Food and Chemical Toxicology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, 4-hydroxy-2,5-dimethyl-3 (2H)-furanone, CAS Registry Number 3658-77-3

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

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

ARTICLE INFO

Keywords:
Genotoxicity
Repeated dose, developmental, and reproductive toxicity
skin sensitization
Phototoxicity/photoallergenicity
Local respiratory toxicity
Environmental safety

Version: 033,120. This version replaces any previous versions.

(*continued on next column*)

(*continued*)

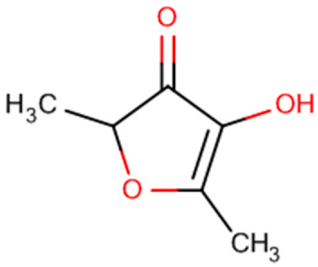
Name: 4-Hydroxy-2,5-dimethyl-3(2H)-furanone CAS Registry Number: 3658-77-3

(*continued on next page*)

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

https://doi.org/10.1016/j.fct.2020.111620
Received 10 April 2020; Received in revised form 11 June 2020; Accepted 11 July 2020
Available online 7 August 2020

(*continued*)



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
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,

(*continued on next column*)

(*continued*)

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.

4-Hydroxy-2,5-dimethyl-3(2H)-furanone was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-hydroxy-2,5-dimethyl-3(2H)-furanone is not genotoxic. Data on 4-hydroxy-2,5-dimethyl-3(2H)-furanone provide a calculated margin of exposure (MOE) > 100 for the repeated dose toxicity endpoint. Data on read-across material maltol (CAS # 118-71-8) provide a calculated MOE >100 for the developmental and reproductive toxicity endpoint. Data from 4-hydroxy-2,5-dimethyl-3(2H)-furanone provided a No Expected Sensitization Induction Level (NESIL) of 590 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 4-hydroxy-2,5-dimethyl-3(2H)-furanone 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 II material, and the exposure to 4-hydroxy-2,5-dimethyl-3(2H)-furanone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 4-hydroxy-2,5-dimethyl-3(2H)-furanone 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, 1978b; RIFM, 2008b; ECHA, 2018)
Repeated Dose Toxicity: NOAEL = 194.25 mg/kg/day.	RIFM (2003)
Developmental and Reproductive Toxicity: NOAEL = 400 mg/kg/day.	(JECFA: Maltol and Related Substances; JECFA, 2018)
Skin Sensitization: NESIL = 590 μg/cm^2.	RIFM (2015b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic	(UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence: Critical Measured Value: 96%	RIFM (1981)
Bioaccumulation: Screening-level: 3.16 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 1837 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: 1837 mg/L	(RIFM Framework; Salvito, 2002)
RIFM PNEC is: 1.837 μg/L	
•**Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** 4-Hydroxy-2,5-dimethyl-3(2H)-furanone
2. **CAS Registry Number:** 3658-77-3
3. **Synonyms:** 2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one; Furaneol; 3(2H)-Furanone, 4-hydroxy-2,5-dimethyl-; Dimethylhydroxy furanone; 3-ヒドロキシ-2,5-ジメチル4(5H)-フラノン; 4-Hydroxy-2,5-dimethyl-furan-3(2H)-one; Pineapple compound; Neofuraneol; 4-Hydroxy-2,5-dimethyl-3(2H)-furanone
4. **Molecular Formula:** $C_6H_8O_3$
5. **Molecular Weight:** 128.12
6. **RIFM Number:** 5025
7. **Stereochemistry:** Isomer not specified. One chiral center present and 2 enantiomers possible

2. Physical data

1. **Boiling Point:** 258.62 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System), >200 °F; CC (Fragrance Materials Association [FMA] Database)
3. **Log K_{OW}:** 0.82 (EPI Suite)
4. **Melting Point**: 77–79 °C (RIFM, 1981), 56.94 °C (EPI Suite)
5. **Water Solubility:** 18,500 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.000293 mm Hg @ 20 °C (EPI Suite v4.0), 0.3 mm Hg 20 °C (FMA Database), 0.00058 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorption between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** Colorless or white crystals with an intensely caramellic-fruity, jam-like odor with some resemblance to the odor of Palatone; the odor is also reminiscent of cooked pineapple

3. Volume of use (worldwide band)

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

4. Exposure

1. 95th Percentile Concentration in Hydroalcoholics: 0.0057% (RIFM, 2014)
2. Inhalation Exposure*: 0.000087 mg/kg/day or 0.0062 mg/day (RIFM, 2014)
3. Total Systemic Exposure**: 0.00051 mg/kg/day (RIFM, 2014)

*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 II, Intermediate

Expert Judgment	Toxtree v2.6	OECD QSAR Toolbox v3.2
Class II, Intermediate	Class II, Intermediate	Class II, Intermediate

2. Analogs Selected:
   a. Genotoxicity: None
   b. Repeated Dose Toxicity: None
   c. Developmental and Reproductive Toxicity: Maltol (CAS # 118-71-8)
   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

Roscher (1997): Fresh strawberries were used as a natural source of free and bound 4-hydroxy-2,5-dimethyl-3(2H)-furanone (DMHF). Six adult volunteers were divided into 2 groups of 2 males and 1 female. The volunteers ate 2.5 kg of Spanish strawberries or 2.5–3.0 kg of Italian strawberries. The urinary excretion of metabolites was monitored for 24 h after administration. Analysis of the 2 batches of strawberries for free DMHF and glycoside conjugate content revealed that Spanish strawberries contained DMHF at a concentration of 26.2 mg/kg (ratio of free: glycoside DMHF = 2:13), while Italian strawberries contained DMHF at a concentration of 60.2 mg/kg (ratio of free:glycoside DMHF = 3:2). This corresponds to a total intake of DMHF of 65.5 mg for volunteers who ingested Spanish strawberries (approximately equivalent to 1.1 mg/kg) and 150.5–180.6 mg of DMHF for those who ingested Italian strawberries (approximately equivalent to 2.51–3.0 mg/kg). Samples of urine collected 24 h after the initial ingestion of strawberries revealed that all volunteers excreted 59%–94% of the ingested DMHF as the glucuronic acid conjugate (4-hydroxy-2,5-dimethyl-3(2H)-furanone b-D-glucuronide). Urinary excretion of the DMHF glucuronic acid conjugate was greater in females (81%–94% of the administered dose) than in males (59%–69% of the administered dose), independent of the dose. No unchanged DMHF or glycosidically-bound forms, DMHF glucoside, or the 6′-malonyl derivative were detected in the urine of any of the subjects. The metabolic scheme is as shown below (Fig. 1):

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

4-Hydroxy-2,5-dimethyl-3(2H)-furanone is reported to occur in the following foods by the VCF*:

Pumpkin seed oil
Arctic bramble (*Rubus arcticus* L.)
Strawberry (*Fragaria* species)
Cocoa category
Licorice (*Glycyrrhiza* species)
Raspberry, blackberry, and boysenberry
Pineapple (*Ananas comosus*)
Coffee
Guava and feyoa
Wheaten bread

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data. This is a partial list.

9. REACH dossier

Available; accessed on 03/31/20.

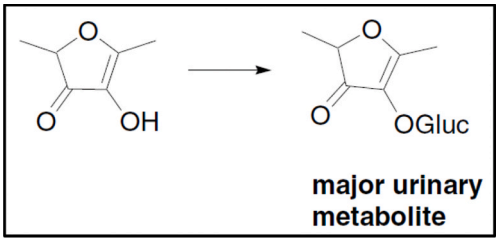


Fig. 1. Adapted from WHO, 2005.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 4-hydroxy-2,5-dimethyl-3(2H)-furanone are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products
1	Products applied to the lips (lipstick)	0.045
2	Products applied to the axillae	0.014
3	Products applied to the face/body using fingertips	0.27
4	Products related to fine fragrances	0.25
5a	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.064
5b	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.064
5c	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.064
5d	Baby cream, oil, talc	0.021
6	Products with oral and lip exposure	0.15
7	Products applied to the hair with some hand contact	0.52
8	Products with significant ano-genital exposure (tampon)	0.021
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.49
10a	Household care products with mostly hand contact (hand dishwashing detergent)	0.49
10b	Aerosol air freshener	1.8
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.021
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 4-hydroxy-2,5-dimethyl-3(2H)-furanone, the basis was the reference dose of 1.94 mg/kg/day, a predicted skin absorption value of 10%, and a skin sensitization NESIL of 590 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).

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data and use levels, 4-hydroxy-2,5-dimethyl-3(2H)-furanone does not present a concern for genetic toxicity.

11.1.1.1. Risk assessment. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone was assessed in the BlueScreen assay and found positive for both cytotoxicity (positive: <80% relative cell density) 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 were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 4-hydroxy-2, 5-dimethyl-3(2H)-furanone (furaneol) was assessed in an Ames study conducted in accordance with OECD TG 471. *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100, and *Escherichia coli* strain WP2uvrA were treated with furaneol at concentrations up to 3333 μg/plate in the presence and absence of metabolic activation. Toxicity was observed in all strains with and without S9 activation at 3333 μg/plate. A significant increase in the number of revertant colonies was detected in strain TA100 at the lowest effective dose without S9 activation, demonstrating the ability of the test material to induce base-pair substitutions. This strain is, however, sensitive to certain frameshift mutagenic effects. Decompositions in the presence of air and humidity were a possibility for this material, so an experiment was designed to compare the effects of varying lengths of exposure to the test material. There were no observable changes in the mutagenic activity of the material, and any decomposition products were deemed equally mutagenic (RIFM, 1978b). Positive effects were also reported in other *in vitro* studies conducted on furaneol. A study concluded furaneol as a mutagen in *Salmonella typhimurium* strains TA97, TA100, and TA102; furaneol also induced an increase in micronucleated reticulocytes. These results were in agreement with later experiments carried out by Hiramoto et al. and Xing (Hiramoto, 1996; Xing, 1988). Evidence of genotoxic effects on sperm cells was also obtained from a poorly documented study in which the study protocol was not well specified (Tian, 1992). Hiramoto et al. also showed that furaneol induced DNA single-strand breaks; these effects were linked to the formation of active oxygen species and an ultimate hydroxyl radical (Hiramoto, 1996).

A mammalian cell gene mutation assay (mouse lymphoma assay) was conducted both with and without metabolic activation. No toxicologically significant increases in the frequency of mutant colonies were observed with any dose of the test material, either with or without metabolic activation (ECHA, 2018). Under the conditions of the study, furaneol was not mutagenic to mammalian cells *in vitro*. In their Scientific Opinion on Flavoring Group Evaluation (EFSA, 2015), the EFSA Panel on Food Contact Materials, Enzymes, Flavorings, and Processing Aids concluded that the results from *in vitro* toxicity studies indicated that the α,β-unsaturated 3(2H)-furanones can be involved in keto-enol tautomerism and that the *in vitro* genotoxicity results observed may have been caused by indirect mechanisms of action, in particular, the generation of reactive oxygen species.

Based on the *in vivo* metabolic data in humans, furaneol is rapidly absorbed in the gastrointestinal tract, conjugated with glucuronic acid in the liver primarily during first-pass phase II metabolism and excreted in the urine. Free furaneol was not detected in the blood of human volunteers to whom it was administered as a constituent of strawberries; its glucuronic acid conjugate is the principal urinary metabolite (Roscher, 1997). Therefore, at low levels of use, it is highly unlikely that it would be available at any significant level to exert a genotoxic effect on germ cells. Considering that furaneol is readily excreted unchanged in a conjugated form in animals and that levels of intake by humans are minute compared to those concentrations and dose levels leading to hydroxyl radical-induced damage, there is no significant risk to humans from the intended use of furaneol.

Furthermore, the results of a valid fertility and dominant lethal study have shown that 4-hydroxy-2,5-dimethyl-3(2H)-furanone did not induce adverse effects on male rat reproductive capacity or dominant lethality (ECHA, 2018). This assay included 2 separate mating trials following 2 and 9 weeks of male dose administration (phases I and II, respectively). The first mating phase was conducted to detect potential early genotoxic effects on the embryo with reduced risk of treatment-related exposure throughout a complete spermatogenic cycle using a second set of untreated females. The dominant lethal test is generally recommended to be conducted when the mammalian spermatogonial chromosomal aberration test (OECD TG 483) gives positive results (Eastmond, 2009). Although an EFSA panel believed a robust GLP-controlled cytogenetic investigation (OECD TG 483) in mouse spermatocytes would be required to evaluate the germ cell genotoxicity of the material in the previous EFSA opinion (EFSA, 2015), the EFSA scientific panel later concluded that 4-hydroxy-2,5-dimethyl-3(2H)-furanone imparts no concern for its potential to induce heritable genetic

damage or adverse effects on male reproductive capacity (EFSA, 2016).

The material was also tested for its ability to induce micronuclei in male and female NMRI mice. At doses up to 1250 mg/kg, 4-hydroxy-2,5-dimethyl-3(2H)-furanone administered orally did not induce polychromatic erythrocytes in the bone marrow compared to the control vehicle (RIFM, 2008b). Furaneol also produced oxidative DNA damage in fetal turkey livers but did not directly interfere with DNA as evident from the results of a turkey egg genotoxicity assay (Kobets, 2018). Also, a 2-year carcinogenicity study conducted according to OECD TG 451 did not show any evidence of furaneol inducing carcinogenic effects (RIFM, 2003).

Based on the available data on an *in vivo* micronucleus study, a dominant lethal assay, and a 2-year carcinogenicity study, 4-hydroxy-2,5-dimethyl-3(2H)-furanone does not present a concern for genotoxic potential.

Additional References: Yamashita (1998); Hiramoto (1998); RIFM, 2012.

Literature Search and Risk Assessment Completed On: 11/20/18.

11.1.2. Repeated dose toxicity

The MOE for 4-hydroxy-2,5-dimethyl-3(2H)-furanone is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. The repeated dose toxicity data on 4-hydroxy-2,5-dimethyl-3(2H)-furanone are sufficient for the repeated dose toxicity endpoint. An OECD 451 dietary 24-month carcinogenicity study was conducted in rats. Groups of 60 rats/sex/dose were administered a dietary admixture of 4-hydroxy-2,5-dimethyl-3(2H)-furanone in doses of 0, 100, 200, or 400 mg/kg/day in a 0.2% ascorbic acid in propylene glycol vehicle for 24 months. The NOAEL was determined to be 200 mg/kg/day (194.25 and 195.90 mg/kg/day in males and females, respectively), based on reduced bodyweight gains and survival among the high-dose group animals (RIFM, 2003). **Therefore, the 4-hydroxy-2,5-dimethyl-3(2H)-furanone MOE for the repeated dose toxicity endpoint can be calculated by dividing the 4-hydroxy-2,5-dimethyl-3(2H)-furanone NOAEL in mg/kg/day by the total systemic exposure to 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 194.25/0.00051 or 380,882.**

In addition, the total systemic exposure to 4-hydroxy-2,5-dimethyl-3(2H)-furanone (0.51 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

11.1.2.1.1. 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, 2008a; IDEA [International Dialogue for the Evaluation of Allergens] project Final Report on the QRA2: Skin Sensitization Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016, http://www.ideaproject.info/uploads/Modules/Documents/qra2-dossier-final–september-2016.pdf) and a reference dose of 1.94 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 RfD for 4-hydroxy-2,5-dimethyl-3(2H)-furanone was calculated by dividing the lowest NOAEL (from the Repeated Dose and Developmental and Reproductive Toxicity sections) of 194.25 mg/kg/day by the uncertainty factor, 100 = 1.94 mg/kg/day.

Additional References: Posternak (1969); Roscher (1997); RIFM, 1978a; RIFM, 1971c; RIFM, 1971a; RIFM, 1971b; RIFM, 1973; Gralla (1969); Kim (2004); Bhathal (1984); Olson (1967); Rennhard (1971); Kimura (1980); Barrand (1987).bib_Barrand_et_al_1987

Literature Search and Risk Assessment Completed On: 11/14/16.

11.1.3. Developmental and reproductive toxicity

The MOE for 4-hydroxy-2,5-dimethyl-3(2H)-furanone is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

11.1.3.1. Risk assessment. There are no developmental toxicity data on 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Read-across material maltol (CAS # 118-71-8; see Section VI) has sufficient developmental toxicity data. A dietary 3-generation reproductive toxicity study was conducted in rats. Groups of 20 rats/sex/dose were fed diets containing the test material maltol at doses of 0, 100, 200, or 400 mg/kg/day. On day 134 of the study, F1 generation rats showed signs of sialodacryoadenitis virus infection, which diminished within 10 days; no deaths occurred. There were no effects on copulation rates, mating viability index, lactation, offspring sex ratio, or pup survival to 21 days. Discrepancies in F1 growth rates were attributed to sialodacryoadenitis infection. There were no treatment-related abnormalities or lesions in the F1 pups. The F1 generation rats were weaned, maintained on the same diets, and then mated on days 189 and 245 of the study to produce the F2a and F2b generations. On day 418 of the study, sialodacryoadenitis infection was observed in all animals but diminished within 10 days; no deaths occurred. F2a and F2b pup survival rates were similar to controls. There were no effects on organs or tissues and no effects on tumor incidence in the F2a and F2b generations. The NOAEL for developmental and reproductive toxicity was considered to be 400 mg/kg/day, the highest dose tested (JECFA, 2018).

There are sufficient male reproductive toxicity data on 4-hydroxy-2,5-dimethyl-3(2H)-furanone. An oral gavage 2-phase male reproductive study was conducted in male Crl:CD(SD) rats to determine the potential effects of 4-hydroxy-2,5-dimethyl-3(2H)-furanone on mating, fertility, and gonadal function. Groups of 25 male rats/dose were administered the test material 4-hydroxy-2,5-dimethyl-3(2H)-furanone via oral gavage at doses of 0, 100, 500, or 1000 mg/kg/day in propylene glycol. Males received 14 daily doses prior to mating with untreated phase I females and 63 daily doses prior to mating with untreated phase II females until females were euthanized (total of 91–93 doses). The females with evidence of mating were euthanized on gestation day (GD) 15, and females without evidence of mating were euthanized 8 days following completion of the cohabitation period; the males were euthanized following completion of postmortem examination of the phase II females with evidence of mating. There were no significant treatment-related adverse effects at any dose level. In the absence of any effects observed on spermatogenic parameters, organ weights, reproductive performance, and embryonic survival, the NOAEL for male reproductive toxicity was considered to be 1000 mg/kg/day, the highest dose tested (RIFM, 2008c). There are no female reproductive toxicity data on 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Read-across material maltol (CAS # 118-71-8; see Section VI) has sufficient female reproductive toxicity data. A dietary 3-generation reproductive toxicity study conducted in male and female rats considered the NOAEL for reproductive toxicity to be 400 mg/kg/day, the highest dose tested (JECFA, 2018). The most conservative NOAEL of 400 mg/kg/day from the 3-generation study was selected for the reproductive toxicity endpoint.

Therefore, the 4-hydroxy-2,5-dimethyl-3(2H)-furanone MOE for the developmental and reproductive toxicity endpoints can be calculated by dividing the maltol NOAEL in mg/kg/day by the total systemic exposure to 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 400/0.00051 or 784,314.

In addition, the total systemic exposure to 4-hydroxy-2,5-dimethyl-3(2H)-furanone (0.51 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the developmental and reproductive toxicity endpoints of a Cramer Class II material at the current level of use.

Additional References: Posternak (1969); Kataoka (1997); Roscher (1997); RIFM, 2003; RIFM, 2000.

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

18.

11.1.4. Skin sensitization

Based on the existing data, 4-hydroxy-2,5-dimethyl-3(2H)-furanone is a skin sensitizer with a defined NESIL of 590 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, 4-hydroxy-2,5-dimethyl-3(2H)-furanone is a skin sensitizer. The chemical structure indicates that this material is not predicted to react directly with skin proteins (Toxtree 3.1.0; OECD Toolbox v4.2). 4-Hydroxy-2,5-dimethyl-3(2H)-furanone was positive in the DPRA and h-CLAT assays but negative in a KeratinoSens assay (RIFM, 2016a; RIFM, 2016c; RIFM, 2016b). In a murine local lymph node assay (LLNA), the material was found to be sensitizing with an EC3 value of 1.8% or 450 μg/cm^2 (RIFM, 2001b; ECHA, 2018). In confirmatory HRIPTs with 1181 μg/cm^2 of this material in 3:1 diethyl phthalate:ethanol (DEP:EtOH), both positive and negative results were reported (RIFM, 2015a; RIFM, 2010). In a separate HRIPT in 108 subjects using 590 μg/cm^2 of this material in 3:1 DEP:EtOH, no sensitization reactions were observed (RIFM, 2015b).

Based on weight of evidence (WoE) from structural analysis and *in vitro*, animal, and human studies, 4-hydroxy-2,5-dimethyl-3(2H)-furanone is a moderate sensitizer with a WoE NESIL of 590 μg/cm^2 (see 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, 2008a; IDEA [International Dialogue for the Evaluation of Allergens] project Final Report on the QRA2: Skin Sensitization Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016, http://www.ideaproject.info/uploads/Modules/Documents/qra2-dossier-final–september-2016.pdf, and a reference dose of 1.94 mg/kg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/25/18.

11.1.5. Phototoxicity/photoallergenicity

Based on available UV/Vis spectra, 4-hydroxy-2,5-dimethyl-3(2H)-furanone does not present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 4-hydroxy-2,5-dimethyl-3(2H)-furanone. 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. Based on the lack of significant absorbance in the critical range, 4-hydroxy-2,5-dimethyl-3(2H)-furanone does not present a concern for phototoxicity or photoallergenicity.

Table 1
Data summary for 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

LLNA Weighted Mean EC3 Value μg/cm[b] (No. Studies)	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-HRIPT (Induction) μg/cm[b]	NOEL-HMT (Induction) μg/cm[b]	LOEL[b] (Induction) μg/cm[b]	WoE NESIL[c] μg/cm[b]
450 [1]	Moderate	590	NA	1181	590

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch 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 HRIPT or HMT.

[c] WoE NESIL limited to 2 significant figures.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for 4-hydroxy-2,5-dimethyl-3(2H)-furanone 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: 07/30/18.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 4-hydroxy-2,5-dimethyl-3(2H)-furanone 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 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Based on the Creme RIFM Model, the inhalation exposure is 0.0062 mg/day. This exposure is 75.8 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.

*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: 03/20/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 4-hydroxy-2,5-dimethyl-3(2H)-furanone was performed following the RIFM Environmental Framework (Salvito, 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, 4-hydroxy-2,5-dimethyl-3(2H)-furanone was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 4-hydroxy-2,5-dimethyl-3(2H)-furanone as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a

WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current VoU (2015), 4-hydroxy-2,5-dimethyl-3(2H)-furanone does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.3. Key studies

11.2.3.1. Biodegradation. **RIFM, 1981:** Biodegradation of the test material was evaluated using 30 ppm standard active sludge. Oxygen consumption and total organic carbon were measured. Biodegradation of 96% was observed after 28 days.

11.2.3.2. Ecotoxicity. **RIFM, 2001a:** A *Daphnia magna* acute immobilization test was conducted according to the OECD 202I method. Under the condition of this study, the 48-h EC50 was 6.8 mg/L (nominal).

11.2.4. Other available data

4-Hydroxy-2,5-dimethyl-3(2H)-furanone has been registered for REACH with no additional data at this time.

11.2.5. Risk assessment refinement

Since 4-hydroxy-2,5-dimethyl-3(2H)-furanone 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	0.82	0.82
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 1.837 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 volumes of use.

Literature Search and Risk Assessment Completed On: 03/14/19.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 03/31/20.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111620.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1837			1000000	1.837	

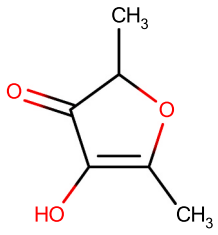
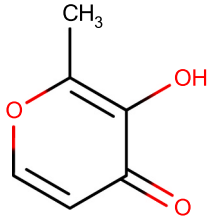
Appendix

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, 2016).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Maltol
CAS No.	3658-77-3	118-71-8
Structure		
Similarity (Tanimoto Score)		0.05
Read-across Endpoint		• Developmental and Reproductive Toxicity
Molecular Formula	$C_6H_8O_3$	$C_6H_6O_3$
Molecular Weight	128.12	126.11
Melting Point (°C, EPI Suite)	56.94	161.50
Boiling Point (°C, EPI Suite)	258.62	267.24
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.0773	0.00571
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	0.82	0.09
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.85e+004	7.976e+004
J_{max} (μg/cm^2/h, SAM)	377.53	18.14
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.49E+000	6.63E-001
Reproductive and Developmental Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, impaired OH or NH2 group	• Non-binder, impaired OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	• Non-toxicant (moderate reliability)	• Non-toxicant (low reliability)
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 4-hydroxy-2,5-dimethyl-3(2H)-furanone (CAS # 3658-77-3). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, maltol (CAS # 118-71-8) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Maltol (CAS # 118-71-8) was used as a read-across analog for the target material 4-hydroxy-2,5-dimethyl-3(2H)-furanone (CAS # 3658-77-3) for the developmental and reproductive toxicity endpoint.

- o The target material and the read-across analog are structurally similar and belong to a class of oxygen-containing heterocycles.
- o The target material and the read-across analog are structural isomers and share an unsaturated oxygen heterocycle structure with keto, methyl, and hydroxyl substituents.
- o The key difference between the target material and the read-across analog is that the target material is a furanone, whereas the read-across analog is a pyrone. This structural difference is toxicologically insignificant.
- o 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 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

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.

Barrand, M.A., Callingham, B.R., Hider, R.C., 1987. Effects of the pyrones, maltol and ethyl maltol, on iron absorption from the rat small intestine. J. Pharm. Pharmacol. 39, 203–211.

Bhathal, P.S., Ho, S.L., Hegarty, M.P., Harris, R.L.N., 1984. Chronic active hepatitis in mice induced by 3-hydroxy-4-pyrone. Experientia 40 (8), 894–896.

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.

Eastmond, D.A., Hartwig, A., Anderson, D., Anwar, W.A., Cimino, M.C., Dobrev, I., Douglas, G.R., Nohmi, T., Phillips, D.H., Vickers, C., 2009. Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS harmonized scheme. Mutagenesis 24 (4), 341–349.

ECHA, 2012. Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment. November 2012 v1.1. http://echa.europa.eu/.

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

ECHA, 2018. 4-Hydroxy-2,5-dimethylfuran-2(3H)-one registration dossier. Retrieved from. https://echa.europa.eu/lv/registration-dossier/-/registered-dossier/22015/1.

EFSA, 2015. Scientific opinion on flavouring group evaluation 99 revision 1 (FGE.99Rev1): consideration of furanone derivatives evaluated by the JECFA (63rd, 65th and 69th meetings). Retrieved from. https://doi.org/10.2903/j.efsa.2015.4286.

EFSA, 2016. EFSA CEF panel (EFSA panel on food Contact materials, Enzymes, flavourings and processing Aids). Scientific opinion on flavouring group evaluation 313, (FGE.313): a,b-unsaturated 3(2H)-furanone derivatives from chemical group 13, 2016 EFSA Journal 14 (7), 4531. Retrieved from: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2016.4531.

Gralla, E.J., Stebbins, R.B., Coleman, G.L., Delahunt, C.S., 1969. Toxicity studies with ethyl maltol. Toxicol. Appl. Pharmacol. 15 (3), 604–613.

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.

Hiramoto, K., Aso-O, R., Ni-Iyama, H., Hikage, S., Kato, T., Kikugawa, K., 1996. DNA strand break by 2,5-dimethyl-4-hydroxy-3(2H)-furanone, a fragrant compound in various foodstuffs. Mutation Research. Environmental Mutagenesis and Related Subjects 359 (1), 17–24.

Hiramoto, K., Kato, T., Takahashi, Y., Yugi, K., Kikugawa, K., 1998. Absorption and induction of micronucleated peripheral reticulocytes in mice after oral administration of fragrant hydroxyfuranones generated in the Maillard reaction. Mutation Research. Genetic Toxicology and Environmental Mutagenesis 415 (1–2), 79–83.

IFRA (International Fragrance Association), 2015. Volume of Use Survey. February 2015.

JECFA, 2018. Safety Evaluations of Groups of Related Flavouring Agents: Maltol and Related Substances. http://www.inchem.org/documents/jecfa/jecmono/v56je07.pdf.

Kataoka, S., Liu, W., Albright, K., Storkson, J., Pariza, M., 1997. Inhibition of benzo[a] pyrene-induced mouse forestomach neoplasia and reduction of H2O2 concentration in human polymorphonuclear leucoytes by flavour components of Japanese-style fermented soy sauce. Food Chem. Toxicol. 35 (5), 449–457.

Kim, Y.-B., Oh, S.H., Sok, D.-E., Kim, M.R., 2004. Neuroprotective effect of maltol against oxidative stress in brain of mice challenged with kainic acid. Nutr. Neurosci. 7 (1), 33–39.

Kimura, R., Matsui, S., Ito, S., Aimoto, T., Murata, T., 1980. Central depressant effects of maltol analogs in mice. Chem. Pharm. Bull. 28 (9), 2570–2579.

Kobets, T., Duan, J.-D., Brunnemann, K.D., Iatropoulos, M.J., Etter, S., Hickey, C., Smith, B., Williams, G.M., 2018. In ovo testing of flavor and fragrance materials in Turkey Egg Genotoxicity Assay (TEGA), comparison of results invitro and in vivo data. Food Chem. Toxicol. 115, 228–243.

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.

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/.

Olson, W.A., 1967. Animal feeds containing maltol. Patent. United States 3 (338), 718 appl. 317,085, 10/17/63.

Posternak, J.M., Linder, A., Vodoz, C.A., 1969. Summaries of toxicological data. Toxicological tests on flavoring matters. Food Chem. Toxicol. 7, 405–407.

Rennhard, H.H., 1971. The metabolism of ethyl maltol and maltol in the dog. J. Agric. Food Chem. 19 (1), 152–154.

RIFM (Research Institute for Fragrance Materials, Inc), 1971a. Biological Evaluation of 4-Hydroxy-5-Methyl-3(2h)-Furanone. I. Palatability Study in Rats. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Wilson, C. RIFM report number 21728.

RIFM (Research Institute for Fragrance Materials, Inc), 1971b. Biological Evaluation of 4-Hydroxy-5-Methyl-3(2h)-Furanone. II. 13-Week Feeding Study in Rats. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Wilson, C. RIFM report number 21729.

RIFM (Research Institute for Fragrance Materials, Inc), 1971c. Biological Evaluation of a Flavor Cocktail. II. 13-Week Study in Rats. Private Communication to FEMA. Unpublished Report from Munday, R. & Kirkby, W.W. RIFM Report Number 29333. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1973. Biological Evaluation of 4-Hydroxy-5-Methyl-3(2h)-Furanone. III. 1-Year Feeding Study in Rats. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Wilson, C. RIFM report number 21730.

RIFM (Research Institute for Fragrance Materials, Inc), 1978a. Day Feeding Study on 2-Ethyl-4-Hydroxy-5-Methyl-3(2H)furanone in Sprague-Dawley Rats. Private Communication to FEMA. RIFM, Woodcliff Lake, NJ, USA, p. 90. Unpublished report from Cox, G.E. & Re, T.A. RIFM report number 21593.

RIFM (Research Institute for Fragrance Materials, Inc), 1978b. Testing for Mutagenic Activity of Fragrance Materials with Salmonella typhimurium. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich Incorporated. RIFM report number 29843.

RIFM (Research Institute for Fragrance Materials, Inc), 1981. Biodegradability Test of 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich SA. RIFM report number 34099.

RIFM (Research Institute for Fragrance Materials, Inc), 2000. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone: A 3-Month Dietary Range-Finding Toxicity Study in Rats. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich. RIFM report number 74075.

RIFM (Research Institute for Fragrance Materials, Inc), 2001a. Study on the Acute Toxicity towards Daphnia of 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Symrise. RIFM report number 60387.

RIFM (Research Institute for Fragrance Materials, Inc), 2001b. Murine Local Lymph Node Assay with 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol). [Amendment Attached]. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich SA. RIFM report number 60432.

RIFM (Research Institute for Fragrance Materials, Inc), 2003. Month Dietary Carcinogenicity Study in Rats for 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol). RIFM, Woodcliff Lake, NJ, USA, p. 24. Unpublished report from Firmenich. RIFM report number 52789.

RIFM (Research Institute for Fragrance Materials, Inc), 2008a. Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 55663.

RIFM (Research Institute for Fragrance Materials, Inc), 2008b. Micronucleus Assay in Bone Marrow Cells of the Mouse with 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Neofuraneol). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich SA. RIFM report number 55067.

RIFM (Research Institute for Fragrance Materials, Inc), 2008c. Oral Male Fertility Study of 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol) in Rats. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from RIFM report number 71181.

RIFM (Research Institute for Fragrance Materials, Inc), 2010. Repeated Insult Patch Test of 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol) at 1.0% in 75% Diethyl Phthalate (DEP)/25% Ethanol. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich SA. RIFM report number 60433.

RIFM (Research Institute for Fragrance Materials, Inc), 2012. Report on the Testing of 21 Compounds in BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Firmenich. RIFM report number 65264.

RIFM (Research Institute for Fragrance Materials, Inc), 2013. Report on the Testing of 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM, Woodcliff Lake, NJ, USA. RIFM report number 65103.

RIFM (Research Institute for Fragrance Materials, Inc), 2014. Exposure Survey 03. April 2014.

RIFM (Research Institute for Fragrance Materials, Inc), 2015a. Repeat Insult Patch Test with 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 68926.

RIFM (Research Institute for Fragrance Materials, Inc), 2015b. Repeated Insult Patch Test (RIPT) with 4-Hydroxy-2,5-Dimethyl-3(2h)-Furanone (Furaneol). RIFM, Woodcliff Lake, NJ, USA. RIFM report number 69745.

RIFM (Research Institute for Fragrance Materials, Inc), 2016a. Direct Peptide Reactivity Assay (DPRA) in Fragrance Materials. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 71870.

RIFM (Research Institute for Fragrance Materials, Inc), 2016b. Induction of Antioxidant-Response-Element Dependent Gene Activity and Cytotoxicity (Using MTT) in the Keratinocyte ARE-Reporter Cell Line KeratinoSens. RIFM, Woodcliff Lake, NJ, USA. RIFM report number 72231.

RIFM (Research Institute for Fragrance Materials, Inc), 2016c. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone: in Vitro Sensitization: Dendritic Cell Line Activation Assay Human Cell Line Activation Test (H-CLAT). RIFM, Woodcliff Lake, NJ, USA. RIFM report number 72749.

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

Roscher, R., Koch, H., Herderich, M., Schreier, P., Schwab, W., 1997. Identification of 2,5-dimethyl-4-hydroxy-3[2H]-furanone beta-delta-glucuronide as the major metabolite of a strawberry flavour constituent in humans. Food Chem. Toxicol. 35 (8), 777–782.

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

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

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

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

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

Tian, Q., Li, X., Shen, J., Wang, Y., 1992. Genotoxicity of dimethylfuranone on reproductive cells in male mice. Weisheng Dulixue Zazhi 6, 26–28.

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.

WHO, 2005. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA): 4-Acetoxy-2,5-Dimethyl-3(2h)furanone. Retrieved from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=5293.

Xing, B., 1988. Mutagenic studies on furaneol (HDMF). Chinese J. Preventative Medicine 22 (2), 85–87.

Yamashita, N., Murata, M., Inoue, S., Hiraku, Y., Yoshinaga, T., Kawanishi, S., 1998. Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper (II). Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis 397 (2), 191–201.