Food and Chemical Toxicology 134 (2019) 110825

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, methyl 2-octynoate, CAS Registry Number 111-12-6

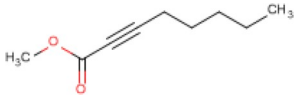
A.M. Api[a], D. Belsito[b], S. Biserta[a], D. Botelho[a], M. Bruze[c], G.A. Burton Jr.[d], J. Buschmann[e], M.A. Cancellieri[a], M.L. Dagli[f], M. Date[a], W. Dekant[g], C. Deodhar[a], A.D. Fryer[h], S. Gadhia[a], L. Jones[a], K. Joshi[a], A. Lapczynski[a], M. Lavelle[a], D.C. Liebler[i], M. Na[a], D. 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*

A B S T R A C T

Methyl 2-octynoate was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that methyl 2-octynoate is not genotoxic. Data provided methyl 2-octynoate a NESIL of 110 μg/cm^2 for the skin sensitization endpoint. The repeated dose, developmental and reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class II material, and the exposure to methyl 2-octynoate is below the TTC (0.009 mg/kg/day, 0.009 mg/kg/day, and 0.47 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; methyl 2-octynoate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; methyl 2-octynoate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Version: 061019. This version replaces any previous versions.



Name: Methyl 2-octynoate
CAS Registry Number: 111-12-6
Abbreviation/Definition List:

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

https://doi.org/10.1016/j.fct.2019.110825
Received 8 July 2019; Accepted 16 September 2019
Available online 19 September 2019
0278-6915/

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., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines

PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.
This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.
Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).
*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

Summary: The existing information supports the use of this material as described in this safety assessment.
Methyl 2-octynoate was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that methyl 2-octynoate is not genotoxic. Data provided methyl 2-octynoate a NESIL of 110 μg/cm^2 for the skin sensitization endpoint. The repeated dose, developmental and reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class II material, and the exposure to methyl 2-octynoate is below the TTC (0.009 mg/kg/day, 0.009 mg/kg/day, and 0.47 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; methyl 2-octynoate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; methyl 2-octynoate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment
Genotoxicity: Not genotoxic. (Wild et al., 1983)
Repeated Dose Toxicity: No NOAEL Available. Exposure is below the TTC.
Developmental and Reproductive Toxicity: No NOAEL Available. Exposure is below the TTC.
Skin Sensitization: NESIL = 110 μg/cm^2. (RIFM, 1989; RIFM, 1990)
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: 85% (OECD 302C) RIFM (2001)
Bioaccumulation: Screening-level: 24.28 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 62.53 mg/L (RIFM Frameworkv; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards
Risk Assessment:
Screening-level: PEC/PNEC (North America and Europe) < 1 (RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 62.53 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.06253 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** Methyl 2-octynoate
2. **CAS Registry Number:** 111-12-6
3. **Synonyms:** Folione; Methyl heptine carbonate; Methyl heptyne carbonate; MHC; 2-Octynoic acid, methyl ester; AB 565; アルキン（C = 7 ~ 8）カルボン酸メチル; 1-ヘプβチン-1-カルボン酸メチル; Methyl oct-2-ynoate; Methyl 2-octynoate
4. **Molecular Formula:** $C_9H_{14}O_2$
5. **Molecular Weight:** 154.2
6. **RIFM Number:** 274

2. Physical data

1. **Boiling Point:** 217 °C (FMA database), 205.31 °C (EPI Suite)
2. **Flash Point:** 88 °C (GHS), 190 °F; CC (FMA database)
3. **Log K_{OW}:** 2.6 (EPI Suite)
4. **Melting Point:** 26.3 °C (EPI Suite)
5. **Water Solubility:** 433.9 mg/L (EPI Suite)
6. **Specific Gravity:** 0.921 (FMA database)
7. **Vapor Pressure:** 0.0829 mm Hg @ 20 °C (EPI Suite v4.0), 0.07 mm Hg @ 20 °C (FMA database), 0.138 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorption between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** Colorless to yellow liquid with powerful, unpleasant odor; very powerful, penetrating vegetable green foliage type odor (Arctander, 1969)

3. Volume of use (worldwide band)

1. **Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.0070% (RIFM, 2015b)
3. **Inhalation Exposure*:** 0.000041 mg/kg/day or 0.0030 mg/day (RIFM, 2015b)
4. **Total Systemic Exposure**:** 0.00023 mg/kg/day (RIFM, 2015b)

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

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

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

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

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See Appendix below for further details.

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Developmental and Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** none
3. **Read-across Justification:** None

6. Metabolism

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

7. Natural occurrence (discrete chemical) or Composition (NCS)

Methyl 2-octynoate is not reported to occur in food by the VCF*.

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

8. REACH dossier

Available; accessed on 06/03/19.

9. Conclusion

The maximum acceptable concentrations[a] in finished products for methyl 2-octynoate are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.0085
2	Products applied to the axillae	0.0025
3	Products applied to the face/body using fingertips	0.051
4	Products related to fine fragrances	0.047
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.012
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.012
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.012
5D	Baby cream, oil, talc	0.012
6	Products with oral and lip exposure	0.028
7	Products applied to the hair with some hand contact	0.096
8	Products with significant ano-genital exposure (tampon)	0.0050
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.092
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.33
10B	Aerosol air freshener	0.33

11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.18
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted

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 methyl 2-octynoate, the basis was a predicted skin absorption value of 80% and a skin sensitization NESIL of 110 μg/cm^2.
[b]For a description of the categories, refer to the IFRA RIFM Information Booklet. (www.rifm.org/doc).

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, methyl 2-octynoate does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. Methyl 2-octynoate was assessed in an Ames assay conducted similar to OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 were treated with methyl 2-octynoate in dimethyl sulfoxide at concentrations up to 3.6 mg/plate in the presence and absence of exogenous metabolically-active microsomal mix (S9 mix). No increase in the number of revertant colonies was observed in the tester strains at any concentration (Wild et al., 1983). Under the conditions of the study, methyl 2-octynoate was considered not mutagenic in the Ames test.

The clastogenic potential of methyl 2-octynoate was assessed in an *in vivo* micronucleus test conducted equivalent to OECD TG 474. Groups of male and female NMRI mice were treated with methyl 2-octynoate in olive oil via a single intraperitoneal injection at the concentrations of 168, 336, and 505 mg/kg. After 30 h, the bone marrow of each animal was removed and samples prepared. Compared to vehicle controls, no significant increase in the number of micronucleated polychromatic erythrocytes was observed (Wild et al., 1983). Under the conditions of the study, methyl 2-octynoate was considered not clastogenic in the *in vivo* micronucleus test.

Based on the available data, methyl 2-octynoate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/15/16.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on methyl-2-octynoate or on any read-across materials. The total systemic exposure to methyl-2-octynoate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

10.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on methyl-2-octynoate or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to methyl-2-octynoate (0.23 μg/kg bw/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/25/18.

10.1.3. Developmental and reproductive toxicity

There are insufficient developmental and reproductive toxicity data on methyl-2-octynoate or on any read-across materials. The total systemic exposure to methyl-2-octynoate is below the TTC for the developmental and reproductive toxicity endpoints of a Cramer Class II material at the current level of use.

10.1.3.1. Risk assessment. There are insufficient developmental and reproductive toxicity data on methyl-2-octynoate or on any read-across materials that can be used to support the developmental and reproductive toxicity endpoints. The total systemic exposure to methyl-2-octynoate (0.23 μg/kg bw/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental and reproductive toxicity endpoints of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/25/18.

10.1.4. Skin sensitization

Based on the existing data, methyl 2-octynoate is considered a strong skin sensitizer with a defined NESIL of 110 μg/cm^2.

10.1.4.1. Risk assessment. The chemical structure of methyl 2-octynoate indicates that it is expected to react with skin proteins directly via the Michael addition mechanism (Roberts et al., 2007; Toxtree 2.6.6; OECD Toolbox v3.3, 2018). In non-animal alternative assays representing key biological events of the Adverse Outcome Pathway (AOP) for skin sensitization, methyl 2-octynoate was found to be positive (Urbisch et al., 2015). Methyl 2-octynoate was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA), KeratinoSens, and human cell line activation test (h-CLAT) (RIFM, 2015a; Urbisch et al., 2015). In 2 murine local lymph node assays (LLNAs), methyl 2-octynoate was found to be sensitizing with an EC3 value of 0.45% (112.5 μg/cm^2) (RIFM, 2005; RIFM, 2006). Additionally, in a confirmatory human repeat insult patch test (HRIPT) with 118 μg/cm^2 of methyl 2-octynoate in 3:1 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 104 volunteers (RIFM, 1989; RIFM, 1990).

Based on weight of evidence (WoE) from structural analysis and animal and human studies, methyl 2-octynoate is a strong sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 110 μg/cm^2 (Table 1). Section IX provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk

Table 1
Data summary for methyl 2-nonynoate.

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]
112.5 [1]	Strong	118	NA	194	110

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 3 significant figures.

Assessment (QRA2) described by Api et al. (RIFM, 2008; 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).

Additional References: RIFM, 1980; RIFM, 1979a; RIFM, 1979b; RIFM, 1979c; RIFM, 1983; Sharp (1978); Klecak et al., 1977; RIFM, 1973; RIFM, 1985a; RIFM, 1985b; RIFM, 1985c; RIFM, 1986; Klecak (1979); RIFM, 1979d; RIFM, 1976a; RIFM, 1976b; RIFM, 1976c; RIFM, 1976d; RIFM, 1977a; Johnson and Goodwin, 1985; RIFM, 1985d; Griepentrog (1959); RIFM, 1965; RIFM, 1972; RIFM, 1977b; RIFM, 1974; RIFM, 1975a; RIFM, 1975b; RIFM, 1976e.

Literature Search and Risk Assessment Completed On: 09/21/15.

10.1.5. Phototoxicity/Photoallergenicity

Based on UV/Vis absorption spectra, methyl 2-octynoate would not be expected to present a concern for phototoxicity or photoallergenicity.

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

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, $1000\,L\,mol^{-1}\cdot cm^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/29/16.

10.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for methyl 2-octynoate is below the Cramer Class III* TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are limited data available on methyl 2-octynoate. Based on the Creme RIFM Model, the inhalation exposure is 0.0030 mg/day. This exposure is 156.7 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

*As per Carthew et al., 2009, Cramer Class II materials default to Cramer Class III.

Additional References: Troy (1977): Gilbert and Kemp, 1996

Literature Search and Risk Assessment Completed On: 03/28/19.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of methyl 2-octynoate was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class–specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, methyl 2-octynoate 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 methyl 2-octynoate as possibly being persistent nor bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

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

10.2.2. Key studies

10.2.2.1. Biodegradation. RIFM, 2001: The inherent biodegradability of the test material was evaluated by the manometric respirometry test according to the OECD 302C method. Biodegradation of 85% was observed after 28 days.

RIFM, 2000: The ready biodegradability of the test material was evaluated by the manometric respirometry test following the OECD 301F method. Under the conditions of this study, biodegradation of 59% was observed after 28 days.

RIFM, 2013: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F method. Biodegradation of 80% was observed after 28 days.

10.2.2.2. Ecotoxicity. RIFM, 2017a: A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under semi-static conditions. The 48-h EC50, based on the mean measured concentration, was reported to be 0.62 mg/L.

RIFM, 2017b: An algae growth inhibition study was conducted according to the OECD 201 method. The 72-h EyC10, EyC50, and NOECy, based on the measured time-weighed mean concentration, were 0.12 mg/L, 0.32 mg/L, and 0.012 mg/L, respectively. The 72-h ErC10, ErC50, and NOECr were 0.2 mg/L 0.33 mg/L, and 0.063 mg/L, respectively.

10.2.2.3. Other available data. Methyl 2-octynoate has been registered for REACH with no additional data at this time.

10.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	62.53			1000000	0.06253	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.6	2.6
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 0.06253 μg/L. The revised PEC/PNECs for EU and NA are not applicable and cleared at screening-level; therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 04/02/19.

11. 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
- **TOXNET:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 05/03/19.

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

Explanation of Cramer Classification

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

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q7. Heterocyclic? No
Q16. Common terpene (see Cramer et al., 1978 for detailed explanation)? ?No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? No
Q20. Aliphatic with some functional groups (see Cramer et al., 1978 for detailed explanation)? No
Q22. Common component of food? No, Class II (Class intermediate)

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.

Arctander, S., 1969. Perfume and Flavor Chemicals (Aroma Chemicals), vols. I and II Published by the author: Montclair, NJ (USA).

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of Cramer classification between Toxtree, the OECD QSAR Toolbox and expert judgment. Regulatory Toxicology and Pharmacology 71 (1), 52–62.

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

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.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food and cosmetics toxicology 16 (3), 255–276 Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., ... & Benfenati, E. (2010, July). CAESAR models for developmental toxicity. In Chemistry Central Journal (Vol. 4, No. S1, p. S4). Springer International Publishing.

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

Gilbert, A.N., Kemp, S.E., 1996. Odor perception phenotypes: multiple, specific hyperosmias to musks. Chem. Senses 21 (4), 411–416.

Griepentrog, F., 1959. Allergiestudien mit einfachen chemischen substanzen [Allergy studies with simple chemical substances]. Allerg. Asthma 5 (4/5), 224–226.

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.

Johnson, A.W., Goodwin, B.F.J., 1985. The draize test and modifications. In: Curr. Probl. Derm. in: Current Problems in Dermatology, vol. 14. pp. 31–38.

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. Int. Fed. Soc. Cosmet. Chem 9/18/79.

Klecak, G., Geleick, H., Frey, J.R., 1977. Screening of fragrance materials for allergenicity in the Guinea pig. I. Comparison of four testing methods. J. Soc. Cosmet. Chem. Jpn. 28, 53–64.

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, 2018. The OECD QSAR Toolbox, v3.2–4.2. Retrieved from. http://www.qsartoolbox.org/.
RIFM (Research Institute for Fragrance Materials, Inc.), 1965. Repeated Insult Patch Test with Methyl 2-octynoate (Methyl Heptine Carbonate). Unpublished report from International Flavors and Fragrances. RIFM report number 53565. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1972. The Contact-Sensitization Potential of Fragrance Materials by Maximization Testing in Humans. Report to RIFM. RIFM report number 1804. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1973. Maguire Hypersensitivity Test of Fragrance Materials in the guinea Pig. Report to RIFM. RIFM report number 5745. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1974. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1779. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1975. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1798. RIFM, Woodcliff Lake, NJ, USA.
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.), 1976. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1796. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Guinea Pig Skin Sensitization Test with Methyl 2-octynoate. Unpublished report from Quest International. RIFM report number 46495. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Guinea Pig Skin Sensitization Test with Methyl 2-octynoate. Unpublished report from Quest International. RIFM report number 46496. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Guinea Pig Skin Sensitization Test with Methyl 2-octynoate. Unpublished report from Quest International. RIFM report number 46499. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Methyl 2-octynoate (Folione): Skin Irritation and Capacity for Allergic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55855. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1977. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1702. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1977. Guinea Pig Skin Sensitization Test with Methyl 2-octynoate. Unpublished report from Quest International. RIFM report number 46493. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1979. Methyl 2-octynoate (Folione): Skin Irritation and Capacity of Allergenic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55857. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1979. Methyl 2-octynoate (Folione): Skin Irritation and Capacity of Allergenic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55859. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1979. Methyl 2-octynoate (Folione): Skin Irritation and Capacity of Allergenic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55860. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1979. Methyl 2-octynoate (Folione): Skin Irritation and Capacity of Allergenic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55861. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1980. Methyl 2-octynoate (Folione): Skin Irritation and Capacity of Allergenic Sensitization Determined by the Open Epicutaneous Test on guinea Pigs. Unpublished report from Givaudan. RIFM report number 55858. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1983. Guinea Pig Skin Sensitization Test with Methyl 2-octynoate. Unpublished report from Quest International. RIFM report number 46494. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1985. Closed Epicutaneous Test of Methyl-2-Octynoate, Methyl-2-Nonynoate, Benzyl Acetate, Trans,trans-2,4-Hexadienal, 2-hexylidene Cyclopentanone, Hexen-2-Al, Trans-2-hexenal Diethyl Acetal and Isoeugenol in guinea Pigs. Report to RIFM. RIFM report number 4474. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1985. Guinea Pig Maximization Test. Report to RIFM. RIFM report number 4899. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1985. Closed Epicutaneous Test of 2-hexylidenecyclopentanone, Methyl 2-nonynoate and Methyl 2-octynoate in guinea Pigs. Report to RIFM. RIFM report number 5175. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1985. Open and Closed Epicutaneous and Maximization Tests of Fragrance Materials in guinea Pigs. Unpublished report from Givaudan Corporation. RIFM report number 6068. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1986. Delayed Contact Hypersensitivity Study of Methyl Heptine Carbonate in guinea Pigs. Report to RIFM. RIFM report number 4465. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1989. Human Repeated Insult Patch Test of Methyl 2-octynoate. RIFM report number 12368. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 1990. Repeated Insult Patch Test of Methyl 2-octynoate in Human Subjects. RIFM report number 12452. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2000. Ready Biodegradability of Methyl 2-octynoate (Folione). Unpublished report from Givaudan. RIFM report number 55854. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2001. Inherent Biodegradability of Methyl 2-octynoate (Folione). Unpublished report from Givaudan. RIFM report number 55853. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2005. Methyl 2-octynoate Diluted with Vehicle 1:3 EtOH:DEP: Local Lymph Node Assay. RIFM report number 48753. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2006. Methyl 2-octynoate: Local Lymph Node Assay. RIFM report number 51627. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2008. Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients. RIFM report number 55663. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2013. Ready Biodegradability of Methyl 2-octynoate (Folione). Unpublished report from Givaudan. RIFM report number 65636. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015. Induction of Antioxidant-Response Element Dependent Gene Activity Cytotoxicity (Using MTT) in the Keratinocyte ARE- Reporter Cell Line Keratinosens. RIFM report number 69647. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2015. Exposure Survey 07, May 2015.
RIFM (Research Institute for Fragrance Materials, Inc.), 2017. Methyl 2-octynoate (Folione): Acute Toxicity to Daphnia Magna in a 48-hour Immobilization Test. Unpublished report from RIFM report number 72318. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc.), 2017. Methyl 2-octynoate (Folione): Effect on Pseudokirchneriella Subcapitata in a 72-hour Algal Growth Inhibition Test. Unpublished report from RIFM report number 72319. RIFM, Woodcliff Lake, NJ, USA.
Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Sharp, D.W., 1978. The sensitization potential of some perfume ingredients tested using a modified Draize procedure. Toxicology 9 (3), 261–271.
Troy, W.R., 1977. Doctoral Dissertation: the Comparative Respiratory Irritation Potential of Fourteen Fragrance Raw Materials (Unpublished). .
Urbisch, D., Mehling, A., Guth, K., Ramirez, T., Honarvar, N., et al., 2015. Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul. Toxicol. Pharmacol. 71 (2), 337–351.
US EPA, 2012. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.
Wild, D., King, M.T., Gocke, E., Eckhardt, K., 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, Basc and micronucleus tests. Food Chem. Toxicol. 21 (6), 707–719.