Food and Chemical Toxicology 163 (2022) 112925

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

RIFM fragrance ingredient safety assessment, nopol, CAS Registry Number 128-50-7

A.M. Api [a], D. Belsito [b], D. Botelho [a], M. Bruze [c], G.A. Burton Jr. [d], M.A. Cancellieri [a], H. Chon [a], M.L. Dagli [e], M. Date [a], W. Dekant [f], C. Deodhar [a], A.D. Fryer [g], L. Jones [a], K. Joshi [a], M. Kumar [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], D.C. Liebler [h], H. Moustakas [a], M. Na [a], T.M. Penning [i], G. Ritacco [a], J. Romine [a], N. Sadekar [a], T.W. Schultz [j], D. Selechnik [a], F. Siddiqi [a], I.G. Sipes [k], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [l]

[a] *Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel for Fragrance Safety, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel for Fragrance Safety, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member Expert Panel for Fragrance Safety, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member Expert Panel for Fragrance Safety, 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*
[f] *Member Expert Panel for Fragrance Safety, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[g] *Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[h] *Member Expert Panel for Fragrance Safety, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[i] *Member of Expert Panel for Fragrance Safety, 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*
[j] *Member Expert Panel for Fragrance Safety, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN 37996- 4500, USA*
[k] *Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[l] *Member Expert Panel for Fragrance Safety, 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*

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 111721. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrancematerialsafetyresource.elsevier.com.

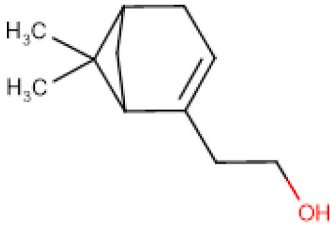
(*continued on next page*)

* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

https://doi.org/10.1016/j.fct.2022.112925
Received 18 November 2021; Accepted 13 March 2022
Available online 16 March 2022
0278-6915/© 2022 Elsevier Ltd. All rights reserved.

(*continued*)

Name: Nopol
CAS Registry Number: 128-50-7
Additional CAS Numbers*: 35836-73-8 (1R)-Nopol (No Reported Use)
*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
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; Safford et al., 2015a, 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
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., 2015a), 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.
Nopol was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog myrtenol (CAS # 515-00-4) show that nopol is not expected to be genotoxic. Data from read-across analog (1R)-nopyl acetate (CAS # 35836-72-7) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints. Data from the target material and analog 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5) provide nopol a No Expected Sensitization Induction Level (NESIL) of 3800 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; nopol is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was completed using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material; exposure is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; nopol was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment
Genotoxicity: Not expected to be genotoxic. (RIFM, 2015c; RIFM, 2015b)
Repeated Dose Toxicity: NOAEL = 60 mg/kg/day.

(*continued on next page*)

(*continued*)

	(ECHA REACH Dossier: Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, 2-acetate, (1R,5S)-; ECHA, 2013b)
Reproductive Toxicity: NOAEL = 478.5 mg/kg/day.	(ECHA REACH Dossier: Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, 2-acetate, (1R,5S)-; ECHA, 2013b)
Skin Sensitization: NESIL = 3800 μg/cm^2.	RIFM (2005c)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	
Environmental Safety Assessment	
Hazard Assessment:	
Persistence: Critical Measured Value: 5% (OECD 301D) for CAS # 35836-73-8	(ECHA REACH Dossier: Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, 2-acetate, (1R,5S)-; ECHA, 2013b)
Bioaccumulation: Screening-level: 69.3 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 16.92 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 16.92 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.01692 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level	

1. Identification

Chemical Name: Nopol	**Chemical Name:** (1R)-Nopol
CAS Registry Number: 128-50-7	**CAS Registry Number:** 35836-73-8
Synonyms: Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-; 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol; Homomyrtenol; 2-Hydroxyethyl-6,6-dimethyl-bicyclo[3,1,1]-hept-2-ene; 2-Norpinene-2-ethanol,6,6-dimethyl-; 10-Hydroxymethylene-2-pinene; 2-ﾋﾄﾞﾛｷｼｴﾁﾙ-7,7-ｼﾞﾒﾁﾙ-ﾋﾞｼｸﾛ[3.1.1]ﾍﾌﾟﾄ-2-ｴﾝ; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl) ethanol; Nopol	**Synonyms:** (1R)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-ethanol
Molecular Formula: $C_{11}H_{18}O$	**Molecular Formula:** $C_{11}H_{18}O$
Molecular Weight: 166.26	**Molecular Weight:** 166.26
RIFM Number: 9178	**RIFM Number:** 7366
Stereochemistry: Two stereocenters and 4 possible stereoisomers.	**Stereochemistry:** Two stereocenters and 4 possible stereoisomers.

2. Physical data

1. **Boiling Point**: 230 °C (Fragrance Materials Association [FMA]), 250.35 °C (EPI Suite)	1. **Boiling Point**: Not available
2. **Flash Point:** >93 °C (Globally Harmonized System [GHS]), >200 °F; CC (FMA)	2. **Flash Point:** 107 °C (GHS)
3. **Log K_{OW}:** 3.29 (EPI Suite)	3. **Log K_{OW}:** Not available
4. **Melting Point**: 49.6 °C (EPI Suite)	4. **Melting Point**: Not available
5. **Water Solubility:** 318.1 mg/L (EPI Suite)	5. **Water Solubility:** Not available
6. **Specific Gravity:** 0.973 (FMA)	6. **Specific Gravity:** Not available
7. **Vapor Pressure:** 0.002 mm Hg at 20 °C (FMA), 0.00252 mm Hg at 20 °C (EPI Suite v4.0), 0.00474 mm Hg at 25 °C (EPI Suite)	7. **Vapor Pressure:** Not available
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	8. **UV Spectra:** Not available
9. **Appearance/Organoleptic:** Colorless, slightly viscous liquid with a mild woody camphoraceous odor	9. **Appearance/ Organoleptic:** Not available

3. Volume of use (Worldwide band)

1. 1–10 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.1.4)*

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.20% (RIFM, 2020b)
2. **Inhalation Exposure**:** 0.00080 mg/kg/day or 0.050 mg/day (RIFM, 2020b)
3. **Total Systemic Exposure***:** 0.010 mg/kg/day (RIFM, 2020b)

*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 fine fragrance, inhalation exposure, and total exposure.

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

***95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 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 (RIFM, 2015a; Safford et al., 2015a; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

1. **Dermal:** 80%
2. **Oral:** Assumed 100%
3. **Inhalation:** Assumed 100%

6. Computational toxicology evaluation

Cramer Classification: Class II, Intermediate (Expert Judgment).

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
II	III	I

*See the Appendix below for further details.

1. Analogs Selected:

a. **Genotoxicity:** Myrtenol (CAS # 515-00-4)
b. **Repeated Dose Toxicity:** (1R)-Nopyl acetate (CAS # 35836-72-7)
c. **Reproductive Toxicity:** (1R)-Nopyl acetate (CAS # 35836-72-7)
d. **Skin Sensitization:** 2,4,6-Trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

2. Read-across Justification: See Appendix below

7. Metabolism

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

7.1. Additional References

None.

8. Natural occurrence

Nopol 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

Nopol has been pre-registered for 11/30/10; no dossier available as of 12/23/20. Available for additional material (1R)-nopol (CAS # 35836-73-8); accessed 02/23/21 (ECHA, 2013a).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for nopol 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)	0.29
2	Products applied to the axillae	0.087
3	Products applied to the face/body using fingertips	0.76
4	Products related to fine fragrances	1.6
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.41
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.41
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.41
5D	Baby cream, oil, talc	0.14
6	Products with oral and lip exposure	0.96
7	Products applied to the hair with some hand contact	0.38
8	Products with significant ano-genital exposure (tampon)	0.14
9	Products with body and hand exposure, primarily rinse-off (bar soap)	1.9
10A	Household care products with mostly hand contact (hand dishwashing detergent)	2.7
10B	Aerosol air freshener	1.9
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.14
12	Other air care products not intended for direct skin contact, minimal, or insignificant transfer to skin	64

(continued on next column)

(continued)

Note: [a]Maximum 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 nopol, the basis was the reference dose of 0.60 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 3800 μg/cm^2.
[b]For 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; December 2019).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.4.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, nopol does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Nopol was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no studies assessing the mutagenic activity of nopol; however, read-across can be made to myrtenol (CAS # 515-00-4; see Section VI). The mutagenic activity of myrtenol has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with myrtenol in solvent dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. Increases in the mean number of revertant colonies were not observed at any tested dose in the presence or absence of metabolic activation (S9) (RIFM, 2015c). Under the conditions of the study, myrtenol was not mutagenic in the Ames test, and this can be extended to nopol.

There are no studies assessing the clastogenic activity of nopol; however, read-across can be made to myrtenol (CAS # 515-00-4; see Section VI). The clastogenic activity of myrtenol 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 myrtenol in solvent DMSO at concentrations up to 500 μg/mL in the presence and absence of S9 for 3 and 24 h. Myrtenol did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2015b). Under the conditions of the study, myrtenol was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to nopol.

Based on the data available, myrtenol does not present a concern for genotoxic potential, and this can be extended to nopol.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/27/21.

11.1.2. Repeated dose toxicity

The MOE for nopol 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 nopol. Read-across material (1R)-nopyl acetate (CAS # 35836-72-7; see Section VI) has sufficient repeated dose toxicity data. In an OECD/GLP 422 study, 3 groups of Sprague Dawley Crl: CD BR rats were administered via dietary admixture of test material, nopyl acetate at concentrations of 0, 1000, 3000, or 9000 ppm (equivalent to doses of 0, 56.5, 180.2, or 478.5 mg/kg/day) for up to 63 days (including 3 weeks of maturation phase, pairing, gestation, and early lactation for females). The dose range was determined in a preliminary dose range finding study. Groups of 3 Sprague Dawley Crl:CD® IGS BR rats/sex/dose were administered daily via dietary admixture at concentrations of 0, 1500, 7500, or 15000 ppm (equivalent to 0, 106, 490, or 952 mg/kg/day, respectively) nopyl acetate for 21 days. The dose range was determined based on reductions in bodyweight gains in the 7500 ppm and above treatment groups and changes in clinical chemistry parameters in 7500 ppm treated males and in 1500 ppm treated females. In the OECD 422 study, each dose group was subdivided into 2 phases: main phase (at 1000 and 3000 ppm: 10 rats/sex/dose; at 0 and 9000 ppm: 5 males and 10 females/dose) and toxicity phase (5 female/dose). A control group was treated with a basal laboratory diet with 2% corn oil. Two recovery groups (5 rats/sex/dose) were treated with 9000 ppm or basal laboratory diet alone for 42 consecutive days and then maintained without treatment for a further 14 days. The NOAEL for systemic toxicity was considered to be 3000 ppm or 180.2 mg/kg/day, based on a statistically significant decrease in bodyweight gains at the highest dose tested (ECHA, 2013b).

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study (ECHA, 2012). The safety factor has been approved by The Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 180.2/3, or 60 mg/kg/day.

Therefore, the nopol MOE for the repeated dose toxicity endpoint can be calculated by dividing the (1R)-nopyl acetate NOAEL in mg/kg/day by the total systemic exposure to nopol, 60/0.010, or 6000.

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 0.60 mg/kg/day.

Derivation of reference dose (RfD):

The RIFM Criteria Document (Api et al., 2015a) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The RfD for nopol was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 60 mg/kg/day by the uncertainty factor, 100 = 0.60 mg/kg/day.

*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: 01/06/21.

11.1.3. Reproductive toxicity

The MOE for nopol is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no developmental toxicity data on nopol. Read-across material, (1R)-nopyl acetate (CAS # 35836-72-7; see Section VI) has sufficient developmental toxicity data. In a GLP/OECD 422 study, 3 groups of Sprague Dawley Crl: CD BR rats were administered via dietary admixture of test material, nopyl acetate at concentrations of 0, 1000, 3000, or 9000 ppm (equivalent to doses of 0, 56.5, 180.2, or 478.5 mg/kg/day) for up to 63 days (including 3 weeks of maturation phase, pairing, gestation, and early lactation for females). The dose range was determined in a preliminary dose range finding study. Groups of 3 Sprague Dawley Crl:CD IGS BR rats/sex/dose were administered daily via dietary admixture at concentrations of 0, 1500, 7500, or 15000 ppm (equivalent to 0, 106, 490, or 952 mg/kg/day) nopyl acetate for 21 days. The dose range was determined based on reductions in bodyweight gains in the 7500 ppm and above treatment groups and changes in clinical chemistry parameters in 7500 ppm treated males and in 1500 ppm treated females. In the OECD 422 study, each dose group was subdivided into 2 phases: main phase (at 1000 and 3000 ppm: 10 rats/sex/dose; at 0 and 9000 ppm: 5 males and 10 females/dose) and toxicity phase (5 female/dose). A control group was treated with a basal laboratory diet with 2% corn oil. Two recovery groups (5 rats/sex/dose) were treated with 9000 ppm or basal laboratory diet alone for 42 consecutive days and then maintained without treatment for a further 14 days. The clinical condition of offspring, litter size and survival, sex ratio, and offspring body weight were assessed, and macroscopic pathology evaluations were conducted. No treatment-related significant effects were observed on offspring litter size, sex ratio, viability, growth, and development. Thus, the NOAEL for developmental toxicity was considered to be 9000 ppm or 478.5 mg/kg/day, the highest dose tested (ECHA, 2013b). **Therefore, the nopol MOE for the developmental toxicity endpoint can be calculated by dividing the (1R)-nopyl acetate NOAEL in mg/kg/day by the total systemic exposure to nopol, 478.5/0.010, or 47850.**

There are no fertility data on nopol. Read-across material, (1R)-nopyl acetate (CAS # 35836-72-7; see Section VI) has sufficient fertility data. In an OECD/GLP 422 study, 3 groups of Sprague Dawley Crl:CD BR rats were administered via dietary admixture of test material, nopyl acetate at concentrations of 0, 1000, 3000, or 9000 ppm (equivalent to doses of 0, 56.5, 180.2, or 478.5 mg/kg/day) for up to 63 days (including 3 weeks of maturation phase, pairing, gestation, and early lactation for females). Each dose group was subdivided into 2 phases: main phase (at 1000 and 3000 ppm: 10 rats/sex/dose; at 0 and 9000 ppm: 5 males and 10 females/dose) and toxicity phase (5 female/dose). A control group was treated with a basal laboratory diet with 2% corn oil. Two recovery groups (5 rats/sex/dose) were treated with 9000 ppm or basal laboratory diet alone for 42 consecutive days and then maintained without treatment for a further 14 days. No treatment-related effects in mating performance, fertility, and gestation lengths were observed up to the highest dose tested. Thus, the NOAEL for fertility was considered to be

Table 1
Data summary for 2,4,6-trimethyl-3-cyclohexene-1-methanol as read-across analog for nopol.

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
>6250 [1]	Weak	3897	NA	5000	3800

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.

9000 ppm or 478.5 mg/kg/day (ECHA, 2013b). **Therefore, the nopol MOE for the fertility endpoint can be calculated by dividing the (1R)-nopyl acetate NOAEL in mg/kg/day by the total systemic exposure to nopol, 478.5/0.010, or 47850.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/08/21.

11.1.4. Skin sensitization

Based on the existing data and read-across to 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5), nopol is considered a skin sensitizer with a defined NESIL of 3800 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available on nopol. Based on the existing data and read-across to 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5; see Section VI), nopol is considered a skin sensitizer. The chemical structure of these materials indicates that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). 2,4,6-trimethyl-3-cyclohexene-1-methanol was found to be negative in *in vitro* Direct Peptide Reactivity Assay (DPRA) and KeratinoSens, but positive in the h-CLAT (RIFM, 2014; RIFM, 2015d; RIFM, 2018). In a murine local lymph node assay (LLNA), nopol (CAS # 35836-73-8) was found to be sensitizing with an EC3 of 6000 μg/cm^2 (ECHA, 2013a). In another LLNA, the read-across material, 2,4,6-trimethyl-3-cyclohexene-1-methanol, was found to be non-sensitizing up to 25% (6250 μg/cm^2) (RIFM, 2005b). In a Buehler study, 60% 2,4,6-trimethyl-3-cyclohexene-1-methanol presented 7/10 reactions indicative of sensitization (RIFM, 1981). In a human maximization test, no sensitization reactions were observed when 8% or 5520 (μg/cm^2) nopol in petrolatum was used for induction and challenge (RIFM, 1976). In a Confirmation of No Induction in Humans test (CNIH) with 3897 μg/cm^2 of the read-across material, 2,4,6-trimethyl-3-cyclohexene-1-methanol in 1:3 EtOH:DEP, no reactions indicative of sensitization was observed in any of the 103 volunteers (RIFM, 2005c). In additional CNIHs with less than 100 subjects, 2,4,6-trimethyl-3-cyclohexene-1-methanol did induce sensitization reactions at 10% or (5000 μg/cm^2) (RIFM, 1982) but not at 5% (2500 μg/cm^2) (RIFM, 1983).

Based on the available data on read-across 2,4,6-trimethyl-3-cyclohexene-1-methanol, summarized in Table 1 below, nopol is considered to be a weak skin sensitizer with a defined NESIL of 3800 μg/cm^2.

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 0.60 mg/kg/day.

Additional References: RIFM, 2005a; ICCVAM, 2011; RIFM, 2009; Api et al., 2015b; Safford et al., 2015b.

Literature Search and Risk Assessment Completed On: 01/20/21.

11.1.5. Phototoxicity/photoallergenicity

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

11.1.5.1. Risk assessment. There are no phototoxicity studies available for nopol in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, nopol 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 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: 02/09/21.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for nopol 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 nopol. Based on the Creme RIFM Model, the inhalation exposure is 0.050 mg/day. This exposure is 9.4 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.

*As per Carthew et al. (2009), Cramer Class II materials default to Cramer Class III for the local respiratory toxicity endpoint.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/12/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of nopol 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, nopol was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified nopol 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., 2015a). 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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	16.92			1000000	0.01692	

EPI Suite v4.11).

11.2.2. Risk assessment

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

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. No data available.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. Additional material (1R)-nopol (CAS # 35836-73-8) has been registered for REACH, with the following additional data available at this time (ECHA, 2013a):

A ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D guideline. Biodegradation of 5% was observed after 28 days.

An acute fish (*Danio rerio*) toxicity test was conducted according to the OECD 203 guideline under semi-static conditions. The 96-h LC50 value based on nominal test concentration was reported to be 11.1 mg/L.

A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guideline under static conditions. The 48-h EC50 value based on nominal test concentration was reported to be 28.8 mg/L (95% CI: 22.3–38.2 mg/L).

A *Daphnia magna* reproduction test was conducted according to the OECD 211 guideline under semi-static conditions. The 21-day NOEC value based on time-weighted average vale was reported to be 0.4 mg/L.

An algae growth inhibition test was conducted according to the OECD 201 guideline under static conditions. The 72-h EC50 values based on mean measured concentration for growth rate and yield were reported to be 21.14 mg/L (95% CI: 19.3–23.1 mg/L) and 7.224 mg/L (95% CI: 5.9–8.8 mg/L), respectively.

11.2.3. Risk assessment refinement

Since nopol 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.29	3.29
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	1–10	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined Regional Volume of Use for both CAS #s.

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

The RIFM PNEC is 0.01692 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 01/27/21.

*11.3. Literature Search**

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **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 11/17/21.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.112925.

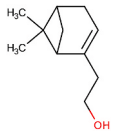
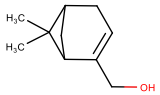
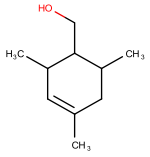
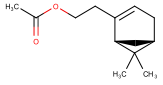
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017).

- 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, oncologic classification, ER binding, and repeat dose categorization predictions 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 material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

Principal Name	Nopol	Myrtenol	2,4,6-Trimethyl-3-cyclohexene-1-methanol	(1R)-Nopyl acetate
CAS No.	128-50-7	515-00-4	68527-77-5	35836-72-7
Structure				
Similarity (Tanimoto Score)		0.72	0.3	NA[1]
Read-across Endpoint		• Genotoxicity	• Skin Sensitization	• Repeated Dose Toxicity • Reproductive Toxicity
Molecular Formula	$C_{11}H_{18}O$	$C_{10}H_{16}O$	$C_{10}H_{18}O$	$C_{13}H_{20}O_2$
Molecular Weight	166.27	152.24	154.25	208.3
Melting Point (°C, EPI Suite)	49.60	38.73	13.51	54.2
Boiling Point (°C, EPI Suite)	250.35	232.42	237.00	259.16
Vapor Pressure (Pa @ 25 °C, EPI Suite)	0.632	1.82	0.996	1.19
Log K_{ow} (KOWWIN v1.68 in EPI Suite)	3.29	3.22	3.30	4.30
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	318.1	426.9	360.2	8.429
J_{max} (μg/cm^2/h, SAM)	94.515	43.314	234.963	0.926
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	9.36E-001	7.05E-001	1.60E+000	6.10E+001
Genotoxicity				
DNA Binding (OASIS V 1.4 QSAR Toolbox 4.2)	• No alert found	• No alert found		
DNA Binding by OECD QSAR Toolbox (4.2)	• No alert found	• No alert found		
Carcinogenicity (Genotox and Non-genotox) Alerts (ISS)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)		
DNA Alerts for Ames, MN, CA by OASIS V 1.1	• No alert found	• No alert found		
In Vitro Mutagenicity (Ames Test) Alerts by ISS	• No alert found	• No alert found		
In Vivo Mutagenicity (Micronucleus) Alerts by ISS	• No alert found	• No alert found		
Oncologic Classification	• Not classified	• Not classified		
Repeated Dose Toxicity				
Repeated Dose (HESS)	• Not categorized			• Not categorized

(*continued on next page*)

(*continued*)

Principal Name	Nopol	Myrtenol	2,4,6-Trimethyl-3-cyclohexene-1-methanol	(1R)-Nopyl acetate
Reproductive Toxicity				
ER Binding by OECD QSAR Tool Box (4.2)	• Non-binder, without OH, or NH2 group			• Non-binder, without OH, or NH2 group
Developmental Toxicity Model by CAESAR v2.1.6	• Toxicant (good reliability)			• Toxicant (good reliability)
Skin Sensitization				
Protein Binding by OASIS V1.1	• No alert found		• No alert found	
Protein Binding by OECD	• No alert found		• No alert found	
Protein Binding Potency	• Not possible to classify (GSH)		• Not possible to classify (GSH)	
Protein Binding Alerts for Skin Sensitization by OASIS V1.1	• No alert found		• No alert found	
Skin Sensitization Model (CAESAR) (Version 2.1.6)	• Sensitizer (good reliability)		• Sensitizer (good reliability)	
Metabolism				
OECD QSAR Toolbox (4.2) Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3	See Supplemental Data 4

Summary

There are insufficient toxicity data on the target material, nopol (CAS # 128-50-7). Hence *in silico* evaluation was conducted to determine a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, analogs myrtenol (CAS # 515-00-4), 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5), and (1R)-nopyl acetate (CAS # 35836-72-7) were identified as read-across materials with sufficient data for toxicological evaluation.

Metabolism

The metabolism of the target material was not evaluated in the risk assessment. Therefore, metabolism data were not reviewed, except when it may pertain in the read-across for specific endpoint sections above. Metabolism of the read-across material (1R)-nopyl acetate (CAS # 35836-72-7) was predicted using the rat liver S9 Metabolism Simulator (OECD QSAR Toolbox v4.2. Read-across analog (1R)-nopyl acetate (CAS # 35836-72-7) is predicted to be metabolized to target material nopol (CAS # 128-50-7) and acetic acid in the first step with 0.95 pre-calculated probability. Hence (1R)-nopyl acetate can be used as a read-across for target material. The target material was out of domain for the *in vivo* rat and *in vitro* rat S9 simulator (OASIS TIMES v2.27.19). However, based on expert judgment, the model's domain exclusion was overridden, and a justification is provided.

Conclusion

- Myrtenol (CAS # 515-00-4) was used as a read-across analog for the target material nopol (CAS # 128-50-7) for the genotoxicity endpoint.
  - The target material and the read-across analog belong to the structural class of primary cyclic unsaturated aliphatic alcohol.
  - The key difference between the target material and the read-across analog is that the target material is a substituted ethanol, whereas the read-across analog is a substituted methanol. This structure difference between the target material and the read-across analog does not affect consideration of the toxicity endpoint.
  - The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. Differences between the structures that affect the Tanimoto score do not affect consideration of the toxicity endpoint.
  - The physical−chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- 2,4,6-Trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5) was used as a read-across analog for the target material nopol (CAS # 128-50-7) for skin sensitization.
  - The target material and the read-across analog belong to the structural class of primary cyclic unsaturated aliphatic alcohol.
  - The key difference between the target material and the read-across analog is that the target material has a bicyclic ring structure that is not found in the read-across analog 2,4,6-trimethyl-3-cyclohexene-1-methanol. This structure difference between the target material and the read-across analog does not affect consideration of the toxicity endpoint.
  - The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. The Tanimoto score is 0.3 for 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5). This is due to the lack of bridge ring structure in the read-across analog as compared to the target material. Differences between the structures that affect the Tanimoto score do not affect consideration of the toxicity endpoint.
  - The physical−chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.

- The target material and the read-across analog material are predicted to be sensitizers by the CAESAR model for skin sensitization. There are no other protein binding alerts for both of the substances. The data described in the skin sensitization section shows that the read-across analog does not pose a concern for the skin sensitization endpoint. Therefore, this alert will be superseded by the availability of the data.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

o (1R)-nopyl acetate (CAS # 35836-72-7) was used as a read-across analog for the reproductive toxicity and repeated dose toxicity endpoints.

- The read-across analog, (1R)-nopyl acetate (CAS # 35836-72-7), is an ester formed from the target material nopol (CAS # 128-50-7).
- Structural differences between the target material and the read-across analog are mitigated by the fact that read-across analog (1R)-nopyl acetate (CAS # 35836-72-7) could be metabolically hydrolyzed to the target material nopol (CAS # 128-50-7). Therefore, the toxicity profile of the target is expected to be that of metabolites.
- The differences between the physical–chemical properties of the target material and the read-across analog can be mitigated by the fact that the target material is a direct metabolite of the read-across analog.
- According to the QSAR OECD Toolbox (v4.2), structural alerts for toxic endpoints are consistent between the target material and the read-across analog.
- The target material and the read-across analogs are predicted to be toxicants by the developmental toxicity model by CAESAR. There are no other reproductive toxicity alerts. The data described shows that the read-across analog does not pose a concern for the reproductive endpoint. Therefore, this alert will be superseded by the availability of the data. The structural alerts for toxic endpoints are consistent between the metabolites of the read-across analogs and the target material.

Explanation of Cramer Classification

Due to potential discrepancies between the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment, based on the Cramer Decision Tree.

Q1. A normal constituent of the body? **No**
Q2. Contains functional groups associated with enhanced toxicity? **No**
Q3. Contains elements other than C, H, O, N, and divalent S? **No**
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? **No**
Q6. Benzene derivative with certain substituents? **No**
Q7. Heterocyclic? **No**
Q16. Common terpene (see Cramer et al., 1978 for detailed explanation)? **No**
Q17. Readily hydrolyzed to a common terpene? **No**
Q19. Open chain? **No**
Q23. Aromatic? **No**
Q24. Monocarbocyclic with simple substituents? **No**
Q25. Cyclopropane, cyclobutane with substituents in Q24 or mono or bicyclic sulfide or mercaptan? **Yes, Class III (Class High)**

References

Api, A.M., Basketter, D., Lalko, J., 2015a. Correlation between experimental human and murine skin sensitization induction thresholds. Cutan. Ocul. Toxicol. 34 (4), 298–302.

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., 2015b. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of cramer classification between toxtree, the OECD QSAR Toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

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., 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.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food Chem. Toxicol. 16 (3), 255–276.

ECHA, 2012. Guidance on information requirements and chemical safety assessment, November 2012 v2.1. http://echa.europa.eu/.

ECHA, 2013a. (1R)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-ethanol registration dossier. Retrieved from. https://echa.europa.eu/en/registration-dossier/-/registered-dossier/5969/1/2.

ECHA, 2013b. Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, 2-acetate, (1R,5S)- registration dossier. Retrieved from. https://echa.europa.eu/registration-dossier/-/registered-dossier/5563.

ECHA, 2017. Read-across assessment framework (RAAF). Retrieved from. https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a.

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.

Interagency Coordinating Committee on the Validation of Alternative Methods, 2011. ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans (Unpublished.

Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A., Basketter, D., 2020. Fragrance Skin Sensitization Evaluation and Human Testing, Dermatitis. https://doi.org/10.1097/DER.0000000000000684. November 16, 2020. Volume Publish Ahead of Print Issue. Retrieved from.

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), 1976. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1796. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1981. Delayed Contact Hypersensitivity Study on 2,4,6-Trimethyl-3-Cyclohexene-1-Methanol (Isocyclogeraniol) in guinea Pigs. Unpublished report from IFF. RIFM report number 21789. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1982. Evaluation of Potential Irritation and Sensitization Hazards by Dermal Contact of 2,4,6-Trimethyl-3-Cyclohexene-1-Methanol (Isocyclogeraniol) in Humans. Unpublished report from International Flavors and Fragrances. RIFM report number 21790. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1983. Evaluation of Potential Hazards by Dermal Contact of 2,4,6-Trimethyl-3-Cyclohexene-1-Methanol (Isocyclogeraniol) in Humans. Unpublished report from International Flavors and Fragrances. RIFM report number 21792. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2005a. Potency Estimations for Several Fragrance Materials in the Local Lymph Node Assay and Their Correlation to Human Studies. RIFM report number 47900. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2005b. 2,4,6-Trimethyl-3-cyclohexene-1-methanol (Isocyclogeraniol) Diluted with Vehicle 1:3 EtOH:DEP: Local Lymph Node Assay. RIFM report number 48755. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2005c. Repeated Insult Patch Test with 2,4,6-Trimethyl-3-Cyclohexene-1-Methanol. RIFM report number 49110. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2009. The Use of Human Data when Conducting Dermal Sensitization Quantitative Risk Assessment for Fragrance Ingredients: How Well Does the LLNA Predict Human NOELs? RIFM Report Number 56491. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2013. Report on the Testing of Nopol in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM report number 65866. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2014. Fragrance Material in Vitro Sensitization: Direct Peptide Reactivity Assay (DPRA). RIFM report number 68623. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2015a. Novel Database for Exposure to Fragrance Ingredients in Cosmetics and Personal Care Products. RIFM report number 68681. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2015b. Myrtenol: in Vitro Micronucleus Assay in Human Peripheral Blood Lymphocytes. RIFM report number 68456. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2015c. Myrtenol: Bacterial Reverse Mutation Assay Plate Incorporation Method with a Confirmatory Assay. RIFM report number 68658. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2015d. Induction of Antioxidant-Response Element Dependent Gene Activity Cytotoxicity (Using MTT) in the Keratinocyte ARE- Reporter Cell Line Keratinosens. RIFM report number 69647. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2018. Non-animal Methods to Predict Skin Sensitization (I): the Cosmetics Europe Database. RIFM report number 73469. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2020a. Clustering a Chemical Inventory for Safety Assessment of Fragrance Ingredients: Identifying Read-Across Analogs to Address Data Gaps. RIFM report number 76272. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2020b. Exposure Survey 29, September 2020.
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.
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., 2015b. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015a. Extension of the dermal sensitization threshold (DST) approach to incorporate chemicals classified as reactive. Regul. Toxicol. Pharmacol. 72 (3), 694–701.
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, v2.0. United States Environmental Protection Agency, Washington, DC, USA.