM, 1975c). In 2 separate CNIH tests with 3488 μg/cm^2 and 2325 μg/cm^2 of read-across material benzyl acetate in petrolatum, no reactions indicative of sensitization were observed in any of the 39 and 44 volunteers, respectively (RIFM, 1975d; RIFM, 1975b). Additionally, in 5 separate CNIH tests with 9449 μg/cm^2 of read-across material benzyl acetate in 3:1 ethanol:diethyl phthalate, no reactions indicative of sensitization were observed in any of the 197, 108, 34, 27, and 38 volunteers (RIFM, 1987; RIFM, 1988a; RIFM, 1988b; RIFM, 1988c; RIFM, 1988d).

Based on WoE from structural analysis and animal and human studies on the read-across material benzyl acetate and WoE material 2-phenoxyethyl isobutyrate as well as the target material, 2-methyl-4-phenyl-2-butyl isobutyrate does not present a concern for skin sensitization.

Additional References: RIFM, 1967; RIFM, 1968; RIFM, 1985a; Klecak (1985); RIFM, 1985c; Ishihara et al., 1986; Klecak (1979); RIFM, 1962; RIFM, 1961.

Literature Search and Risk Assessment Completed On: 07/18/23.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, 2-methyl-4-phenyl-2-butyl isobutyrate would not be expected to present a concern for photoirritation or photoallergenicity.

11.1.5.1. Risk assessment. There are no photoirritation studies available for 2-methyl-4-phenyl-2-butyl isobutyrate in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 2-methyl-4-phenyl-2-butyl isobutyrate does not 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 no absorbance in the range of 290–700 nm. As such, it is not a concern for photoirritant or photoallergenic effects (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/19/23.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2-methyl-4-phenyl-2-butyl isobutyrate is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 2-methyl-4-phenyl-2-butyl isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0000008 mg/day. This exposure is 1750000 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/26/23.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2-methyl-4-phenyl-2-butyl 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 of 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, 2-methyl-4-phenyl-2-butyl 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) identified 2-methyl-4-phenyl-2-butyl isobutyrate as possibly persistent but not 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 $\geq$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).

11.2.2. Risk assessment

Based on the current VoU (2019), 2-methyl-4-phenyl-2-butyl isobutyrate does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. Biodegradation

No data available.

Ecotoxicity

No data available.

Other available data

2-Methyl-4-phenyl-2-butyl isobutyrate has been pre-registered for REACH with no additional data at this time.

11.2.3. 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)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1.069 mg/L			1000000	0.001069 µg/L	

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	4.84	4.84
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	Not reported	<1
Risk Characterization: PEC/PNEC	**N/A**	**<1**

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

The RIFM PNEC is 0.001069 µg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 07/24/23.

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 04/19/24.

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 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.2024.114757.

Appendix

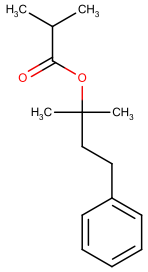
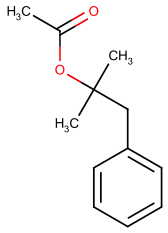
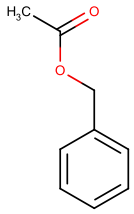
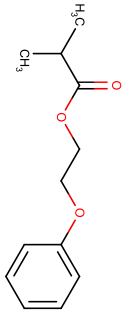
Read-across Justification

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 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 the 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 the 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	Read-across Material	WoE Material
Principal Name	2-Methyl-4-phenyl-2-butyl isobutyrate	1,1-Dimethyl-2-phenylethyl acetate	Benzyl acetate	2-Phenoxyethyl isobutyrate
CAS No.	10031-71-7	151-05-3	140-11-4	103-60-6
Structure				
Similarity (Tanimoto Score)		0.53	0.32	0.26
SMILES	CC(C)C(=O)OC(C)(C)CCc1ccccc1	CC(=O)OC(C)(C)Cc1ccccc1	CC(=O)OCc1ccccc1	CC(C)C(=O)OCCOc1ccccc1
Endpoint		Genotoxicity (mutagenicity) Repeated dose toxicity Reproductive toxicity	Skin sensitization	Skin sensitization
Molecular Formula	$C_{15}H_{22}O_2$	$C_{12}H_{16}O_2$	$C_9H_{10}O_2$	$C_{12}H_{16}O_3$
Molecular Weight (g/mol)	234.339	192.258	150.177	208.257
Melting Point (°C, EPI Suite)	49.10	28.29	−51.30	37.71
Boiling Point (°C, EPI Suite)	291.23	252.16	213.00	276.18
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.49E-01	2.97E+00	2.36E+01	7.01E-01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	2.11E+00	5.49E+01	3.10E+03	1.06E+02
Log K_{OW}	4.84	3.44	1.96	3.01
J_{max} (μg/cm^2/h, SAM)	0.24	3.51	64.04	2.69
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	7.85E+00	3.35E+00	1.14E+00	1.80E-01
Genotoxicity				
DNA Binding (OASIS v1.4, QSAR Toolbox v4.5)	No alert found	AN2|AN2 ≫ Schiff base formation after aldehyde release|AN2 ≫ Schiff base formation after aldehyde release ≫ Specific Acetate Esters|SN1|SN1 ≫ Nucleophilic attack after carbenium ion formation|SN1 ≫ Nucleophilic attack after carbenium ion formation ≫ Specific Acetate Esters|SN2|SN2 ≫ Acylation|SN2 ≫ Acylation ≫ Specific Acetate Esters|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom ≫ Specific Acetate Esters		
DNA Binding (OECD QSAR Toolbox v4.5)	Michael addition|Michael addition ≫ P450-mediated Activation to Quinones and Quinone-type Chemicals|Michael addition ≫ P450-mediated Activation to Quinones and Quinone-type Chemicals ≫ Arenes	Michael addition|Michael addition ≫ P450-mediated Activation to Quinones and Quinone-type Chemicals|Michael addition ≫ P450-mediated Activation to Quinones and Quinone-type Chemicals ≫ Arenes		
Carcinogenicity (ISS)	No alert found	No alert found		
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found		

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material	WoE Material
***In Vitro* Mutagenicity (Ames, ISS)**	No alert found	No alert found		
***In Vivo* Mutagenicity (Micronucleus, ISS)**	No alert found	No alert found		
Oncologic Classification	Not classified	Not classified		
Repeated Dose Toxicity				
Repeated Dose (HESS)	Not categorized	Toluene (Renal toxicity) Alert		
Reproductive Toxicity				
ER Binding (OECD QSAR Toolbox v4.5)	Non-binder, without OH or NH_2 group	Non-binder, without OH or NH_2 group		
Developmental Toxicity (CAESAR v2.1.6)	Non-toxicant (low reliability)	Non-toxicant (low reliability)		
Skin Sensitization				
Protein Binding (OASIS v1.1)	No alert found		SN2|SN2 ≫ SN2 Reaction at a sp3 carbon atom|SN2 ≫ SN2 Reaction at a sp3 carbon atom ≫ Activated alkyl esters and thioesters	No alert found
Protein Binding (OECD)	No alert found		SN2|SN2 ≫ SN2 reaction at sp3 carbon atom|SN2 ≫ SN2 reaction at sp3 carbon atom ≫ Allyl acetates and related chemicals	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)		Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found		SN2|SN2 ≫ SN2 Reaction at a sp3 carbon atom|SN2 ≫ SN2 Reaction at a sp3 carbon atom ≫ Activated alkyl esters and thioesters	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified		Alert for Acyl Transfer agent was identified	No skin sensitization reactivity domain alerts were 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	See Supplemental Data 3	See Supplemental Data 4

Summary

There are insufficient toxicity data on 2-methyl-4-phenyl-2-butyl isobutyrate (CAS # 10031-71-7). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, 1,1-dimethyl-2-phenylethyl acetate (CAS # 151-05-3) and benzyl acetate (CAS # 140-11-4) were identified as read-across analogs, and 2-phenoxyethyl isobutyrate (CAS # 103-60-6) was identified as a WoE material with sufficient data for toxicological evaluation.

Conclusions

- 1,1-Dimethyl-2-phenylethyl acetate (CAS # 151-05-3) was used as a read-across analog for the target material, 2-methyl-4-phenyl-2-butyl isobutyrate (CAS # 10031-71-7), for the genotoxicity (mutagenicity), repeated dose toxicity, and reproductive toxicity endpoints.
  - o The target material and the read-across analog share a commonality in that they are both phenylethyl esters with geminal dimethyl alkylation next to oxygen.
  - o The key difference between the target material and the read-across analog is that the target material is an isopropyl ester, whereas the read-across analog is a methyl ester. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - 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 contain alerts for Michael addition and P450-mediated activation to quinones. The read-across analog also contains additional alerts for SN1 nucleophilic addition and Schiff base formation (genotoxicity). The read-across analog also contains an alert for renal toxicity (repeated dose toxicity). However, data for the read-across analog indicates that it is not a concern for genotoxicity and that the MOE for the target material is adequate under the current use. According to these predictions, the read-across analog is expected to be more reactive compared to the target material. Data superseded predictions in this case.

- o Both the target material and read-across analog contain alerts for non-binder and non-toxicant (reproductive toxicity). The data from the reproductive toxicity section indicate that the MOE for the target material is adequate under the current use. *In silico* alerts are consistent with 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.

- Benzyl acetate (CAS # 140-11-4) was used as a read-across analog, and 2-phenoxyethyl isobutyrate (CAS # 103-60-6) was used as a WoE material for the target material, 2-methyl-4-phenyl-2-butyl isobutyrate (CAS # 10031-71-7), for the skin sensitization endpoint.
  - o The target material and the read-across analog share a commonality in that they are both esters with an aryl moiety.
  - o The key difference between the target material and the read-across analog is that the target material is an isopropyl ester, whereas the read-across analog is a methyl ester. Therefore, to satisfy the structural domain of the target material, substance 2-phenoxyethyl isobutyrate (CAS # 103-60-6) is used as WoE. This chemical has an isopropyl ester group in the same position as the target material. The read-across analog, combined with the WoE material, contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - 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 Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for the target material corresponds to skin absorption $\leq$40%, and J_{max} for the read-across analog corresponds to skin absorption $\leq$80%. While the percentage of skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - 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 read-across analog contains additional *in silico* alerts compared to the target material for acyl transfer and SN2 nucleophilic addition (skin sensitization). The data from the skin sensitization indicates that the read-across analog is not a concern for skin sensitization. According to these predictions, the read-across analog is expected to be more reactive compared to the target material. Data superseded predictions in this case.
  - 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.

Explanation of Cramer Classification

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

Q1. A normal constituent of the body? No.
Q2. Contains functional groups associated with enhanced toxicity? No.
Q3. Contains elements other than C, H, O, N, and divalent S? No.
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No.
Q6. Benzene derivative with certain substituents? No.
Q7. Heterocyclic? No.
Q16. Common terpene? (see Cramer et al., 1978 for a detailed explanation). No.
Q17. Readily hydrolyzed to a common terpene? No.
Q23. Aromatic? Yes.
Q27. Rings with substituents? Yes.
Q28. More than one aromatic ring? No.
Q30. Aromatic ring with complex substituents? No.
Q19. Open chain? Yes.
Q20. Aliphatic with some functional groups (see Cramer et al., 1978 for detailed explanation)? Yes.
Q21. Three or more different functional groups? No.
Q18. One of the list? (see Cramer et al., 1978 for a detailed explanation on the list of categories). No. Class Low (Class I).

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