Food and Chemical Toxicology 144 (2020) 111548

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

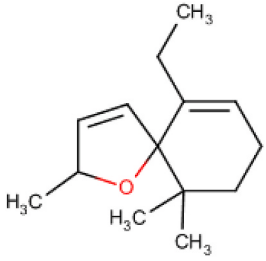
RIFM fragrance ingredient safety assessment, 1-oxaspiro[4.5]deca-3,6--diene, 6-ethyl-2,10,10-trimethyl-, CAS Registry Number 79893-63-3

A.M. Api [a], D. Belsito [b], S. Biserta [a], D. Botelho [a], M. Bruze [c], G.A. Burton Jr. [d], J. Buschmann [e], M. A. Cancellieri [a], M.L. Dagli [f], M. Date [a], W. Dekant [g], C. Deodhar [a], A.D. Fryer [h], S. Gadhia [a], L. Jones [a], K. Joshi [a], A. Lapczynski [a], M. Lavelle [a], D.C. Liebler [i], M. Na [a], D. O'Brien [a], A. Patel [a], T.M. Penning [j], G. Ritacco [a], F. Rodriguez-Ropero [a], J. Romine [a], N. Sadekar [a], D. Salvito [a], T. W. Schultz [k], F. Siddiqi [a], I.G. Sipes [l], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [m], S. Tsang [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of Expert Panel, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996- 4500, USA*
[l] *Member Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member Expert Panel, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman, Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

Version: 050319. This version replaces any previous versions.

Name: 1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-
CAS Registry Number: 79893-63-3



Abbreviation/Definition List:

(*continued on next column*)

(*continued*)

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

(*continued on next page*)

* Corresponding author.
E-mail addresses: gsullivan@rifm.org, garypsullivan@gmail.com (G. Sullivan).

https://doi.org/10.1016/j.fct.2020.111548
Received 12 December 2019; Received in revised form 14 May 2020; Accepted 19 June 2020
Available online 4 July 2020

(*continued*)

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.

1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- (CAS # 71078-31-4) show that 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is not expected to be genotoxic. Data on read-across analog 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5) provide a calculated MOE >100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class III material, and the exposure to 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is below the TTC (0.0015 mg/kg/day and 0.47 mg/day, respectively). Data from 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- and read-across material 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5) show that 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- does not present a concern for skin sensitization. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- 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 expected to be genotoxic. (RIFM, 2016b; RIFM, 2016c)

Repeated Dose Toxicity: NOAEL = 167 mg/kg/day. RIFM (1993)

(*continued on next column*)

(*continued*)

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

Skin Sensitization: Not a concern for skin sensitization at the current, declared use levels. RIFM (1992)

Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM Database; RIFM, 1981)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence:
Screening-level: 2.31 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation:
Screening-level: 1022 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:
Screening-level: 48-h *Daphnia* LC50: 0.104 mg/L (ECOSAR; US EPA, 2012b)

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: 48-h *Daphnia* LC50: 0.104 mg/L (ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.0104 μg/L

- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

1. **Chemical Name:** 1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-
2. **CAS Registry Number:** 79893-63-3
3. **Synonyms:** 6-Ethyl-2,10,10-trimethyl-1-oxaspiro[4.5]deca-3,6-diene; 1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-
4. **Molecular Formula:** $C_{14}H_{22}O$
5. **Molecular Weight:** 206.32
6. **RIFM Number:** 6399
7. **Stereochemistry:** Two stereocenters and 4 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 257.33 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** 5.07 (EPI Suite)
4. **Melting Point:** 51.18 °C (EPI Suite)
5. **Water Solubility:** 1.915 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0173 mm Hg @ 25 °C (EPI Suite), 0.00998 mm Hg @ 20 °C (EPI Suite v4.0)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. 0.1–1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.0051% (RIFM, 2016a)
2. **Inhalation Exposure*:** 0.000018 mg/kg/day or 0.0013 mg/day (RIFM, 2016a)
3. **Total Systemic Exposure**:** 0.00020 mg/kg/day (RIFM, 2016a)

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

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

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 III, High

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
III	III	III

2. Analogs Selected:
   a. **Genotoxicity:** 1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- (CAS # 71078-31-4)
   b. **Repeated Dose Toxicity:** 1-Oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (neocaspirene; CAS # 89079-92-5)
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** 1-Oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References: None.

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

1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- 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

Pre-registered for 2010; no dossier available as of 05/03/19.

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, 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. There are no data assessing the mutagenic and clastogenic activity of 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-; however, read-across can be made to 1-oxaspiro [4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- (CAS # 71078-31-4; see Section VI).

The mutagenic activity of 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- 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/preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 1-oxaspiro [4.5] deca-3,6-diene, 2,6,9,10-tetramethyl- 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, 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- was not mutagenic in the Ames test, and this can be extended to 1-oxaspiro[4.5] deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-.

The clastogenic activity of 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- 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 1-oxaspiro[4.5] deca-3,6-diene, 2,6,9,10-tetramethyl- in acetone at concentrations up to 1920 μg/mL in a DRF study; micronuclei analysis was conducted at concentrations up to 90 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h 1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2016c). Under the conditions of the study, 1-oxaspiro [4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-.

Based on the available data, 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- does not present a concern for genotoxic potential, and this can be extended to 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/13/19.

11.1.2. Repeated dose toxicity

The MOE for 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- 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 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-. Read-across material, 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5; see Section VI) has sufficient repeated dose toxicity data that can be used to support the repeated dose toxicity endpoint. A GLP 28-day oral gavage subchronic toxicity study was conducted in CD strain rats. Groups of 5 rats/sex/dose were administered 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- via oral gavage at doses of 0, 10, 100, or 500 mg/kg/day in maize oil for 4 weeks. One male and 1 female rat from the control group were found dead on day 2 of the study, and 1 female rat from the high-dose group

was euthanized on the same day. At necropsy, ruptures of the esophagus associated with accidental dosing were noted as the factor contributing to the death of all 3 rats. As this occurred early in the study, these animals were replaced. At 500 mg/kg/day, statistically significant findings included a decrease in bodyweight gain during weeks 1–2 (males only), a decrease in the mean cell volume and mean cell hemoglobin (males only), an increase in the plasma activity of 5′-nucleotidase (females only), an increase in the plasma activity of alanine aminotransferase (males only), and an increase in the serum protein concentration (males only). Although there were no changes in the total serum protein concentration among female animals, there was a statistically significant increase in α2-globulin and β-globulin in high-dose females. An increase in the activity of 5′-nucleotidase is generally associated with hepatobiliary disease when seen in parallel with an increase in alkaline phosphatase activity, but there was no evidence to support this. Statistically significant increases in the absolute and relative liver weights were observed among animals of the highest-dose group. The increase in the relative liver weights extended to males of the mid-dose group. The absolute and relative kidney weights were statistically significantly increased among males of the highest-dose group. Enlargement of the liver and kidneys was observed in male rats dosed at 500 mg/kg/day, and pallor of these organs was noted in a few male and female rats dosed at 100 and 500 mg/kg/day. In all high-dose male rats, the cytoplasm of the epithelial cells of the proximal tubules contained eosinophilic hyaline droplets, and in 2 of these rats, this accumulation was associated with degeneration of the epithelial cells. In addition, 3 out of 5 male rats treated at 100 mg/kg/day showed an accumulation of hyaline droplets within the renal tubular epithelium. These changes were not apparent in female rats. The kidney changes in males were consistent with documented changes of α-2u-globulin nephropathy, which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman and Caudill, 1992; Lehman-McKeeman et al., 1990). All high-dose animals exhibited hypertrophy of hepatocytes, which may be associated with the increased serum proteins since they are synthesized in the liver; therefore, the increased plasma concentration is most likely related to the increased liver weights. Since there was no histopathological or clinical chemistry evidence of liver degeneration or necrosis, the liver weight increases were considered to be adaptive (Hall et al., 2012). The NOAEL for systemic toxicity was considered to be 500 mg/kg/day, the highest dose tested (RIFM, 1993).

A default safety factor of 3 was used when deriving a NOAEL from a 28-day study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

The derived NOAEL for the repeated dose toxicity data is 500/3 or 167 mg/kg/day.

Therefore, the 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- MOE for the repeated dose toxicity endpoint can be calculated by dividing the 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- NOAEL in mg/kg/day by the total systemic exposure to for 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-, 167/0.00020, or 835000.

In addition, the total systemic exposure for 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- (0.20 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint at the current level of use.

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/30/19.

11.1.3. Reproductive toxicity

There are no reproductive toxicity data on 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- or on any read-across materials. The total systemic exposure to 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is below the TTC for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- (0.20 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/07/19.

11.1.4. Skin sensitization

Based on the existing data and read-across 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5), 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- presents no concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-. Based on the existing data and read-across material 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5; see Section VI), 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is not considered a skin sensitizer. The chemical structure of these materials indicate that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2). In 2 guinea pig maximization tests with read-across material 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)-, no reactions indicative of skin sensitization were observed (RIFM, 1992; RIFM, 1983). However, in a human repeat insult patch test, no reactions indicative of sensitization were observed under the conditions of the study when 4% 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- in white petrolatum was used for induction and challenge (RIFM, 1981).

Based on WoE from structural analysis, animal and human studies, and read-across material 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)-, 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/03/19.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra and existing data, 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). In a photo-HRIPT, there were no reactions observed following the application of 4% 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- in either the irradiated or non-irradiated sites during induction or challenge (RIFM, 1981). Based on the human study data and the lack of absorbance, 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. 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} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/29/19.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-. Based on the Creme RIFM Model, the inhalation exposure is 0.0013 mg/day. This exposure is 361.5 times lower than the Cramer Class III TTC value of 0.47 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: 05/15/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- 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, 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- 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, 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).

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. No data available.

11.2.1.2.2. Ecotoxicity. No data available.

11.2.1.3. Other available data. 1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- has been pre-registered for REACH with no additional data at this time.

11.2.2. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in μg/L).

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>0.59</u>			1000000	0.00059	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.295	<u>0.104</u>	0.163	10000	0.0104	Vinyl/Allyl Ethers
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.299	0.225	0.541			Neutral Organics SAR

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	5.07	5.07
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 additional assessment is necessary.

The RIFM PNEC is 0.0104 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported Volume of Use.

Literature Search and Risk Assessment Completed On: 06/11/19.

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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **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 HPVIS:** https://ofmpub.epa.gov/oppthpv/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 appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 05/14/20.

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.2020.111548.

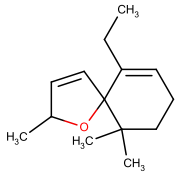
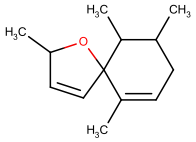
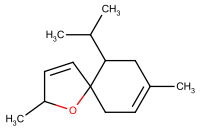
Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also 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, 2016).

- 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 v4.11 (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 v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	1-Oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl-	1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl-	1-Oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)-
CAS No.	79893-63-3	71078-31-4	89079-92-5
Structure			
Similarity (Tanimoto Score)		0.97	0.63
Read-across Endpoint		• Genotoxicity	• Repeated Dose Toxicity • Skin Sensitization
Molecular Formula	$C_{14}H_{22}O$	$C_{13}H_{20}O$	$C_{14}H_{22}O$
Molecular Weight	206.32	192.30	206.32
Melting Point (°C, EPI Suite)	51.18	34.88	38.65
Boiling Point (°C, EPI Suite)	257.33	244.03	256.04
Vapor Pressure (Pa @ 25 °C, EPI Suite)	2.31E+00	6.28E+00	3.23E+00
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	5.07	4.54	5.03
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	1.915	6.365	2.055
J_{max} (μg/cm^2/h, SAM)	93.732	174.172	25.702
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.65E+02	3.50E+02	4.65E+02
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• No alert found	• No alert found	
DNA Binding (OECD QSAR Toolbox v4.2)	• No alert found	• No alert found	
Carcinogenicity (ISS)	• No alert found	• No alert found	
DNA Binding (Ames, MN, CA, OASIS v1.1)	• No alert found	• No alert found	
***In Vitro* Mutagenicity (Ames, ISS)**	• No alert found	• No alert found	
***In Vivo* Mutagenicity (Micronucleus, ISS)**	• No alert found	• No alert found	
Oncologic Classification	• Not classified	• Not classified	
Repeated Dose Toxicity			
Repeated Dose (HESS)	• Not categorized		• Not categorized
Skin Sensitization			
Protein Binding (OASIS v1.1)	• No alert found		• No alert found
Protein Binding (OECD)	• No alert found		• No alert found
Protein Binding Potency	• Not possible to classify according to these rules (GSH)		• Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• No alert found		• No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No alert found		• No alert found
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• See Supplemental Data 1	• See Supplemental Data 2	• See Supplemental Data 3

Summary

There are insufficient toxicity data on 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- (CAS # 79893-63-3). 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, read-across analogs 1-oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5) and 1-oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- (CAS # 71078-31-4) were identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl- (CAS # 71078-31-4) was used as a read-across analog for the target material 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- (CAS # 79893-63-3) for the genotoxicity endpoint.
  - The target material and the read-across analog are structurally similar and belong to a class of substituted dihydrofurans.
  - The target material and the read-across analog share a dihydrofuran in spiro connection with cyclohexene as a common substructure.
  - The key difference between the target material and the read-across analog is that the target material has a 6 ethyl and a 2,10,10 trimethyl substitution while the read-across analog has a 2,6,9,10 tetramethyl substitution. This structural difference is toxicologically insignificant.
  - 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.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - There are no toxicity alerts for the target material or the read-across analog. Data are consistent with *in silico* alerts.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

- 1-Oxaspiro[4.5]deca-3,6-diene, 2,7-dimethyl-10-(1-methylethyl)- (CAS # 89079-92-5) was used as a read-across analog for the target material 1-oxaspiro[4.5]deca-3,6-diene, 6-ethyl-2,10,10-trimethyl- - (CAS # 79893-63-3) for the repeated dose toxicity and skin sensitization endpoints.
  - The target material and the read-across analog share a dihydrofuran in spiro connection with cyclohexene as a common substructure.
  - The key difference between the target material and the read-across analog is that the target material has a 6 ethyl and a 2,10,10 trimethyl substitution while the read-across analog has a 2,7 dimethyl 1 isopropyl substitution. This structural difference is toxicologically insignificant.
  - 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.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - There are no toxicity alerts for the target material or the read-across analog. Data are consistent with *in silico* alerts.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

Api, A.M., 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.

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

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

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

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

ECHA, 2016. Read-across Assessment Framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., et al., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes–conclusions from the 3rd International ESTP expert workshop. Toxicol. Pathol. 40 (7), 971–994.

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.

Lehman-McKeeman, L.D., Caudill, D., 1992. a-2u-globulin is the only member of the lipocalin protein superfamily that binds to hyaline droplet inducing agents. Toxicol. Appl. Pharmacol. 116 (2), 170–176.

Lehman-McKeeman, L.D., Rivera-Torres, M.I., Caudill, D., 1990. Lysosomal degradation of alpha2u-globulin and alpha2u-globulin-xenobiotic conjugates. Toxicol. Appl. Pharmacol. 103 (3), 539–548.

OECD, 2015. Guidance Document on the Reporting of Integrated Approaches to Testing and Assessment (IATA). ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.

OECD, 2018. The OECD QSAR Toolbox, v3.2–4.2. Retrieved from. http://www.qsartoolbox.org/.

RIFM (Research Institute for Fragrance Materials, Inc), 1981. Repeated Insult Patch Test/photosensitization Study of 1-oxaspiro[4.5]deca-3,6-Diene, 6-Ethyl-2,10,10-Trimethyl- in Human Subjects. Unpublished report from Firmenich SA. RIFM report number 39879. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1983. Sensitization Test with 1-Oxaspiro[4.5]deca-3,6-Diene, 2,7-Dimethyl-10-(1-Methylethyl)- in guinea Pigs. Unpublished report from Quest International. RIFM report number 46563. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1992. 1-oxaspiro[4.5]deca-3,6-diene, 2,7-Dimethyl-10-(1-Methylethyl)-: Skin Sensitization Study in guinea Pigs. Unpublished report from Quest International. RIFM report number 46581. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1993. 1-oxaspiro[4.5]deca-3,6-diene, 2,7-Dimethyl-10-(1-Methylethyl)-: 28 Day Subacute Oral Toxicity Study in Rats. Unpublished report from Quest International. RIFM report number 46588. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2016a. Exposure Survey 10, March 2016.

RIFM (Research Institute for Fragrance Materials, Inc), 2016b. 1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl: Reverse Mutation Assay 'ames Test' Using Salmonella typhimurium and Escherichia coli. RIFM report number 69950. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2016c. 1-Oxaspiro[4.5]deca-3,6-diene, 2,6,9,10-tetramethyl-: Micronucleus Test in Human Lymphocytes in Vitro. RIFM report number 70475. RIFM, Woodcliff Lake, NJ, USA.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C. A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.

Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74, 164–176.

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.