RIFM fragrance ingredient safety assessment, dihydrocoumarin, CAS Registry Number 119-84-6

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

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

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 060421. 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.
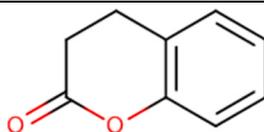
(continued on next column)

(continued)

Name: Dihydrocoumarin
CAS Registry Number: 119-84-6

Abbreviation/Definition List:

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

AF - Assessment Factor

BCF - Bioconcentration Factor

(continued on next page)

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

(*continued*)

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, 2017) compared to a deterministic aggregate approach

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

DRF - Dose Range Finding

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

ECOSAR - Ecological Structure-Activity Relationships Predictive Model

EU - Europe/European Union

GLP - Good Laboratory Practice

IFRA - The International Fragrance Association

LOEL - Lowest Observable Effect Level

MOE - Margin of Exposure

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

NA - North America

NESIL - No Expected Sensitization Induction Level

NOAEC - No Observed Adverse Effect Concentration

NOAEL - No Observed Adverse Effect Level

NOEC - No Observed Effect Concentration

NOEL - No Observed Effect Level

OECD - Organisation for Economic Co-operation and Development

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

PBT - Persistent, Bioaccumulative, and Toxic

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

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

QRA - Quantitative Risk Assessment

QSAR - Quantitative Structure-Activity Relationship

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

RfD - Reference Dose

RIFM - Research Institute for Fragrance Materials

RQ - Risk Quotient

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

TTC - Threshold of Toxicological Concern

UV/Vis spectra - Ultraviolet/Visible spectra

VCF - Volatile Compounds in Food

VoU - Volume of Use

vPvB - (very) Persistent, (very) Bioaccumulative

WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api, 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.

Dihydrocoumarin was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that dihydrocoumarin is not genotoxic. Data on dihydrocoumarin provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint. Data on read-across analog coumarin (CAS # 91-64-5) provide a calculated MOE >100 for the reproductive toxicity endpoint. Data provided dihydrocoumarin a No Expected Sensitization

(*continued*)

Induction Level (NESIL) of 490 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; dihydrocoumarin is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to dihydrocoumarin is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; dihydrocoumarin was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 1982; Heck, 1989; NTP, 1993)
Repeated Dose Toxicity: NOAEL = 150 mg/kg/day.	NTP (1993)
Reproductive Toxicity: Developmental Toxicity NOAEL = 150 mg/kg/day. Fertility NOAEL = 96 mg/kg/day.	(Roll, 1967; PreussUeberschar, 1984)
Skin Sensitization: NESIL = 490 μg/cm^2.	RIFM (2019)
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: 90% (OECD 301F)	(ECHA REACH Dossier: 3,4-Dihydrocoumarin; ECHA, 2018)
Bioaccumulation:	
Screening-level: 3.1 L/kg	(EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 1571 mg/L	(RIFM Framework; Salvito, 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, 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 1571 mg/L	(RIFM Framework; Salvito, 2002)
RIFM PNEC is: 1.571 μg/L	

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

1. Identification

1. **Chemical Name:** Dihydrocoumarin
2. **CAS Registry Number:** 119-84-6
3. **Synonyms:** 1,2-Benzodihydropyrone; 2H-1-Benzopyran-2-one, 3,4-dihydro-; 2-Chromanone; 3,4-Dihydro-2H-1-benzopyran-2-one; o-Hydroxydihydrocinnamic acid lactone; Melilotic acid lactone; Chroman-2-one; Dihydrocoumarin
4. **Molecular Formula:** $C_9H_8O_2$
5. **Molecular Weight:** 148.16
6. **RIFM Number:** 295
7. **Stereochemistry:** No isomer specified. No stereocenters.

2. Physical data

1. **Boiling Point:** 272 °C (Fragrance Materials Association [FMA]), 288.73 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System), >200 °F; CC (FMA)
3. **Log K_{OW}:** 0.97 (EPI Suite)
4. **Melting Point:** 22 °C (FMA), 39.96 °C (EPI Suite)
5. **Water Solubility:** 11540 mg/L (EPI Suite)
6. **Specific Gravity:** 1.188 (FMA)

7. **Vapor Pressure:** 0.00507 mm Hg at 20 °C (EPI Suite v4.0), 0.02 mm Hg at 20 °C (FMA), 0.00827 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Viscous, almost colorless to light, mobile liquid (at warm temperatures) with the powerful, sweet, herbaceous, nut-like odor of new-mown hay

3. Volume of use (Worldwide band)

1. **Volume of Use:** 1–10 metric tons per year (IFRA, 2015)

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

1. **95th Percentile Concentration in Hydroalcoholics:** 0.026% (RIFM, 2018)
2. **Inhalation Exposure*:** 0.000013 mg/kg/day or 0.00092 mg/day (RIFM, 2018)
3. **Total Systemic Exposure**:** 0.00074 mg/kg/day (RIFM, 2018)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class III, High

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

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** Coumarin (CAS # 91-64-5)
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

There are no metabolism data on dihydrocoumarin. Adams et al. (Adams, 1998) outline the metabolism pathway for dihydrocoumarin based on its structural analog, coumarin. Humans are expected to metabolize dihydrocoumarin to form the corresponding hydroxy acid (ortho-hydroxyphenylpropionic acid, o-HPPA). The acid may either be conjugated with glycine prior to excretion or β-oxidized and cleaved to yield o-hydroxybenzoic acid (see Fig. 1).

8. Natural occurrence

Dihydrocoumarin is reported to occur in the following foods by the VCF*:

Sweetgrass oil (*Hierochloe odorata*).

*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

Available; accessed 06/04/21 (ECHA, 2018).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for dihydrocoumarin 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)	0.038
2	Products applied to the axillae	0.011
3	Products applied to the face/body using fingertips	0.23
4	Products related to fine fragrances	0.21
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.053
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.053
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.053
5D	Baby cream, oil, talc	0.018
6	Products with oral and lip exposure	0.12
7	Products applied to the hair with some hand contact	0.43
8	Products with significant ano-genital exposure (tampon)	0.018
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.41
10A	Household care products with mostly hand contact (hand dishwashing detergent)	1.5
10B	Aerosol air freshener	1.5
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.018
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

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 dihydrocoumarin, the basis was the reference dose of 0.96 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 490 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).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.0.5.
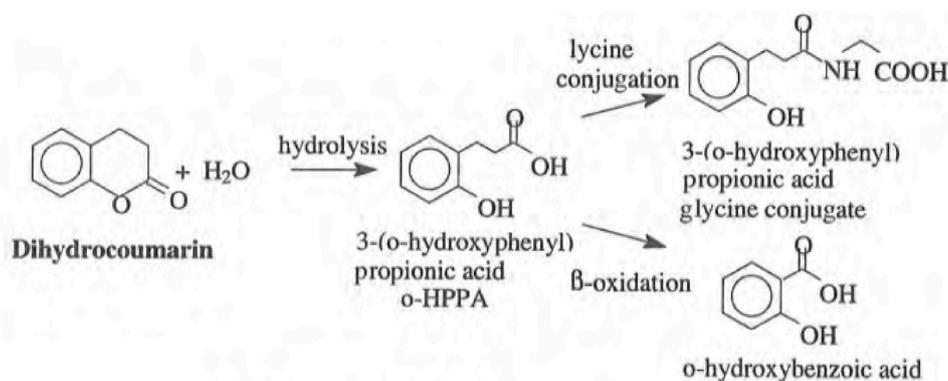
Fig. 1. Adapted from Adams (1998)..

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment.

The mutagenic activity of dihydrocoumarin has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and according to guidelines similar to OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with dihydrocoumarin in dimethyl sulfoxide (DMSO) at concentrations up to 150 μL/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (RIFM, 1982). Under the conditions of the study, dihydrocoumarin was not mutagenic in the Ames test. Additionally, a mammalian cell gene mutation assay (mouse lymphoma assay) was conducted on dihydrocoumarin. Mouse lymphoma cells were treated with dihydrocoumarin in DMSO at concentrations up to 2500 nL/mL for 4 h in the presence and absence of metabolic activation. Negative results were observed in the absence of metabolic activation. A fold induction of 4.1 was observed at the highest concentration assessed in the presence of metabolic activation (Heck, 1989). Despite the observed induction, statistical analysis was not carried out on the data, and the presence of a dose-response was not assessed. The authors noted that these findings should be viewed with caution considering the known effects of non-physiological medium conditions on the outcome of the MLA assay. Additionally, a positive response was only observed in the presence of S9, and there has been evidence that S9 degradation products may affect certain test systems such as mouse lymphoma and other *in vitro* cytogenetic assays. However, these effects are less likely to be observed in Ames and HGPRT test systems. The positive response observed could have resulted as a consequence of cytotoxicity (details not included in the study), because it was only observed at the highest dose concentration. Hence the increases in the mouse lymphoma study are not considered to be biologically relevant. Based on the negative response in a well-conducted bacterial reverse mutation test, dihydrocoumarin was not considered to be a concern for mutagenicity.

The clastogenicity of dihydrocoumarin was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster ovary cells were treated with dihydrocoumarin in DMSO at concentrations up to 1600 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any dose of the test material, either with or without S9 metabolic activation (NTP, 1993). Under the conditions of the study, dihydrocoumarin was considered to be non-clastogenic to mammalian cells. In cytogenetic tests with Chinese hamster ovary (CHO) cells, dihydrocoumarin (effective doses, 50–300 μg/mL) induced a dose-related increase in SCE in the absence of S9; with S9, a significant increase in SCE was observed only at the highest doses tested (1600 and 2000 μg/mL) in each of 2 trials. The response in the second trial with S9 was dose-related. In the second SCE trial with S9, cytotoxicity was apparent at the 2000 μg/mL dose level, and only 36 cells could be scored. In order to verify the results of *in vitro* studies, a 13-week *in vivo* micronucleus study was conducted. No induction of micronuclei was noted in peripheral blood erythrocyte samples obtained from male and female B6C3F1 mice at the end of the 13-week *in vivo* micronucleus study (NTP, 1993).

Based on the data available, dihydrocoumarin does not present a concern for genotoxic potential.

Additional References: Prival (1982); Edenharder (1995); Edenharder (1997); RIFM, 1986; RIFM, 1983; RIFM, 1984.

Literature Search and Risk Assessment Completed On: 06/01/21.

11.1.2. Repeated dose toxicity

The MOE for dihydrocoumarin is adequate 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 dihydrocoumarin. An NTP carcinogenicity study was conducted in male and female F344/N rats administered dihydrocoumarin by gavage (doses of 0, 150, 300, or 600 mg/kg/day) over a period of 2 years. There was a decrease in survival among treated male rats as compared to controls. This was considered to be due to a male rat species-specific nephropathy reported during the study that leads to decreases in body weight, nephropathy, parathyroid gland hyperplasia, renal tubular adenomas, and transition cell carcinomas among mid- and high-dose males. Hemoglobin concentrations, mean erythrocyte volumes, or mean erythrocyte hemoglobin concentrations in the top 2 dose group females were slightly but significantly lower than those of the controls, whereas in males, only hemoglobin concentrations were statistically significantly decreased as compared to controls. Alterations in clinical chemistry parameters included statistically significant increases in ALP, ALT, SDH, or GGT among mid- and high-dose males. High-dose females were reported to have statistically significant increases in ALP and GGT. Incidences of forestomach ulcers among treated males were significantly higher than those of controls. Incidences of focal hyperplasia and renal tubule adenomas were significantly increased in dosed male rats, which were also outside the historical control range of treatment facility. Treatment-related nephropathy was considered to be related to the strain of the rat and route of administrations. Forestomach ulcers reported among males were considered to be due to direct toxicity of dihydrocoumarin at the site of contact after long-term administration and possible alterations in the physiological state of administration due

to kidney disease and/or stress since no such effects were reported among animals during the 13-week study. Thus, the NOAEL was considered to be 150 mg/kg/day based on alterations in hematological and clinical chemistry parameters among higher dose group animals (NTP, 1993).

In another instance, in a 2-year carcinogenicity study, test material dihydrocoumarin was administered to groups of 70 B6C3F1 mice/sex/dose via gavage at doses of 0, 200, 400, or 800 mg/kg/day. At the end of 15 months, 5–10 animals from each group were euthanized to conduct hematological and clinical chemistry evaluations. At the end of the 2-year treatment, incidences of hepatocellular adenomas were significantly increased in female mice (29/50, 23/51, 36/51, and 31/50) in all dose groups. There was a marginal increase in the incidence of alveolar/bronchiolar adenomas in the low- (15/50) and mid-dose (15/50) group males, but it was not considered to be treatment-related since the incidence was slight and no increase was reported among high-dose males. Focal kidney hyperplasia and adenoma or carcinoma of the renal tubule were reported among males. With an extended evaluation of step sections, focal hyperplasia or renal tubule adenomas were identified among treated males. Since the incidences were not greater than the control and without any dose-response, these changes were not considered to be treatment-related. Thus, the NOAEL was considered to be 800 mg/kg/day, the highest dose tested.

Therefore, the most conservative NOAEL of 150 mg/kg/day from the 2-year rat study was considered for the current safety assessment.

Therefore, the dihydrocoumarin MOE for the repeated dose toxicity endpoint can be calculated by dividing the dihydrocoumarin NOAEL in mg/kg/day by the total systemic exposure to dihydrocoumarin, 150/0.00074, or 202703.

In addition, the total systemic exposure to dihydrocoumarin (0.74 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/20/21.

11.1.3. Reproductive toxicity

The MOE for dihydrocoumarin 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 dihydrocoumarin. Read-across material coumarin (CAS # 91-64-5; see Section VI) has sufficient developmental toxicity data that can be used to support the developmental toxicity endpoint. A dietary developmental toxicity study was conducted in pregnant NMRI mice in 2 phases (breeding and Caesarean section). In the breeding phase, groups of pregnant NMRI mice (31–39) were fed diets containing 0%, 0.05%, 0.1%, or 0.25% coumarin from post coitum (p.c.) days 6–17. At concentrations of 0.1% and 0.5%, only the descendants of the dams were examined. At the concentration of 0.25%, the testing was performed in a breeding test of the descendants of 3 treated generations up to the F2 generation (N = 39, 10, and 20 dams for the P, F1, and F2 generations, respectively). The 6.1% stillbirth rate in the 3 generations at 0.25% was significantly higher than that of the controls, while no significant variations were observed in the number of stillbirths between the controls and 0.1% and 0.05%. In the Caesarean section phase, groups of pregnant NMRI mice (26–30) were fed diets containing 0%, 0.05%, or 0.25% coumarin from p.c. days 6–17. The fetuses were delivered on day 18 or 19 p.c. by Caesarean section and examined microscopically for skeletal anomalies. Coumarin at 0.05% had no direct effect on embryonic and fetal development. At 0.25%, increased late resorptions (8.4% compared to 4.3% for controls) and the weights of the removed fetuses on day 18 or 19 p.c. were reduced. On day 18 p.c. at 0.25%, significantly more bony nuclei of the calcaneus were lacking. Similarly, there were

significant differences in the ossification of the talus. Although the lack of ossification should not be viewed as skeletal anomalies (the cartilaginous features were already present), the different development levels of the controls at 0.25% suggest a development inhibiting effect of coumarin, which was confirmed by the reduced fetal weights. Therefore, the NOAEL for developmental toxicity was considered to be 0.1% or 150 mg/kg/day, based on delays in the development of fetuses and increased stillbirths at 0.25% (Roll, 1967).

Additionally, no developmental toxicity was observed in studies with a mixture of coumarin and rutin conducted in rats, rabbits, or minipigs (Grote, 1971, 1973, 1977) and in a reproduction study with a mixture of coumarin and troxerutin conducted in rats (PreussUeberschar, 1984). **Therefore, the dihydrocoumarin MOE for the developmental toxicity endpoint can be calculated by dividing the coumarin NOAEL in mg/kg/day by the total systemic exposure to dihydrocoumarin, 150/0.00074, or 202703.**

There are insufficient fertility data on dihydrocoumarin. Read-across material coumarin (CAS # 91-64-5) has sufficient fertility data that can be used to support the fertility endpoint. An oral gavage multigeneration reproductive toxicity study was conducted in rats with Venalot (a mixture of 15 mg coumarin and 90 mg troxerutin). The 0 (control), 1-, 8-, 64-, and 128-fold of the daily therapeutic doses for humans were suspended in tap water and administered orally by gavage to groups of 23 male and 46 female Wistar rats. Males were pre-treated for 10 weeks, and females were pre-treated for 3 weeks. These treatments then continued during the mating phase (maximum 3 weeks). Half of the females were scheduled for Caesarean section and received the test material until the day of laparotomy (gestation day 20). The remaining females, those selected for littering, received treatment through lactation day 24 postpartum. From the littered offspring of the 0, 64-, and 128-fold groups, 34, 33, and 38 mating pairs were randomly chosen for continued breeding. No adverse reproductive effects (parental fertility, deformity rates in the fetuses, or postnatal developments of pups) were observed on either the treated P generation or the untreated F1 and F2 generations up to the highest dose of 128-fold of the daily therapeutic dose for humans or approximately 96–192 mg/kg/day of coumarin. The most conservative NOAEL for fertility was considered to be 96 mg/kg/day (PreussUeberschar, 1984). **Therefore, the dihydrocoumarin MOE for the fertility endpoint can be calculated by dividing the coumarin NOAEL in mg/kg/day by the total systemic exposure to dihydrocoumarin, 96/0.00074, or 129730.**

In addition, the total systemic exposure to dihydrocoumarin (0.74 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

11.1.4. 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, 2020) and a reference dose of 0.96 mg/kg/day.

The RIFM Criteria Document (Api, 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 dihydrocoumarin was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 96 mg/kg/day by the uncertainty factor, 100 = 0.96 mg/kg/day.

Additional References: NTP, 1993; Grote (1973); Grote (1977); Grote (1971); Carlton (1996).

Literature Search and Risk Assessment Completed On: 05/31/21.

11.1.5. Skin sensitization

Based on the existing data, dihydrocoumarin is considered to be a skin sensitizer with a defined NESIL of 490 μg/cm^2.

11.1.5.1. Risk assessment. Based on the existing data, dihydrocoumarin is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Dihydrocoumarin was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA) (Gerberick, 2004a; Natsch, 2007, 2013), and human cell line activation test (h-CLAT) (Sakaguchi, 2006; Nukada, 2011). However, it was found to be negative in KeratinoSens (Natsch, 2013) and U937-CD86 tests (Natsch, 2013; Piroird, 2015). In a murine local lymph node assay (LLNA), dihydrocoumarin was found to be non-sensitizing up to 10% (Kimber, 1989a). However, in 3 murine LLNAs, dihydrocoumarin was found to be sensitizing with an EC3 values of 5.6% (1400 µg/cm^2); 3.2% (812.5 µg/cm^2), and 4% (1000 µg/cm^2) (Gerberick, 2004b; RIFM, 2003; RIFM, 2012). In guinea pig maximization tests, dihydrocoumarin presented reactions indicative of sensitization at 20% (Guillot, 1983, 1985). In 2 guinea pig Open Epicutaneous Tests (OET), dihydrocoumarin presented reactions indicative of sensitization at 20% (Guillot, 1983, 1985; Klecak, 1985). In 3 human maximization tests, skin sensitization reactions were observed with dihydrocoumarin at 1% (690 µg/cm^2) and 20% (13800 µg/cm^2) (RIFM, 1977a; RIFM, 1975). However, in another human maximization test, no skin sensitization reactions were observed at 2% (1380 µg/cm^2) (RIFM, 1977b). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 2% (2000 µg/cm^2) dihydrocoumarin in alcohol SDA39C, no reactions indicative of sensitization were observed in any of the 49 volunteers (RIFM, 1978). In a CNIH with 490 µg/cm^2, dihydrocoumarin in 1:3 ethanol:diethyl phthalate, no reactions indicative of sensitization were observed in any of the 115 volunteers (RIFM, 2019).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, dihydrocoumarin is a sensitizer with a WoE NESIL of 490 µg/cm^2 (Table 1). 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, 2020) and a reference dose of 0.96 mg/kg/day.

Additional References: Kimber (1989b); RIFM, 1972; Kimber (1991); Klecak (1979); ECHA, 2018; NICNAS, 2016; Marzulli (1980a); Brulos (1977); Guillot (1983); Guillot (1985); Hausen (1986); Maisey (1986); Marzulli (1980b).

Literature Search and Risk Assessment Completed On: 05/26/21.

11.1.6. Phototoxicity/photoallergenicity

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

11.1.6.1. Risk assessment. There are no phototoxicity studies available for dihydrocoumarin 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, 2009). Based on the lack of significant absorbance in the critical range, dihydrocoumarin does not present a concern for phototoxicity or photoallergenicity.

11.1.7. UV spectra analysis

UV/Vis absorption spectra (OECD TG 101) for dihydrocoumarin were obtained. The spectra indicate minor 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, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/19/21.

11.1.8. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for dihydrocoumarin is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.8.1. Risk assessment. There are no inhalation data available on dihydrocoumarin. Based on the Creme RIFM Model, the inhalation exposure is 0.00092 mg/day. This exposure is 511 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/28/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of dihydrocoumarin was performed following the RIFM Environmental Framework (Salvito, 2002), which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K$_{OW}$ and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor, as discussed in Salvito et al. (2002). At Tier 2, the model ECOSAR (US EPA, 2012b) (providing chemical class-specific ecotoxicity estimates) is used, and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage, which is considered proprietary information, is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, dihydrocoumarin was identified as a fragrance material with no potential to present possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.1 did not identify dihydrocoumarin to be 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same criteria used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite models BIOWIN 2 or BIOWIN 6 < 0.5 and BIOWIN 3 < 2.2,

Table 1
Data summary for dihydrocoumarin.

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
1070 [3]	Moderate	NA	2000	690	490

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 (LLNA) using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to one significant figure.

then the material is considered to be potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF $\geq$2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. Should additional assessment be required, based on these model outputs (Step 1), a weight of evidence-based review is 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 v4.1). Data on biodegradation, fate, and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

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

11.2.2. Key studies

11.2.2.1. Biodegradation. RIFM, 1999: The biodegradability of the test material was evaluated using a closed bottle test following the OECD 301D guidelines. The test material was tested at an initial concentration of 3.2 mg/L. The test material was suspended in a mineral medium, inoculated with a mixed population of aquatic microorganisms, and incubated for 28 days under aerobic conditions in the dark at 20 ± 1 °C. Under the condition of this study, the test material showed degradation of 79% within 28 days.

11.2.2.2. Ecotoxicity. RIFM, 1999: *Daphnia magna* acute immobilization was evaluated according to the Directive 92/69/EEC C.2 method under static conditions. Under the condition of this study, the EC0 of the test material was greater or equal to 101 mg/L (arithmetic mean of analytical values).

11.2.2.3. Other available data. Dihydrocoumarin has been registered under REACH, and the following additional data is available (ECHA, 2018):

The ready biodegradability of the test material was evaluated in a manometric respirometry test according to the OECD 301F method. Biodegradation of 90% was observed after 28 days.

A *Daphnia magna* immobilization test was conducted according to the ASTM E729-80 method under static conditions. The 48-h EC50 was greater than 24.3 mg/L but less than 36.9 mg/L.

11.2.2.4. Risk assessment refinement. Since dihydrocoumarin 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, 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	0.97	0.97
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	Not reported	<1
Risk Characterization: PEC/PNEC	**N/A**	**<1**

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

The RIFM PNEC is 1.571 µg/L. The revised PEC/PNECs for EU (Not reported) and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 05/25/21.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** 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 06/04/21.

Conflicts of interest

The authors declare that they have no conflicts of interest.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>1571</u>			1000000	1.571	

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112557.

Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity, as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 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, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	Dihydrocoumarin	Coumarin
CAS No.	119-84-6	91-64-5
Structure		
Similarity (Tanimoto Score)		0.11
Read-across Endpoint		• Reproductive Toxicity
Molecular Formula	$C_9H_8O_2$	$C_9H_6O_2$
Molecular Weight	148.16	146.14
Melting Point (°C, EPI Suite)	25.00	71.00
Boiling Point (°C, EPI Suite)	272.00	301.70
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.10257	0.13066
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	0.97	1.39
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	3.00E+03	1.90E+03
J_{max} (µg/cm^2/h, SAM)	42.465	17.162
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	3.19E+00	1.01E-02
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH, or NH2 group	• Non-binder, without OH, or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (good reliability)	• Toxicant (experimental value)
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• See Supplemental Data 1	• See Supplemental Data 2
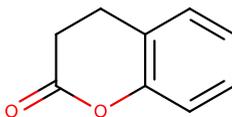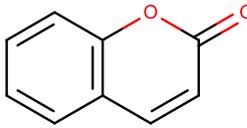
Summary

There are insufficient toxicity data on dihydrocoumarin (CAS # 119-84-6). Hence, *in silico* evaluation was conducted to determine a read-across analog for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, coumarin (CAS # 91-64-5) was

identified as a read-across analog with sufficient data for toxicological evaluation.
Conclusions

- Coumarin (CAS # 91-64-5) was used as a read-across analog for the target material dihydrocoumarin (CAS # 119-84-6) for the reproductive toxicity endpoint.
  o The target material and the read-across analog are structurally similar and belong to a class of aromatic δ-lactones.
  o The target material and the read-across analog share a 1-benzopyran-2-one moiety.
  o The key difference between the target material and the read-across analog is that the read-across analog has an extra unsaturation resulting in an α,β-unsaturated lactone. This structural difference is toxicologically insignificant.
  o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  o Both the target material and the read-across analog have a toxicant alert for Developmental Toxicity (CAESAR v2.1.6). The data described in the reproductive toxicity section shows that the MOE is adequate at the current level of use. The predictions are superseded by the data.
  o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

Adams, T.B., Greer, D.B., Doull, J., Munro, I.C., Newberne, P., Portoghese, P.S., Smith, R.L., Wagner, B.M., Weil, C.S., Woods, L.A., Ford, R.A., 1998. The FEMA GRAS assessment of lactones used as flavour ingredients. Food Chem. Toxicol. 36 (4), 249–278.

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.

Brulos, M.F., Guillot, J.P., Martini, M.C., Cotte, J., 1977. The influence of perfumes on the sensitizing potential of cosmetic bases. I. A technique for evaluating sensitising potential. J. Soc. Cosmet. Chem. 28, 357–365.

Carlton, B.D., Aubrun, J.-C., Simon, G.S., 1996. Effects of coumarin following perinatal and chronic exposure in Sprague-Dawley rats and CD-1 mice. Fund. Appl. Toxicol. 30, 145–151.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

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

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

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

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

ECHA, 2017. Read-across Assessment Framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

ECHA, 2018. 3,4-Dihydrocoumarin Registration Dossier. Retrieved from. https://echa.europa.eu/registration-dossier/-/registered-dossier/22066.

Edenharder, R., Tang, X., 1997. Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by flavonoids, coumarins, quinones and other phenolic compounds. Food Chem. Toxicol. 35 (3/4), 357–372.

Edenharder, R., Speth, C., Decker, M., Kolodziej, H., Kayser, O., Platt, K.L., 1995. Inhibition of mutagenesis of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) by coumarin and furanocoumarins, chromanones and furanochromanones. Mutat. Res. Genet. Toxicol. 345 (1–2), 57–71.

Gerberick, G.F., Ryan, C.A., Kern, P.S., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A., 2004a. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 50 (5), 274–288.

Gerberick, G.F., Vassallo, J.D., Bailey, R.E., Chaney, J.G., Morrall, S.W., Lepoittevin, J.-P., 2004b. Development of a peptide reactivity assay for screening contact allergens. Toxicol. Sci. 81 (2), 332–343.

Grote, V.W., Gunther, R., 1971. Test of a coumarin-rutin combination for teratogenicity by examination of fetal skeletons. Arzneimittel-Forschung [Drug Research] 21 (12), 2016–2022.

Grote, W., Weinmann, I., 1973. Examination of the active substances coumarin and rutin in a teratogenic trial with rabbits. Arzneimittel-Forschung [Drug Research] 23 (9), 1319–1320.

Grote, W., Schulz, L.C., Drommer, W., Uberschar, S., Schafer, E.A., 1977. Test of combination of the agents coumarin and troxerutin for embryotoxic and teratogenic side-effects in Gottingen miniature pigs. Arzneimittel-Forschung [Drug Research] 27 (3), 613–617.

Guillot, J.P., Gonnet, J.F., 1985. The epicutaneous maximization test. In: Current Problems in Dermatology, vol. 14, pp. 220–247.

Guillot, J.P., Gonnet, J.F., Clement, C., Faccini, J.M., 1983. Comparative study of methods chosen by the Association Francaise de Normalisation (AFNOR) for evaluating sensitizing potential in the albino Guinea-pig. Food Chem. Toxicol. 21 (6), 795–805.

Hausen, B.M., Kallweit, M., 1986. The sensitizing capacity of coumarins (II). Contact Dermatitis 15 (5), 289–294.

Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9 (1), 257.

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.

Kimber, I., Weisenberger, C., 1989. A murine local lymph node assay for the identification of contact allergens. Arch. Toxicol. 63 (4), 274–282.

Kimber, I., Weisenberger, C., 1991. Anamnestic responses to contact allergens: application in the murine local lymph node assay. J. Appl. Toxicol. 11 (2), 129–133.

Kimber, I., Hilton, J., Weisenberger, C., 1989. The Murine Local Lymph Node Assay for identification of contact allergens: a preliminary test of in situ measurement of lymphocyte proliferation. Contact Dermatitis 21, 215–220.

Klecak, G., 1979. The open epicutaneous test (OET), a predictive test procedure in the Guinea pig for estimation of allergenic properties of simple chemical compounds, their mixtures and of finished cosmetic preparations. International Federation Societies Cosmetic Chemists, 9/18/79.

Klecak, G., 1985. The freund's complete adjuvant test and the open epicutaneous test. In: Current Problems in Dermatology, vol. 14, pp. 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.

Maisey, J., Miller, K., 1986. Assessment of the ability of mice fed on vitamin A supplemented diet to respond to a variety of potential contact sensitizers. Contact Dermatitis 15 (1), 17–23.

Marzulli, F.N., Maibach, H.I., 1980a. Further studies of effects of vehicles and elicitation concentration in experimental contact sensitization testing in humans. Contact Dermatitis 6, 131–133.

Marzulli, F.N., Maibach, H.I., 1980b. Contact allergy: predictive testing of fragrance ingredients in humans by Draize and maximization methods. J. Environ. Pathol. Toxicol. 3 (5–6), 235–245.

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.

National Toxicology Program, 1993. Toxicology and Carcinogenesis Studies of 3,4-dihydrocoumarin in F344/N Rats and B6C3F1 Mice (Gavage Studies). NTP-TR-423. NIH Publication No, pp. 93–3154.

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.

NICNAS, 2016. Coumarins: Human Health Tier II Assessment. Retrieved from. https://www.nicnas.gov.au/_data/assets/pdf_file/0003/94224/Coumarins_Human-health-tier-II-assessment.pdf.

Nukada, Y., Ashikaga, T., Sakaguchi, H., Sono, S., Mugita, N., Hirota, M., Miyazawa, M., Ito, Y., Sasa, H., Nishiyama, N., 2011. Predictive performance for human skin sensitizing potential of the human cell line activation test (h-CLAT). Contact Dermatitis 65 (6), 343–353.

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. http://www.qsartoolbox.org/.

Piroird, C., Ovigne, J.-M., Rousset, F., Martinozzi-Teissier, S., Gomes, C., Cotovio, J., Alepee, N., 2015. The Myeloid U937 Skin Sensitization Test (U-SENS) addresses the activation of dendritic cell event in the adverse outcome pathway for skin sensitization. Toxicol. Vitro 29 (5), 901–916.

Preuss-Ueberschar, C., Ueberschar, S., Grote, W., 1984. Reproduction toxicological studies following oral administration of benzopyrone preparation in rats. Arzneimittel-Forschung [Drug Research] 34 (10), 1305–1313.

Prival, M.J., Sheldon Jr., A.T., Popkin, D., 1982. Evaluation, using Salmonella typhimurium, of the mutagenicity of seven chemicals found in cosmetics. Food Chem. Toxicol. 20, 427–432.

RIFM (Research Institute for Fragrance Materials, Inc), 1972. The Contact-Sensitization Potential of Fragrance Materials by Maximization Testing in Humans. RIFM, Woodcliff Lake, NJ, USA. Report to RIFM. RIFM report number 1804.

RIFM (Research Institute for Fragrance Materials, Inc), 1975. Report on Human Maximization Studies. Report to RIFM. RIFM Report Number 1799. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1977a. Report on Human Maximization Studies. RIFM, Woodcliff Lake, NJ, USA. Report to RIFM. RIFM report number 1702.

RIFM (Research Institute for Fragrance Materials, Inc), 1977b. Human Maximization Studies. Report to RIFM. RIFM Report Number 1979. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1978. Repeated Insult Patch Test with Dihydrocoumarin. Unpublished Report from International Flavors and Fragrances. RIFM Report Number 50738. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1982. Mutagenicity Evaluation of Dihydrocoumarin in the Ames Salmonella/microsome Plate Test. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Lorillard Tobacco Company. RIFM report number 36506.

RIFM (Research Institute for Fragrance Materials, Inc), 1983. Mutagenicity Evaluation of Dihydrocoumarin in an in Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Lorillard Tobacco Company. RIFM report number 36507.

RIFM (Research Institute for Fragrance Materials, Inc), 1984. Mutagenicity Evaluation of Dihydrocoumarin in the Mouse Lymphoma Forward Mutation Assay. RIFM,

Woodcliff Lake, NJ, USA. Private communication to FEMA. Unpublished report from Lorillard Tobacco Company. RIFM report number 36508.

RIFM (Research Institute for Fragrance Materials, Inc), 1986. Unscheduled DNA Synthesis in Rat Primary Hepatocytes of Dihydrocoumarin. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Lorillard Tobacco Company. RIFM report number 36504.

RIFM (Research Institute for Fragrance Materials, Inc), 1999. Investigation of the Ecological Properties of Dihydrocoumarin. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Symrise. RIFM report number 60209.

RIFM (Research Institute for Fragrance Materials, Inc), 2003. Evaluation of Skin Sensitization Potential of Coumarin in Mice Using the Local Lymph Node Assay (LLNA). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Rhodia Services - RSP. RIFM report number 47072.

RIFM (Research Institute for Fragrance Materials, Inc), 2012. Dihydrocoumarin: Local Lymph Node Assay. RIFM Report Number 63816. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2018. Exposure Survey 19, January 2018.

RIFM (Research Institute for Fragrance Materials, Inc), 2019. Dihydrocoumarin: Repeated Insult Patch Test (RIPT). RIFM, Woodcliff Lake, NJ, USA. RIFM report number 76166.

RIFM (Research Institute for Fragrance Materials, Inc), 2020. Updating Exposure Assessment for Skin Sensitization Quantitative Risk Assessment for Fragrance Materials. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 76775.

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.

Roll, R., Bar, F., 1967. Effect of coumarin on pregnant mice. Arzneimittel-Forschung [Drug Research] 17 (1), 97–100.

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.

Sakaguchi, H., Ashikaga, T., Kosaka, N., Sono, S., Itagaki, H., Suzuki, H., 2006. Predicting and classification of allergic potential using an in vitro skin sensitization test: human cell line activation test (h-CLAT). Toxicologist 90 (1), 466.

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., Cronin, M.T.D., 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 (12), 164–176.

US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.