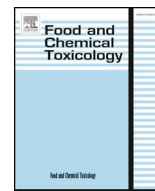




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Short Review

RIFM fragrance ingredient safety assessment, methyl 3-hexenoate, CAS Registry Number 2396-78-3



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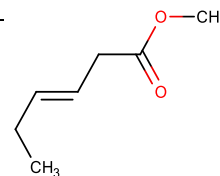
Name: Methyl 3-hexenoate

CAS Registry Number: 2396-78-3

Additional CAS*:

13894-62-7 Methyl cis-3-hexenoate

*This material was included in this assessment because the materials are a mixture of isomers.



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

AF - Assessment Factor

BCF - Bioconcentration Factor

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., 2015a, 2017) compared to a deterministic aggregate approach

DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts

DRF - Dose Range Finding

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

ECOSAR - Ecological Structure-Activity Relationships Predictive Model

EU - Europe/European Union

GLP - Good Laboratory Practice

IFRA - The International Fragrance Association

LOEL - Lowest Observable Effect Level

MOE - Margin of Exposure

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

NA - North America

NESIL - No Expected Sensitization Induction Level

NOAEC - No Observed Adverse Effect Concentration

NOAEL - No Observed Adverse Effect Level

NOEC - No Observed Effect Concentration

NOEL - No Observed Effect Level

OECD - Organisation for Economic Co-operation and Development

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

PBT - Persistent, Bioaccumulative, and Toxic

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

Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.

QRA - Quantitative Risk Assessment

QSAR - Quantitative Structure-Activity Relationship

REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals

RfD - Reference Dose

RIFM - Research Institute for Fragrance Materials

RQ - Risk Quotient

Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test

TTC - Threshold of Toxicological Concern

UV/Vis spectra - Ultraviolet/Visible spectra

VCF - Volatile Compounds in Food

VoU - Volume of Use

vPvB - (very) Persistent, (very) Bioaccumulative

WoE - Weight of Evidence

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

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

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

Methyl 3-hexenoate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that methyl 3-hexenoate is not genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the threshold of toxicological concern (TTC) for a Cramer Class I material, and the exposure to methyl 3-hexenoate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The skin sensitization endpoint was completed using the dermal sensitization threshold (DST) for non-reactive materials (900 $\mu\text{g}/\text{cm}^2$); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; methyl 3-hexenoate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; methyl 3-hexenoate was found not to be persistent, bioaccumulative, and toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current Volume of Use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are < 1 .

Human Health Safety Assessment

Genotoxicity: Not genotoxic.

(RIFM, 2016a; RIFM, 2016b)

Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.

Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.

Skin Sensitization: No safety concerns at current, declared use levels; exposure is below the DST.

Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.

(UV Spectra, RIFM Database)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence: Screening-level: 3.4 (BIOWIN 3)

(US EPA, 2012a; EPI Suite v4.11)

Bioaccumulation: Screening-level: 11.68 L/kg

(US EPA, 2012a; EPI Suite v4.11)

Ecotoxicity: Screening-level: Fish LC50: 135.95 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: 135.95 mg/L

Salvito et al. (2002)

RIFM PNEC is: 0.13595 µg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

Chemical Name: Methyl 3-hexenoate
CAS Registry Number: 2396-78-3
Synonyms: 3-Hexenoic acid, methyl ester; 0-Hexylhexanolide; Methyl hex-3-enoate; Methyl 3-hexenoate
Molecular Formula: C₇H₁₂O₂
Molecular Weight: 128.17
RIFM Number: 6734
Stereochemistry: Isomer not specified. One geometric center and total 2 geometric isomers possible.

Chemical Name: Methyl *cis*-3-hexenoate
CAS Registry Number: 13894-62-7
Synonyms: 3-Hexenoic acid, methyl ester; 0-Hexylhexanolide; Methyl hex-3-enoate; Methyl 3-hexenoate
Molecular Formula: C₇H₁₂O₂
Molecular Weight: 128.17
RIFM Number: 48
Stereochemistry: *Cis* isomer is defined

2. Physical data

CAS # 2396-78-3
Boiling Point: 155.15 °C (EPI Suite)
Flash Point*: 115.00 °F TCC (46.11 °C)
Log K_{ow}: 2.12 (EPI Suite)
Melting Point: 45.17 °C (EPI Suite)
Water Solubility: 1439 mg/L (EPI Suite)
Specific Gravity: Not Available
Vapor Pressure: 2.26 mm Hg @ 20 °C (EPI Suite v4.0), 2.0 mm Hg 20 °C (FMA), 3.15 mm Hg @ 25 °C (EPI Suite)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol⁻¹ · cm⁻¹)
Appearance/Organoleptic*: A colorless, clear liquid with a medium earthy, sweet, fruity, estery, green, fresh, banana odor

CAS # 13894-62-7
Boiling Point: 85 °C at 80 mm Hg (Private communication to FEMA), 155.15 °C (EPI Suite)
Flash Point*: 139.00 °F TCC (59.44 °C)
Log K_{ow}: 2.12 (EPI Suite)
Melting Point: 45.17 °C (EPI Suite)
Water Solubility: 1439 mg/L (EPI Suite)
Specific Gravity: 0.914–0.924 (Private communication to FEMA)
Vapor Pressure: 3.15 mm Hg @ 25 °C (EPI Suite)
Appearance/Organoleptic*: A colorless, clear liquid with a fruity floral odor

*<http://www.thegoodscentscompany.com/data/rw1035941.html#toorgano>, retrieved 02/05/18.

3. Exposure to fragrance ingredient***

1. Volume of Use

(Worldwide Band):
 < 0.1 metric ton per year

IFRA, (2015)

2. **95th Percentile Concentration in Hydroalcohols:** 0.000076%

RIFM, (2015)

3. **Inhalation Exposure*:** 0.0000080 mg/kg/day or 0.00050 mg/day
 RIFM, (2015)

4. **Total Systemic Exposure**:** 0.000078 mg/kg/day
 RIFM, (2015)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section IV. 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., 2015a, 2017).

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in Hydroalcohols, inhalation exposure, and total exposure.

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

Expert Judgment	Toxtree v3.1.0	OECD QSAR Toolbox v3.2
I	I	I

2. **Analogs Selected:**

- a. **Genotoxicity:** None
- b. **Repeated Dose Toxicity:** None
- c. **Reproductive Toxicity:** None
- d. **Skin Sensitization:** None
- e. **Phototoxicity/Photoallergenicity:** None
- f. **Local Respiratory Toxicity:** None
- g. **Environmental Toxicity:** None

3. **Read-across Justification:** None

6. Metabolism

No relevant data available for inclusion in this safety assessment.

Additional References: None.

7. Natural occurrence (discrete chemical) or composition (NCS)

Methyl 3-hexenoate is reported to occur in the following foods by the VCF*:

Mountain papaya (*C. candamarcensis*, *C. pubescens*) Pineapple (*Ananas comosus*) Prickly pear (*Opuntia ficus indica*) Soursop (*Annona muricata* L.)

Methyl *cis*-3-hexenoate is reported to occur in the following food by the VCF*:

Guava and feyoa.
Passion fruit (*Passiflora* species).
Pineapple (*Ananas comosus*).
Soursop (*Annona muricata* L.)
Starfruit (*Averrhoa carambola* L.)
Tea.

*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.

8. REACH dossier

Methyl 3-hexenoate is pre-registered for 2010; no dossier available as of 11/07/19. Methyl *cis*-3-hexenoate is pre-registered for 2018; no dossier available as of 11/07/19.

9. Conclusion

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

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. Methyl 3-hexenoate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: < 80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2014). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of methyl 3-hexenoate 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 *Escherichia coli* strain WP2uvrA were treated with methyl 3-hexenoate 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, 2016b). Under the conditions of the study, methyl 3-hexenoate was not mutagenic in the Ames test.

The clastogenic activity of methyl 3-hexenoate 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 methyl 3-hexenoate in DMSO at concentrations up to 1280 µg/mL in the presence and absence of S9 for 4 h and in the absence of S9 for 24 h. Methyl 3-hexenoate did not induce binucleated cells with micronuclei when tested up to cytotoxic

concentrations in either the presence or absence of an S9 activation system (RIFM, 2016a). Under the conditions of the study, methyl 3-hexenoate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, methyl 3-hexenoate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/05/17.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on methyl 3-hexenoate or on any read-across materials. The total systemic exposure to methyl 3-hexenoate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on methyl 3-hexenoate or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to methyl 3-hexenoate (0.078 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: 01/22/18.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on methyl 3-hexenoate or on any read-across materials. The total systemic exposure to methyl 3-hexenoate is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on methyl 3-hexenoate or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to methyl 3-hexenoate (0.078 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: 01/22/18.

10.1.4. Skin sensitization

Based on the application of DST, methyl 3-hexenoate does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Toxtree 2.6.13; OECD Toolbox v4.1). No predictive skin sensitization studies are available for methyl 3-hexenoate. Acting conservatively, due to the insufficient data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 µg/cm² (Safford, 2008; Safford et al., 2011, 2015b; Roberts et al., 2015). The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for methyl 3-hexenoate that present no appreciable risk for skin sensitization based on the non-reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/24/18.

Table 1
Maximum acceptable concentrations for methyl 3-hexenoate that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category ^a	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	NRU ^b
2	Products applied to the axillae	0.021%	0.0052%
3	Products applied to the face using fingertips	0.41%	$9.0 \times 10^{-7}\%$
4	Fine fragrance products	0.39%	$7.6 \times 10^{-5}\%$
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	$8.8 \times 10^{-5}\%$
6	Products with oral and lip exposure	0.23%	$1.9 \times 10^{-4}\%$
7	Products applied to the hair with some hand contact	0.79%	NRU ^b
8	Products with significant ano-genital exposure	0.041%	No Data
9	Products with body and hand exposure, primarily rinse-off	0.75%	$8.9 \times 10^{-6}\%$
10	Household care products with mostly hand contact	2.7%	$3.2 \times 10^{-5}\%$
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.5%	No Data
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.0097%

Note: Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

^a For a description of the categories, refer to the IFRA/RIFM Information Booklet.

^b No reported use.

10.1.5. Phototoxicity/photoallergenicity

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

10.1.5.1. Risk assessment. There are no phototoxicity studies available for methyl 3-hexenoate in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, methyl 3-hexenoate does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, $1000 \text{ L mol}^{-1} \cdot \text{cm}^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/18/17.

10.1.6. Local Respiratory Toxicity

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

10.1.6.1. Risk assessment. There are no inhalation data available on methyl 3-hexenoate. Based on the Creme RIFM Model, the inhalation exposure is 0.00050 mg/day. This exposure is 2800 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: 01/12/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of methyl 3-hexenoate 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, methyl 3-hexenoate 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 methyl 3-hexenoate as possibly persistent or bioaccumulative based on its structure and physical-chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF $\geq 2000 \text{ L/kg}$. 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).

10.2.2. Risk assessment

Based on the current Volume of Use (2015), methyl 3-hexenoate does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Key studies. **Biodegradation:** No data available.

Ecotoxicity: No data available.

10.2.2.2. Other available data. Methyl 3-hexenoate has been pre-registered for REACH with no additional data at this time.

10.2.3. 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.

	Predicted LC50 (Fish) (mg/L)	EC50 (<i>Daphnia</i>)	EC50 (Algae)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening-level (Tier 1)	<u>135.95</u>			1000000	0.13595	

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

Exposure	Europe (EU)	North America (NA)
Log K _{ow} Used	2.12	2.12
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	< 1	< 1

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

The RIFM PNEC is 0.13595 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 volumes of use.

Literature Search and Risk Assessment Completed On: 01/16/18.

11. 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**
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **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://hvpchemicals.oecd.org/ui/Default.aspx>
- **EPA ACToR:** <https://actor.epa.gov/actor/home.xhtml>
- **US EPA HPVIS:** https://ofmpub.epa.gov/opthpv/public_search_publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission

- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** <https://www.google.com>
- **ChemIDplus:** <https://chem.nlm.nih.gov/chemidplus/>

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as ap-

propriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/22/19.

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.

References

- 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.
- 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.
- 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.
- ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. <http://echa.europa.eu/>.
- 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), 2015. Volume of Use Survey, February 2015.
- 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.
- RIFM (Research Institute for Fragrance Materials, Inc), 2014. Report on the Testing of Methyl 3-hexenoate in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM report number 67398. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2015. Exposure Survey 08, October 2015.
- RIFM (Research Institute for Fragrance Materials, Inc), 2016. Methyl 3-hexenoate: in Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). RIFM report number 71339. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2016. Methyl 3-hexenoate: Bacterial Reverse Mutation Assay. RIFM report number 71340. RIFM, Woodcliff Lake, NJ, USA.
- Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of high potency category chemicals for which the dermal sensitization threshold (DST) approach should not be applied. *Regul. Toxicol. Pharmacol.* 72 (3), 683–693.
- 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., 2015a. 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., 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.
- Safford, R.J., 2008. The dermal sensitisation threshold—A TTC approach for allergic contact dermatitis. *Regul. Toxicol. Pharmacol.* 51 (2), 195–200.
- Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015b. Extension of the dermal sensitization threshold (DST) approach to incorporate chemicals classified as reactive. *Regul. Toxicol. Pharmacol.* 72 (3), 694–701.
- Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. *Regul. Toxicol. Pharmacol.* 60 (2), 218–224.
- 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.
- 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, v1.11. United States Environmental Protection Agency, Washington, DC, USA.