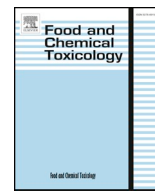




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Short review

RIFM fragrance ingredient safety assessment, ethyl 2-hexylacetoacetate, CAS Registry Number 29214-60-6



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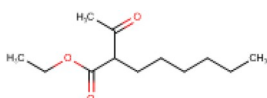
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Version: 080618. This version replaces any previous versions.

Name: Ethyl 2-hexylacetoacetate CAS Registry Number: 29214-60-6

**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

Crete RIFM Model - The Crete 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

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

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

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<https://doi.org/10.1016/j.fct.2019.03.015>

Received 22 August 2018; Received in revised form 1 February 2019; Accepted 11 March 2019

Available online 16 March 2019

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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

QRA - Quantitative Risk Assessment

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 under the limits 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.

Persistence: Screening-level: 3.1 (BIOWIN 3)

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

Bioaccumulation: Screening-level: 26.82 L/kg

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

Ecotoxicity: Screening-level: Fish LC50: 75.53 mg/L

(RIFM Framework; 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

(RIFM Framework; Salvito et al., 2002)

Critical Ecotoxicity Endpoint: Fish LC50: 75.53 mg/L

(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.07553 µg/L

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

1. Identification

- Chemical Name:** Ethyl 2-hexylacetoacetate
- CAS Registry Number:** 29214-60-6
- Synonyms:** Ethyl 2-acetyloctanoate; n-Hexyl ethyl acetoacetate; Octanoic acid, 2-acetyl-, ethyl ester; Gelsone; 外脂肪酸(C = 3 ~ 10) アルキル(C = 1 ~ 5); 2-アセチルカドリル酸エチル; Ethyl 2-acetylcaprylate; 2-Acetyloctanoic acid ethyl ester; Ethyl 2-hexylacetoacetate
- Molecular Formula:** C₁₂H₂₂O₃
- Molecular Weight:** 214.31
- RIFM Number:** 364
- Stereochemistry:** Isomer not specified. One stereocenter and 2 stereoisomers possible.

2. Physical data

- Boiling Point:** 270.69 °C (EPI Suite)
- Flash Point:** > 93 °C (GHS), > 200 °F; CC (FMA Database)
- Log K_{ow}:** 2.67 (EPI Suite)
- Melting Point:** 36.75 °C (EPI Suite)
- Water Solubility:** 193.6 mg/L (EPI Suite)
- Specific Gravity:** 0.933 (FMA Database)
- Vapor Pressure:** 0.00401 mm Hg @ 20 °C (EPI Suite v4.0; US EPA, 2012a), 0.009 mm Hg 20 °C (FMA Database), 0.00715 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 slightly oily liquid which has an oily-herbaceous, somewhat winery odor with distinctly green-herbal notes, reminiscent of undertones in Jasmin complex

3. Exposure

- Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2015)
- 95th Percentile Concentration in Hydroalcohols:** 0.19% (RIFM, 2017)
- Inhalation Exposure*:** 0.00054 mg/kg/day or 0.041 mg/day (RIFM, 2017)
- Total Systemic Exposure**:** 0.0029 mg/kg/day (RIFM, 2017)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 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; Safford et al.,

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 use of this material under current conditions is supported by existing information.

Ethyl 2-hexylacetoacetate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that ethyl 2-hexylacetoacetate is not genotoxic. The skin sensitization endpoint was completed using DST for non-reactive materials (900 µg/cm²); exposure is below the DST. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to ethyl 2-hexylacetoacetate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; ethyl 2-hexylacetoacetate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; ethyl 2-hexylacetoacetate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.

(RIFM, 2014a; RIFM, 2014b)

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 DB)

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

Environmental Safety Assessment

Hazard Assessment:

2015a; Safford et al., 2017; and Comiskey et al., 2017).

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 v 2.6	OECD QSAR Toolbox v 3.2
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

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

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

Ethyl 2-hexylacetoacetate is not reported to occur in food 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.

8. IFRA standard

None.

9. REACH dossier

Pre-registered for 2010, no dossier available as of 08/06/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, ethyl 2-hexylacetoacetate does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. Ethyl 2-hexylacetoacetate 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, 2013). BlueScreen is a screening assay that assesses genotoxic stress through alterations in gene expressions in a human cell line. Additional assays were considered

to fully assess the potential mutagenic or clastogenic effects on the target material.

The mutagenic activity of ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate 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, 2014a). Under the conditions of the study, ethyl 2-hexylacetoacetate was not mutagenic in the Ames test.

The clastogenic activity of ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate in DMSO at concentrations up to 2145 µg/mL in the presence and absence of metabolic activation (S9) for 3 h and in the absence of metabolic activation for 24 h. Ethyl 2-hexylacetoacetate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels either non-activated or S9-activated test systems (RIFM, 2014b). Under the conditions of the study, ethyl 2-hexylacetoacetate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, ethyl 2-hexylacetoacetate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/15/2017.

10.1.2. Repeated dose toxicity

The margin of exposure for ethyl 2-hexylacetoacetate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on ethyl 2-hexylacetoacetate or any read-across materials evaluated that can be used to support the repeated dose toxicity endpoints. The total systemic exposure to ethyl 2-hexylacetoacetate (2.9 µg/kg bw/day) is below the TTC (30 µg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012; of a Cramer Class I material) for the repeated dose toxicity endpoint at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/05/2017.

10.1.3. Reproductive toxicity

There are no reproductive toxicity data on ethyl 2-hexylacetoacetate or any read-across materials evaluated. The total systemic exposure to ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate or any read-across materials evaluated that can be used to support the reproductive toxicity endpoints. The total systemic exposure to ethyl 2-hexylacetoacetate (2.9 µg/kg bw/day) is below the TTC (30 µg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012; of a Cramer Class I material) for the reproductive toxicity endpoint at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/05/2017.

10.1.4. Skin sensitization

Based on the existing data and the application of DST, ethyl 2-hexylacetoacetate 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 (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v3.4). No predictive skin sensitization studies are available for ethyl 2-hexylacetoacetate or read-across materials. However, in a human maximization test, no skin sensitization reactions were observed (RIFM, 1972a). Additionally, in 2 confirmatory human repeat insult patch tests (HRIPs) with 15% (11628 µg/cm²) or 5% (3876 µg/cm²) of ethyl 2-hexylacetoacetate in ethanol, no reactions indicative of sensitization were observed in any of the 39 or 40 volunteers, respectively (RIFM, 1972b; RIFM, 1964).

Acting conservatively, due to the limited data, the reported exposure was benchmarked utilizing the non-reactive Dermal Sensitization Threshold (DST) of 900 µg/cm² (Safford, 2008; Safford et al., 2011; Safford et al., 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 acceptable concentrations for ethyl 2-hexylacetoacetate that present no appreciable risk for skin sensitization based on the non-reactive DST. These concentrations are not limits; they represent acceptable concentrations based on the DST approach.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/20/17.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate 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 lack of absorbance, ethyl 2-hexylacetoacetate 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 L·mol⁻¹·cm⁻¹

Table 1

Acceptable concentrations for ethyl 2-hexylacetoacetate that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category ^a	Description of Product Type	Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Concentration in Finished Products
1	Products applied to the lips	0.07%	0.00%
2	Products applied to the axillae	0.02%	0.02%
3	Products applied to the face using fingertips	0.41%	0.01%
4	Fine fragrance products	0.39%	0.19%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.02%
6	Products with oral and lip exposure	0.23%	0.01%
7	Products applied to the hair with some hand contact	0.79%	0.00% ^b
8	Products with significant ano-genital exposure	0.04%	No Data ^c
9	Products with body and hand exposure, primarily rinse-off	0.75%	0.01%
10	Household care products with mostly hand contact	2.70%	0.07%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.50%	No Data ^c
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	3.15%

Note.

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

^b Negligible exposure (< 0.01%).

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

(Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/11/17.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate. Based on the Creme RIFM Model, the inhalation exposure is 0.041 mg/day. This exposure is 34.1 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: 12/1/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of ethyl 2-hexylacetoacetate 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, ethyl 2-hexylacetoacetate 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 ethyl 2-hexylacetoacetate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥ 2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on the current Volume of Use (2015), ethyl 2-hexylacetoacetate does not present a risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

10.2.2.1. Other available data. Ethyl 2-hexylacetoacetate 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 $\mu\text{g/L}$)

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (<i>Daphnia</i>) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC ($\mu\text{g/L}$)	Chemical Class
RIFM Framework Screening-Level (Tier 1)	<u>75.53</u>			1,000,000	0.07553	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.67	2.67
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 0.07553 $\mu\text{g/L}$. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 11/21/2017.

11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** <http://echa.europa.eu/>
- **NTP:** <https://ntp.niehs.nih.gov/>
- **OECD Toolbox**
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **PubMed:** <http://www.ncbi.nlm.nih.gov/pubmed>
- **TOXNET:** <http://toxnet.nlm.nih.gov/>
- **IARC:** <http://monographs.iarc.fr>
- **OECD SIDS:** <http://webnet.oecd.org/hpv/ui/Default.aspx>
- **EPA ACToR:** <https://actor.epa.gov/actor/home.xhtml>
- **US EPA HPVIS:** https://ofmpub.epa.gov/opphpv/public_search_publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** <http://www.safe.nite.go.jp/english/db.html>
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** <https://www.google.com>
- **ChemIDplus:** <https://chem.nlm.nih.gov/chemidplus/>

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 06/12/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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), 1964. **Repeated Insult Patch Test with Ethyl 2-hexylacetoacetate.** Unpublished Report from International Flavors and Fragrances. RIFM report number 51123. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1972a. **The Contact-Sensitization Potential of Fragrance Materials by Maximization Testing in Humans.** Report to RIFM. RIFM report number 1804. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1972b. **Repeated Insult Patch Test with Ethyl 2-hexylacetoacetate.** Unpublished Report from International Flavors and Fragrances. RIFM report number 51124. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2013. **Report on the Testing of Ethyl 2-hexylacetoacetate in the BlueScreen HC Assay (-/+ S9 Metabolic Activation).** RIFM report number 66267. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2014a. **Ethyl 2-hexylacetoacetate (Ethyl 2-acetyloctanoate): Bacterial Reverse Mutation Assay.** RIFM report number 67271. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2014b. **Ethyl 2-hexylacetoacetate: in Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes.** RIFM report number 67501. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2017. **Exposure Survey 15, March 2017.**
- 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.
- 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.
- 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.