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RIFM fragrance ingredient safety assessment, hexen-2-al, CAS Registry Number 6728-26-3

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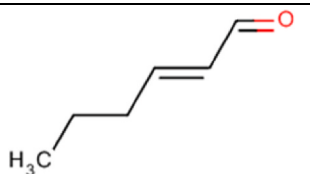
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Name: Hexen-2-al CAS Registry Number: 6728-26-3

Additional CAS Numbers*: 16635-54-4 *cis*-2-Hexenal (No Reported Use)



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505-57-7 2-Hexenal

*Included because the materials are 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

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CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)

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; Safford et al., 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

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

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.

Hexen-2-al was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that hexen-2-al is not genotoxic. Data provide hexen-2-al 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 I material, and the exposure to hexen-2-al is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). Data provided hexen-2-al a NESIL of 18 µg/cm² for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; hexen-2-al is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; hexen-2-al was found not to be PBT as per the IFRA Environmental

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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, 2007; EFSA, 2014)

Repeated Dose Toxicity: NOAEL = 200 mg/kg/day. (Gaunt et al., 1971)

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

Skin Sensitization: NESIL = 18 µg/cm². RIFM, (2020b)

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: Critical Measured Value: RIFM, (2002a)

73% (OECD 301 F) for CAS # 6728-26-3

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

5.15 L/kg

Ecotoxicity: Screening-level: Fish LC50: (RIFM Framework; Salvito et al., 2002)

197.6 mg/L

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: (RIFM Framework; Salvito et al., 2002)

197.6 mg/L

RIFM PNEC is: 0.1976 µg/L

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

1. Identification

Chemical Name: Hexen-2-al	Chemical Name: <i>cis</i> -2-Hexenal	Chemical Name: 2-Hexenal
CAS Registry Number: 6728-26-3	CAS Registry Number: 16635-54-4	CAS Registry Number: 505-57-7
Synonyms: <i>trans</i> -2-Hexenal; 2-Hexenal, (E)-; Leaf aldehyde; β-Propyl acrolein; ٦-٦-٦-٦; Hex-2-enal; Hexen-2-al	Synonyms: 2-Hexenal, (Z)-; <i>cis</i> -2-Hexenal	Synonyms: Hex-2-enal; hexen-2-al; 2-Hexenal
Molecular Formula: C ₆ H ₁₀ O	Molecular Formula: C ₆ H ₁₀ O	Molecular Formula: C ₆ H ₁₀ O
Molecular Weight: 98.14	Molecular Weight: 98.14	Molecular Weight: 98.14
RIFM Number: 452	RIFM Number: None	RIFM Number: 452
Stereochemistry: Trans isomer specified. One stereocenter and 2 stereoisomers	Stereochemistry: Cis isomer specified. One stereocenter and 2 stereoisomers	Stereochemistry: No isomer specified. One stereocenter and 2 stereoisomers

2. Physical data

CAS # 6728-26-3	CAS # 16635-54-4	CAS # 505-57-7
Boiling Point: 293 °F (FMA Database), 139.17 °C (EPI Suite)	Boiling Point: 139.17 °C (EPI Suite)	Boiling Point: Not Available
Flash Point: 100 °F; CC (FMA Database)	Flash Point: Not Available	Flash Point: 38 °C (GHS)
Log K_{ow}: 1.58 (Biobyte Corp.), Log Pow = 1.8 (RIFM, 2002b), 1.58 (EPI Suite)	Log K_{ow}: 1.58 (EPI Suite)	Log K_{ow}: Not Available
Melting Point: -55.63 °C (EPI Suite)	Melting Point: -55.63 °C (EPI Suite)	Melting Point: Not Available
Water Solubility: 5261 mg/L (EPI Suite)	Water Solubility: 5261 mg/L (EPI Suite)	Water Solubility: Not Available
Specific Gravity: 0.846 (FMA Database)	Specific Gravity: Not Available	Specific Gravity: Not Available
Vapor Pressure: 3.42 mm Hg @ 20 °C (EPI Suite v4.0), 3.2 mm		Vapor Pressure: Not Available

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CAS # 6728-26-3	CAS # 16635-54-4	CAS # 505-57-7
Hg 20 °C (FMA Database), 4.72 mm Hg @ 25 °C (EPI Suite)	Vapor Pressure: 4.72 mm Hg @ 25 °C (EPI Suite)	
UV Spectra: Minor absorbance between 290 and 700 nm; molar absorption coefficients (71, 0, 73 L mol ⁻¹ • cm ⁻¹ , under neutral, acidic, and basic conditions, respectively) are below the benchmark (1000 L mol ⁻¹ • cm ⁻¹)	UV Spectra: Not Available	UV Spectra: Not available
Appearance/Organoleptic: Colorless liquid with powerful green-fruity, pungent, vegetable-like odor	Appearance/Organoleptic: Not Available	Appearance/Organoleptic: Not available

3. Volume of use (worldwide band)

1. 1–10 metric tons per year	(IFRA, 2015)
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4. Exposure*** to fragrance ingredient (Creme RIFM Aggregate Exposure Model v3.0.)

1. 95th Percentile Concentration in Fine Fragrance: 0.0012%	RIFM, (2020a)
2. Inhalation Exposure*: 0.000050 mg/kg/day or 0.0035 mg/day	RIFM, (2020a)
3. Total Systemic Exposure**: 0.00013 mg/kg/day	RIFM, (2020a)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer Classification

Class I, Low

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

6.2. 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

7. Metabolism

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

8. Natural occurrence

Hexen-2-al is reported to occur in the following foods by the VCF*:

Apricot (<i>Prunus armeniaca</i> L.)	Pistacia palaestina (<i>Pistacia terebinthus</i> L.)
Citrus fruits	Radish (<i>Raphanus sativus</i> L.)
Mangifera species	Salvia species
Mentha oils	Thyme (<i>Thymus</i> species)
Pistachio oil (<i>Pistacia vera</i>)	Wormwood oil (<i>Artemisia absinthium</i> L.)

cis-2-Hexenal is reported to occur in the following foods by the VCF*:

Chicken	Nectarine
Citrus fruits	Olive (<i>Olea europaea</i>)
Kiwifruit (<i>Actinidia chinensis</i> , syn. A. deliciosa)	Tea
	Thyme (<i>Thymus</i> species)

2-Hexenal is reported to occur in the following foods by the VCF*:

Allium species	Barley
Apple processed (<i>Malus</i> species)	Beans
Asparagus (<i>Asparagus officinalis</i> L.)	

*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. This is a partial list.

9. REACH dossier

Dossier available for hexen-2-al; accessed 04/21/21 (ECHA, 2018). cis-2-Hexenal and 2-hexenal have not been pre-registered; no dossiers available as of 04/21/21.

10. Conclusion

The maximum acceptable concentrations^a in finished products for hexen-2-al are detailed below.

IFRA Category ^b	Description of Product Type	Maximum Acceptable Concentrations ^a in Finished Products (%) ^c
1	Products applied to the lips (lipstick)	0.0014
2	Products applied to the axillae	0.00041
3	Products applied to the face/body using fingertips	0.0083
4	Products related to fine fragrances	0.0077
5a	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.0020
5b	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.0020

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IFRA Category ^b	Description of Product Type	Maximum Acceptable Concentrations ^a in Finished Products (%) ^c
5c	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0020
5d	Baby cream, oil, talc	0.00067
6	Products with oral and lip exposure	0.0045
7	Products applied to the hair with some hand contact	0.016
8	Products with significant anogenital exposure (tampon)	0.00067
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.015
10a	Household care products with mostly hand contact (hand dishwashing detergent)	0.054
10b	Aerosol air freshener	0.054
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.00067
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted

Note: ^aMaximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For hexen-2-al, the basis was the reference dose of 2 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 18 µg/cm².

^bFor a description of the categories, refer to the IFRA RIFM Information Booklet (<https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf>).

^cCalculations by Creme RIFM Aggregate Exposure Model v3.0.5.

11. Summary

11.1. Human Health Endpoint Summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment. Hexen-2-al was tested in the BlueScreen assay and was found negative for genotoxicity in the presence and absence of metabolic activation, indicating a lack of genotoxic concern (RIFM, 2013a). The mutagenicity of hexen-2-al was assessed in an Ames study conducted in compliance with GLP regulations and in accordance with OECD TG 471 using both the standard plate incorporation and modified preincubation methods (RIFM, 2007). No substantial increase in revertant colony number of any of the 5 tester strains was observed following treatment with *trans*-2-hexenal at any concentration level, neither in the presence nor absence of metabolic activation in experiment I. However, a minor but dose-dependent increase in revertant colony numbers was observed in the more sensitive preincubation assay (experiment II) in strain TA100 without metabolic activation. The required threshold of 2 times the number of the corresponding control was slightly exceeded at 100 µg/plate, factor of 2.1). At higher concentrations, the number of revertants was reduced, apparently due to overlapping toxic effects. These effects were verified in an additional preincubation test beginning at 50 µg/plate. At 100 µg/plate, the threshold was again exceeded. Although the increase observed in this second experiment is not really substantial, a possible mutagenic potential of the test item cannot be excluded. Based on the criteria of the assay, hexen-2-al is considered mutagenic in the Ames assay.

On the basis of the *in vitro* bacterial reverse mutation assay results reported above for hex-2(*trans*)-enal, EFSA concluded it was most appropriate to probe its genotoxic potential using a MutaMouse (lacZ/

GalE) assay with an *in vivo* micronucleus component included. The assay was carried out in transgenic mice (RIFM, 2013a). Micronuclei were measured in peripheral blood, and in the mutation arm of the experiment, the liver and the duodenum were chosen as the most appropriate tissues in order to address the potential for mutation at the site of most significant metabolism and at the site of the first contact, respectively. Therefore, groups of MutaMouse CD2-lacZ80/HazfBR mice were administered hex-2(*trans*)-enal via gavage, and the liver, duodenum, and peripheral blood were analyzed for the potential induction of DNA damage in a GLP study performed according to OECD Guidelines 474 and 488. Groups of 6 male MutaMouse mice were treated daily by oral gavage with hex-2(*trans*)-enal at doses of 120, 235, and 350 mg/kg bw/day, including a vehicle control (corn oil) for 28 days with a 3-day recovery period prior to sacrifice. Concurrent positive control animals were not included in this study. Tissue-matched positive control DNA was included in all packaging reactions in order to confirm correct assay functioning. The positive control DNA originated from animals dosed with ethylnitrosourea. All individual packaging reactions resulted in at least 30000 plaque-forming units (PFU) and at least 1 mutant plaque. For all animals, data were generated for at least 200000 PFU per tissue from at least 3 independent packaging reactions. At least 1 million PFU were obtained per group, per tissue, from a minimum of 5 animals. No significant increases in mutation frequency (MF) or significant dose-related trends were observed in the liver or the duodenum. Some of the hex-2(*trans*)-enal treatment groups showed duodenum MF that exceeded historical laboratory controls but were comparable to concurrent vehicle control values (EFSA, 2014). In conclusion, EFSA noted that, overall, the available experimental data from animals and humans, while not showing induction of gene mutations, do not allow assessment of the potential clastogenic activity of hex-2(*trans*)-enal at the first site of contact and the liver where higher levels of DNA adducts were observed than in other tissues investigated. They, therefore, confirmed the need for an *in vivo* comet assay to be conducted in the duodenum and liver for hex-2(*trans*)-enal (EFSA, 2014).

trans-2-Hexenal was tested in male Han Wistar rats (6/dose) for its potential to induce micronuclei (MN) in the polychromatic erythrocytes (PCE) of the bone marrow and/or to induce DNA damage in the liver and duodenum using the comet assay (RIFM, 2017). In the micronucleus assay, the proportion of PCE was assessed in at least 500 total erythrocytes per animal, and micronucleated (MN) cells were scored in at least 4000 PCE per animal (2000 PCE per slide in duplicate slides). The group mean %PCE in animals treated with *trans*-2-hexenal at the low and middle doses were similar to the concurrent vehicle control group and within the laboratory's historical vehicle control data, indicating no evidence of test article-related bone marrow toxicity. In 3 different comet studies, liver and duodenum tissues were collected and processed for comet analysis in the first and second experiments and histopathology in the first experiment. Only liver tissue was processed for comet analysis in the third experiment and included hOGG1 modification. Histopathology was evaluated only for liver tissue in the second experiment, and no histopathology was performed in the third. There were no test substance-related macroscopic or microscopic findings. In clinical chemistry also there were no treatment-related findings. There were no statistically significant increases in tail intensity at any dose level of *trans*-2-hexenal, compared to the concurrent vehicle control, in the first or second experiments. The group mean %tail intensity and tail moment values for all groups of animals treated with *trans*-2-hexenal were smaller than the group mean of the vehicle control. In the third experiment, there was little or no variability between animals or between slides, as previously seen, either with or without hOGG1 modification. Both with and without the hOGG1 modification, animals treated with *trans*-2-hexenal at all doses had tail intensities and tail moments similar to the concurrent vehicle control group that also fell within the laboratory's historical vehicle control range. There were no statistically significant increases in tail intensity for any of the test groups compared to the concurrent vehicle control, indicating that

trans-2-hexenal did not induce oxidative DNA damage in the liver (RIFM, 2017).

Based on the data available, trans-2-Hexenal does or does not present a concern for genetic toxicity.

Additional References: Florin et al., 1980; Marnett et al., 1985; Griffin and Segall, 1986; Canonero et al., 1990; Kato et al., 1989; Eder et al., 1992; Eder et al., 1993; Eisenbrand et al., 1995; Dittberner et al., 1995; Dittberner et al., 1997; Golzer et al., 1996; Janzowski et al., 2000; Eder and Schuler, 2000; Glaab et al., 2001; Eder and Deininger, 2002; Stout et al., 2003; Stout et al., 2005; Stout et al., 2008; RIFM, 2010; RIFM, 2013b.

Literature Search and Risk Assessment Completed On: 03/12/21.

11.1.2. Repeated dose toxicity

The MOE for hexen-2-al is sufficient for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on hexen-2-al. In a non-GLP and non-guideline subchronic study, 15 CFE rats/sex/dose were fed diets containing 0, 260, 640, 1600, or 4000 ppm hexen-2-al for 13 weeks (equivalent to 0, 13, 32, 80, or 200 mg/kg/day, respectively). No treatment-related mortality was reported for any dose group. No treatment-related changes in food consumption, body weight parameter, hematology, clinical chemistry, organ weights, and histopathology were reported. There was a slight increase in male urine volume with a concurrent decrease in the specific gravity of urine at the highest dose, but there were no alterations in kidney weight or histopathology. In the high-dose group females, ovary weight was significantly increased but without any correlating histopathological changes. Hence, these effects were not considered to be treatment-related adverse effects. Based on the lack of any treatment-related adverse effects at the highest tested dose, the NOAEL for repeated dose toxicity was considered to be 4000 ppm or 200 mg/kg/day (Gaunt et al., 1971). **Therefore, the hexen-2-al MOE can be calculated by dividing the hexen-2-al NOAEL in mg/kg/day by the total systemic exposure to hexen-2-al, 200/0.00013, or 1818182.**

In addition, the total systemic exposure to hexen-2-al (0.13 µg/kg/day) is below the TTC (30 µg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Derivation of reference dose (RfD):

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020c) and a reference dose of 2 mg/kg/day.

The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The reference dose for hexen-2-al was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 200 mg/kg/day by the uncertainty factor, 100 = 2 mg/kg/day.

See Table 1 below for additional studies.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/16/21.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on hexen-2-al or on any read-across materials. The total systemic exposure to hexen-2-al is

below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on hexen-2-al or on any read-across materials. The total systemic exposure to hexen-2-al (0.13 µg/kg bw/day) is below the TTC (30 µg/kg bw/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: Gaunt et al., 1971.

Literature Search and Risk Assessment Completed On: 03/03/21.

11.1.4. Skin sensitization

Based on the existing data, hexen-2-al is considered to be a skin sensitizer with a defined NESIL of 18 µg/cm².

11.1.4.1. Risk assessment. Based on the existing data, hexen-2-al is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD Toolbox v4.2). Hexen-2-al was found to be positive in the *in vitro* Direct Peptide Reactivity Assay (DPRA), KeratinoSens, and human Cell Line Activation Test (h-CLAT) but negative in the U937-CD86 test (Natsch et al., 2007; Natsch and Gfeller, 2008; Natsch et al., 2013; Urbisch et al., 2015). In 2 local lymph node assays (LLNA) with hexen-2-al, the weighted mean EC3 value was 1012 µg/cm² (RIFM, 2005a; Gerberick et al., 2005). In a human maximization test conducted on 25 subjects, no reactions indicative of sensitization were observed with 4% (2760 µg/cm²) hexen-2-al (RIFM, 1973). In a confirmation of no induction in humans test (CNIH) with 236 µg/cm² hexen-2-al in EtOH:DEP (3:1), sensitization effects were observed in 6/25 subjects (RIFM, 1990). However in CNIHs with 38, 37, and 106 subjects, 23 µg/cm² hexen-2-al did not induce sensitization reactions (RIFM, 1990; RIFM, 1989; RIFM, 2005b). In a CNIH with 109 subjects, no reactions indicative of sensitization were observed with 18 µg/cm² hexen-2-al (RIFM, 2020b).

Based on the weight of evidence from structural analysis and animal and human studies, hexen-2-al is a sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 18 µg/cm² (see Table 2 below). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2)

Table 2

Data summary for Hexen-2-al.

LLNA Weighted Mean EC3 Value µg/cm ² (No. Studies)	Potency Classification Based on Animal Data ^a	Human Data			
		NOEL-CNIH (Induction) µg/cm ²	NOEL-HMT (Induction) µg/cm ²	LOEL ^b (Induction) µg/cm ²	WoE NESIL ^c µg/cm ²
1012 [2]	Moderate	18	2760	236	18

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans Test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

^a Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

^b Data derived from CNIH or HMT.

^c WoE NESIL limited to 2 significant figures.

Table 1

Available additional studies within inadequate study design for the treatment material.

Duration	Animals/Sex/Dose	GLP/Guidelines	Route	Doses	Adverse effects	NOAEL	Ref
28 days	5 male F344rats/dose	OECD 407	Oral gavage	0, 10, 30, 100 mg/kg/day	None	100	ECHA (2018)

described by Api et al. (RIFM, 2020c) and a reference dose of 2 mg/kg/day.

Additional References: RIFM, 1986; RIFM, 1985a; RIFM, 1988; RIFM, 1985b; Klecak (1985) McKim et al., 2010.

Literature Search and Risk Assessment Completed On: 02/26/21.

11.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorbance spectra, hexen-2-al does not present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for hexen-2-al in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of significant absorbance in the critical range, hexen-2-al does not present a concern for phototoxicity or photoallergenicity.

Key studies:

There are no predictive phototoxicity studies available for hexen-2-al in experimental models.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for hexen-2-al were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficients (71, 0, 73 L mol⁻¹ • cm⁻¹, under neutral, acidic, and basic conditions, respectively) are 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: 03/02/21.

11.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to the lack of appropriate data. The exposure level for hexen-2-al is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on hexen-2-al. Based on the Creme RIFM Model, the inhalation exposure is 0.0035 mg/day. This exposure is 400 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: 03/12/2021.

2. Environmental Endpoint Summary:

11.2. Environmental Endpoint Summary

11.2.1. Screening-level assessment

A screening-level risk assessment of hexen-2-al 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, hexen-2-al 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 hexen-2-al as either 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 (US EPA, 2012a)). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current VoU (2015), hexen-2-al does not present a risk to the aquatic compartment in the screening-level assessment.

Key studies

11.2.2.1. Biodegradation. For CAS # 6728-26-3.

RIFM, 2002a: The ready biodegradability of the test material was evaluated by the Manometric respirometry test according to the OECD 301 F method. At 100 mg/L, 71% biodegradation was observed on day 28 and 73% on day 36.

RIFM, 2001: The purpose of this study was to assess the ready biodegradability of the test substance in an aerobic aqueous medium using a closed bottle test, according to the OECD 301D method. The test material achieved 25%, 28%, 27%, and 32% degradation after 7, 14, 21, and 28 days, respectively.

Ecotoxicity

For CAS # 6728-26-3.

RIFM, 2000: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 method under static conditions. Under the conditions of this study, the 48-h EC50 value based on measured concentration was reported to be 4.1 mg/L.

Other available data: Hexen-2-al has been registered for REACH with no additional data available at this time.

11.2.3. Risk assessment refinement

Since hexen-2-al has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (<i>Daphnia</i>) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening-level (Tier 1)	197.6			1,000,000	0.1976	

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

Exposure	Europe (EU)	North America (NA)
Log K _{ow} used	1.8	1.8
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	1–10	1–10
Risk Characterization: PEC/PNEC	<1	<1

*Combined Regional Volumes of Use for all CAS numbers.

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

The RIFM PNEC is 0.1976 µg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level.

Literature Search and Risk Assessment Completed On: 03/09/21.

12. 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>
- **National Library of Medicine's Toxicology Information Services:** <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/opthpv/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 04/21/21.

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.
- Canonero, R., Martelli, A., Marinari, U.M., Brambilla, G., 1990. Mutation induction in Chinese hamster lung V79 cells by five alk-2-enals produced by lipid peroxidation. *Mutat. Res. Lett.* 224 (2), 153–156.
- 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.
- Dittberner, U., Eisenbrand, G., Zankl, H., 1995. Genotoxic effects of the alpha,beta-unsaturated aldehydes 2-trans-butenal, 2-trans-hexenal and 2-trans, 6-cis-nonadienal. *Mutat. Res. Environ. Mutagen Relat. Subj.* 335 (3), 259–265.
- Dittberner, U., Schmetzer, B., Golzer, P., Eisenbrand, G., Zankl, H., 1997. Genotoxic effects of 2-trans-hexenal in human buccal mucosa cells in vivo. *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 390 (1–2), 161–165.
- ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. <http://echa.europa.eu/>.
- ECHA, 2018. Registration Dossier *Trans-hex-2-enal*. <https://echa.europa.eu/registration-dossier/-/registered-dossier/25168>.
- Eder, E., Deininger, C., 2002. The influence of the solvents DMSO and ethanol on the genotoxicity of alpha,beta-unsaturated aldehydes in the SOS chromotest. *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 516 (1–2), 81–89.
- Eder, E., Schuler, D., 2000. An approach to cancer risk assessment for the food constituent 2-hexenal on the basis of 1,N(2)-propanodeoxyguanosine adducts of 2-hexenal in vivo. *Arch. Toxicol.* 74 (10), 642–648.
- Eder, E., Deininger, C., Neudecker, T., Deininger, D., 1992. Mutagenicity of beta-alkyl substituted acrolein congeners in the Salmonella typhimurium strain TA100 and genotoxicity testing in the SOS chromotest. *Environ. Mol. Mutagen.* 19, 338–345.
- Eder, E., Scheckenbach, S., Deininger, C., Hoffman, C., 1993. The possible role of alpha, beta-unsaturated carbonyl compounds in mutagenesis and carcinogenesis. *Toxicol. Lett.* 67 (1–3), 87–103.
- EFSA, 2014. Scientific opinion on flavouring group evaluation 200 (FGE.200): 74 α,β-unsaturated aldehydes and precursors from subgroup 1.1.1 of FGE.19. *EFSA J.* 12 (6), 3709, 2014. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3709>.
- Eisenbrand, G., Schuhmacher, J., Golzer, P., 1995. The influence of glutathione and detoxifying enzymes on DNA damage induced by 2-alkenals in primary rat hepatocytes and human lymphoblastoid cells. *Chem. Res. Toxicol.* 8 (1), 40–46.
- Florin, I., Rutberg, L., Curvall, M., Enzell, C.R., 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames Test. *Toxicology* 18 (3), 219–232.
- Gaunt, I.F., Colley, J., Wright, M., Creasey, M., Grasso, P., Gangolli, S.D., 1971. Acute and short-term toxicity studies on trans-2-hexenal. *Food Chem. Toxicol.* 9 (6), 775–786.
- Gerberick, G.F., Ryan, C.A., Kern, P.S., Schlatter, H., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A., 2005. Compilation of historical local lymph node

- data for evaluation of skin sensitization alternative methods. *Dermatitis* 16 (4), 157–202.
- Glaab, V., Collins, A.R., Eisenbrand, G., Janowski, C., 2001. DNA-damaging potential and glutathione depletion of 2-cyclohexene-1-one in mammalian cells, compared to food relevant 2-alkenals. *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 497 (1–2), 185–197.
- Golzer, P., Janzowski, C., Pool-Zobel, B.L., Eisenbrand, G., 1996. (E)-2-Hexenal-induced DNA damage and formation of cyclic 1,N(2)-(1,3-propano)-2'-deoxyguanosine adducts in mammalian cells. *Chem. Rev. Toxicol.* 9 (7), 1207–1213.
- Griffin, D.S., Segall, H.J., 1986. Genotoxicity and cytotoxicity of selected pyrrolizidine alkaloids, a possible alkenal metabolite of the alkaloids, and related alkenals. *Toxicol. Appl. Pharmacol.* 86 (2), 227–234.
- 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.
- Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., Eisenbrand, G., 2000. 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem. Toxicol.* 38 (9), 801–809.
- Kato, F., Araki, A., Nozaki, K., Matsushima, T., 1989. Mutagenicity of aldehydes and diketones. *Mutat. Res. Environ. Mutagen Relat. Subj.* 216, 366–367.
- Klecak, G., 1985. The Freund's complete adjuvant test and the open epicutaneous test. *Curr. Probl. Dermatol.* 14, 152–171.
- Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. *Food Chem. Toxicol.* 45 (12), 2533–2562.
- Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. *Regul. Toxicol. Pharmacol.* 62 (1), 160–182.
- Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N., 1985. Naturally occurring carbonyl compounds are mutagens in salmonella tester strain TA104. *Mutat. Res. Fund Mol. Mech. Mutagen* 148 (1–2), 25–34.
- McKim Jr., J.M., Keller III, D.J., Gorski, J.R., 2010. A new in vitro method for identifying chemical sensitizers combining peptide binding with ARE/EpRE-mediated gene expression in human skin cells. *Cutan. Ocul. Toxicol.* 29 (3), 171–192.
- Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A., Basketter, D., 2020. Fragrance Skin Sensitization Evaluation and Human Testing. *Dermatitis*: November 16, 2020. Volume Publish Ahead of Print Issue. <https://doi.org/10.1097/DER.0000000000000684>.
- Natsch, A., Gfeller, H., 2008. LC-MS-Based characterization of the peptide reactivity of chemicals to improve the in vitro prediction of the skin sensitization potential. *Toxicol. Sci.* 106 (2), 464–478.
- Natsch, A., Gfeller, H., Rothaupt, M., Ellis, G., 2007. Utility and limitations of a peptide reactivity assay to predict fragrance allergens in vitro. *Toxicol. Vitro* 21 (7), 1220–1226.
- Natsch, A., Ryan, C.A., Foertsch, L., Emter, R., Jaworska, J., Gerberick, F., Kern, P., 2013. A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. *J. Appl. Toxicol.* 33 (11), 1337–1352.
- RIFM (Research Institute for Fragrance Materials, Inc), 1973. Report on Human Maximization Studies. Report to RIFM. RIFM Report Number 1802. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1985a. Closed Epicutaneous Test of Methyl-2-Octynoate, Methyl-2-Nonynoate, Benzyl Acetate, Trans,trans-2,4-Hexadienal, 2-hexylidene Cyclopentanone, Hexen-2-Al, Trans-2-hexenal Diethyl Acetal and Isoeugenol in guinea Pigs. Report to RIFM. RIFM Report Number 4474. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1985b. Open and Closed Epicutaneous and Maximization Tests of Fragrance Materials in guinea Pigs. Unpublished Report from Givaudan Corporation. RIFM Report Number 6068 (RIFM, Woodcliff Lake, NJ, USA.).
- RIFM (Research Institute for Fragrance Materials, Inc), 1986. Delayed Contact Hypersensitivity Study of Trans-2-hexenal in guinea Pigs. Report to RIFM. RIFM Report Number 4468. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1988. Delayed Contact Hypersensitivity Study of Hexen-2-Al in guinea Pigs. Report to RIFM. RIFM Report Number 8228. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1989. Repeated Insult Patch Test of T-2-Hexenal in Human Subjects. Report to RIFM. RIFM Report Number 27821. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 1990. Repeated Insult Patch Test of Methyl Octine Carbonate and T-2-Hexenal in Human Subjects. Report to RIFM. RIFM Report Number 27822. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2000. Hexen-2-al (Hexenal Trans-2 nat.): Acute Immobilisation Test (48 H) to Daphnia Magna Straus. Unpublished Report from Symrise. RIFM Report Number 61864. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2002a. Ready Biodegradability (2 nat.): Ready Biodegradability Closed Bottle Test. Unpublished Report from Symrise. RIFM Report Number 61863. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2002b. Ready Biodegradability of Hexen-2-Al (Hexenal-2-trans). Unpublished Report from Givaudan. RIFM Report Number 56016 (RIFM, Woodcliff Lake, NJ, USA.).
- RIFM (Research Institute for Fragrance Materials, Inc), 2002b. Partition Coefficient N-Octanol/water of Hexen-2-Al (Hexenal-2-trans). Unpublished Report from Givaudan. RIFM Report Number 56017 (RIFM, Woodcliff Lake, NJ, USA.).
- RIFM (Research Institute for Fragrance Materials, Inc), 2005a. Hexen-2-al Diluted with Vehicle 1:3 EtOH:DEP: Local Lymph Node Assay. RIFM Report Number 48756. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2005b. Repeated Insult Patch Test with Hexen-2-Al. RIFM Report Number 49111. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2007. Salmonella typhimurium Reverse Mutation with Hexen-2-Al. RIFM Report Number 54280. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2010. Genotoxicity Tests Conducted on a Group of Structurally Related Aldehydes. RIFM Report Number 58568. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2013a. Report on the Testing of Hexen-2-Al in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM Report Number 65462. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2013b. 2-Hexenal (Trans-2-hexenal): Induction of lacZ- Mutations in the Liver and Duodenum of Treated MutaTM Mice. Unpublished Report from Beevers, C. RIFM Report Number 73550. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2017. 2-Hexenal (Trans-2-hexenal): Rat Micronucleus and Alkaline Comet Assay. Unpublished Report from Keig-Shevelin, Z. RIFM Report Number 73551. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2020a. Exposure Survey 26, January 2020.
- RIFM (Research Institute for Fragrance Materials, Inc), 2020b. Hexen-2-al: Repeated Insult Patch Test (RIPT). RIFM Report Number 76090. RIFM, Woodcliff Lake, NJ, USA.
- RIFM (Research Institute for Fragrance Materials, Inc), 2020c. Updating Exposure Assessment for Skin Sensitization Quantitative Risk Assessment for Fragrance Materials. RIFM Report Number 76775. 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.
- 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.
- Stout, M., Nakamura, J., Boysen, G., Powley, M., Swenberg, J., 2005. Correlation of hexenal-derived DNA binding with detoxification and DNA repair status in cultured cells. *Toxicologist* 84 (S-1), 107.
- Stout, M.D., Bodes, E., Schoonhoven, R., Upton, P.B., Travlos, G.S., Swenberg, J.A., 2008. Toxicity, DNA binding, and cell proliferation in male F344 rats following short-term gavage exposures to trans-2-hexenal. *Toxicol. Pathol.* 36 (2), 232–246.
- Stout, M.D., Sangaiah, R., Koc, H., Swenberg, J.A., 2003. LC-ESI-MS/MS Quantitation of hexenal-derived 1, N(2)-propanodeoxyguanosine adducts. *Toxicologist* 72 (S-1), 248.
- Urbisch, D., Mehling, A., Guth, K., Ramirez, T., Honarvar, N., et al., 2015. Assessing skin sensitization hazard in mice and men using non-animal test methods. *Regul. Toxicol. Pharmacol.* 71 (2), 337–351.
- US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
- US EPA, 2012b. The ECOSAR (ECOLOGICAL Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.