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RIFM fragrance ingredient safety assessment, N-[(ethoxycarbonyl) methyl)-p-menthane-3-carboxamide, CAS Registry Number 68489-14-5

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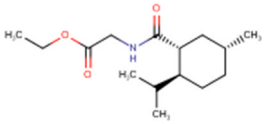

Name: N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide CAS Registry Number: 68489-14-5
39668-74-1 Glycine, N-[[5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester*
*Included because the materials are isomers

(*continued on next page*)

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(*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
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
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 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.

N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is not genotoxic. Data on N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). Data show that there are no safety concerns for N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide 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, 2005b; RIFM, 2017d; RIFM, 2006; RIFM, 2021)
Repeated Dose Toxicity: NOAEL = 225 mg/kg/day. RIFM, (2011)
Reproductive Toxicity: No NOAEL available. Exposure is below TTC.
Skin Sensitization: No concern for skin sensitization under the current, declared levels of use. (ECHA REACH Dossier: N-[(Ethoxycarbonyl)methyl)-p-menthane-3-carboxamide; ECHA, 2017; RIFM, 2017e; RIFM, 2018a)
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

(*continued on next page*)

(*continued*)

Hazard Assessment:	
Persistence:	
Critical Measured Value: 16% (OECD 301F) for CAS # 68489-14-5	RIFM, (2017b)
Bioaccumulation:	
Screening-level: 160.9 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 84.2 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 84.2 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.0842 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe (No VoU): Not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name**: N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide	1. **Chemical Name**: Glycine, N-[[5-methyl-2-(1-methylethyl)cyclohexyl] carbonyl]-, ethyl ester
2. **CAS Registry Number:** 68489-14-5	2. **CAS Registry Number:** 39668-74-1
3. **Synonyms:** [1R-(1α,2β,5.α)]-N-[[5-Methyl-2-(1-methylethyl)cyclohexyl] carbonyl glycine ethyl ester; Winsense; Symcool WS-5; Cooling Agent; Glycine, N-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] carbonyl]-, ethyl ester; N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide	3. **Synonyms:** Ethyl N-[[5-methyl-2-(isopropyl)cyclohexyl]carbonyl] glycinate; Glycine, N-[[5-methyl-2-(1-methylethyl)cyclohexyl] carbonyl]-, ethyl ester
4. **Molecular Formula:** $C_{15}H_{27}NO_3$	4. **Molecular Formula:** $C_{15}H_{27}NO_3$
5. **Molecular Weight:** 269.38 g/mol	5. **Molecular Weight:** 269.38 g/mol
6. **RIFM Number:** 1345	6. **RIFM Number:** 1345
7. **Stereochemistry:** Four stereocenters and 16 possible stereoisomers	7. **Stereochemistry:** Four stereocenters and 16 possible stereoisomers

2. Physical data

1. **Boiling Point**: Not available	1. **Boiling Point**: Not Available
2. **Flash Point:** Not Available	2. **Flash Point:** Not Available
3. **Log K_{OW}:** 2.73 (RIFM, 2005a)	3. **Log K_{OW}:** Not Available
4. **Melting Point**: Not available	4. **Melting Point:** Not Available
5. **Water Solubility:** Not available	5. **Water Solubility:** Not Available
6. **Specific Gravity:** Not Available	6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.00023 Pa at 25 °C (RIFM, 2017a)	7. **Vapor Pressure:** Not Available
8. **UV Spectra:** No absorbance between 290 and 500 nm	8. **UV Spectra**: Not Available
9. **Appearance/Organoleptic:** Not Available	9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

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

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

1. **95**th **Percentile Concentration in Toothpaste:** 0.10% (RIFM, 2017f)

(No reported use in hydroalcoholics).

2. **Inhalation Exposure*:** <0.0001 mg/kg/day or <0.0001 mg/day (RIFM, 2017f)
3. **Total Systemic Exposure**:** 0.0013 mg/kg/day (RIFM, 2017f)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. *Cramer Classification: class I*, Low (Expert judgment)*

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

*See Appendix below for details.

6.2. *Analogs selected*

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** None
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. *Read-across justification*

None.

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References:

None.

8. Natural occurrence

N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is not reported to occur in foods by the VCF*.

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

9. REACH dossier

Available for N-[(ethoxycarbonyl)methyl)-p-menthane-3-carboxamide; ECHA, 2017; accessed 04/11/22; glycine, N-[[5-methyl-2-(1--methylethyl)cyclohexyl]carbonyl]-, ethyl ester has been pre-registered for 2010; no dossier available as of 04/11/22.

10. Conclusion

The existing information supports the use of this material as described in this safety assessment.

11. Summary

11.1. Human Health Endpoint Summaries

11.1.1. Genotoxicity

Based on the current existing data, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. Increases in the mean number of revertant colonies were observed in strains TA100 and TA1535 in the absence of S9 (RIFM, 2005b). Under the conditions of the study, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was mutagenic in the Ames test.

Following the positive results observed in the previous Ames test, the mutagenic activity of N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide has been evaluated in a newer bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide in DMSO at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2017d). Under the conditions of the study, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was not mutagenic in the Ames test.

To verify the results observed in the Ames tests, a mammalian cell gene mutation assay (mouse lymphoma assay) was conducted according to OECD TG 476 and GLP guidelines. MLA cells were treated with N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide in DMSO at concentrations of 1008.75 μg/mL (as determined in a preliminary toxicity assay) for 4 and 24 h. Effects were evaluated both with and without metabolic activation. No statistically significant increases in the frequency of mutant colonies were observed with any concentration of the test material, either with or without metabolic activation (RIFM, 2006). Under the conditions of the study, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was not mutagenic to mammalian cells *in vitro*.

The clastogenic activity of N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide in DMSO at concentrations up to 2000 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 2000 μg/mL in the presence and absence of metabolic activation. N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide did not induce binucleated cells with micronuclei when tested up to cytotoxic levels or the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2021). Under the conditions of the study, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide does not present a concern for genotoxic potential.

Additional References: RIFM, 2004.

Literature Search and Risk Assessment Completed On: 11/06/20.

11.1.2. Repeated dose toxicity

The MOE for N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide. In a GLP and OECD 408-compliant subchronic toxicity study, 10 Crl:CD(SD) rats/sex/dose were administered N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide via gavage at doses of 0, 25, 75 and 225, and 675 mg/kg/day for 91 days. An additional 5 Crl:CD(SD) rats/sex/dose were administered N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide at 0 and 675 mg/kg/day and maintained as recovery groups for 2 weeks after the treatment period. No mortality occurred throughout the study period. No treatment-related effects were observed on body weights, food consumption, body temperature, or ophthalmic findings. Counts of neutrophils, monocytes, and white blood cells were significantly higher in both sexes at the highest dose (675 mg/kg/day). Hematocrit values were lower in males at 225 mg/kg/day, and in both sexes at 675 mg/kg/day. Reduced hematocrit and hemoglobin values, red blood cell counts, and mean corpuscular volume values, as well as increased red blood cell distribution width values, were seen in both sexes of the recovery group. Serum creatinine, urea nitrogen, calcium, and triglyceride levels, as well as total urine volume, were increased in both sexes at 675 mg/kg/day. Serum albumin/globulin ratios, chloride levels, and specific gravity values were decreased in both sexes at 675 mg/kg/day. However, all of these effects were reversed during the recovery period. Enlarged kidneys with a rough surface were seen in males (1/10), while pale kidneys were seen in females (1/10), at 675 mg/kg/day. Kidney and liver weights were increased in both sexes at 675 mg/kg/day, but these effects were reversed during the recovery period. Kidney effects such as tubular degeneration, regeneration, and dilatation, as well as hyaline droplets, interstitial fibrosis, and tubular epithelium vacuolation, were seen in both sexes at 675 mg/kg/day. Liver effects such as periportal hepatocellular vacuolation and centrilobular hepatocellular hypertrophy were seen in both sexes at 675 mg/kg/day. Cardiomyopathy was seen in females at 675 mg/kg/day. Kidney, liver, and heart effects were reduced but not fully reversed during the recovery period. Evidence of α-2u-globulin nephropathy (positive staining) was seen in males in all groups, but the mean severity scores of punctate and globular staining were

slightly higher in treatment groups than in the control. However, this effect is species- and sex-specific, and thus not relevant to human health. Based on adverse tubular degenerative changes in kidneys of both sexes, as well as cardiomyopathy in females at 675 mg/kg/day, the NOAEL for this study was considered to be 225 mg/kg/day (RIFM, 2011).

Therefore, the N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide MOE for the repeated dose toxicity endpoint can be calculated by dividing the N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide NOAEL in mg/kg/day by the total systemic exposure to N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide, 225/0.0013, or 173077.

In addition, the total systemic exposure to N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide (1.3 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/15/20.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide or any read-across materials. The total systemic exposure to N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure (1.3 μg/kg/day) is below the TTC for N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/06/20.

11.1.4. Skin sensitization

Based on the existing data, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide presents no concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Based on the existing data, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; OECD Toolbox v4.2). N-[(Ethoxycarbonyl)methyl)--*p*-menthane-3-carboxamide was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens test, but positive in the human cell line activation test (h-CLAT) (ECHA, 2018; 001 key study; 002 key study; 003 key study; RIFM, 2017e; RIFM, 2018a; RIFM, 2018b).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* studies, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/03/20.

11.1.5. Phototoxicity/Photoallergenicity

Based on the available UV spectra, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide in experimental models. UV absorption spectra indicate no absorption between 290 and 500 nm. As such, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. The available spectra indicate no absorbance in the range of 290–500 nm. As the material does not absorb in the range of interest, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/23/20.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide. Based on the Creme RIFM Model, the inhalation exposure is < 0.0001 mg/day. This exposure is 14000 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/05/20.

11.2. Environmental Endpoint Summary

11.2.1. Screening-level assessment

A screening-level risk assessment of N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide 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, N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide as possibly persistent but not bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	84.2			1000000	0.0842	

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 Volume of Use (2015), N-[(ethoxycarbonyl) methyl)-*p*-menthane-3-carboxamide presents no risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. ***Biodegradation:***

RIFM, 2015a**:** The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guidelines. Biodegradation of 15% was observed after 28 days.

RIFM, 2017b**:** The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guidelines. Biodegradation of 16% was observed after 28 days.

Ecotoxicity:

RIFM, 2015d**:** The *Daphnia magna* acute immobilization test (limit test) was conducted according to the OECD 202 guidelines under static conditions. The 48-h EC50 value based on nominal test concentrations was reported to be greater than 100 mg/L.

RIFM, 2015b**:** The acute fish (*Oncorhynchus mykiss*) toxicity test was conducted according to the OECD 203 guidelines under semi-static conditions. The 96-h LC50 value based on nominal test concentration was reported to be 5.7 mg/L (95% CI: 4.1–7.8 mg/L).

RIFM, 2017c**:** The acute fish (*Gobiocypris rarus*) toxicity test was conducted according to the OECD 203 guidelines under semi-static conditions. The 96-h LC50 value based on nominal test concentration was reported to be 6.90 mg/L (95% CI: 5.93–8.02 mg/L).

RIFM, 2015c**:** The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC50 value based on nominal test concentration for growth rate and yield were reported to be 76 mg/L and 36 mg/L (95% CI: 33–40 mg/L), respectively.

11.10 Other available data

N-[(Ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide (CAS # 68489-14-5) has been registered for REACH with no additional information available at this time.

11.2.3. Risk assessment refinement

Since N-[(ethoxycarbonyl)methyl)-*p*-menthane-3-carboxamide has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	2.73	2.73
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	No VoU	1–10
Risk Characterization: PEC/PNEC	**NA**	**<1**

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

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

The RIFM PNEC is 0.0842 μg/L. The revised PEC/PNECs for EU (No VoU) 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: 11/03/20.

12. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 04/11/22.

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. A normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q43. Possibly harmful divalent sulfur (not detected via Q3)? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q42. Possibly harmful analog of benzene? No
Q7. Heterocyclic? No
Q16. Common terpene? (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? Yes
Q18. One of the list? (see Cramer et al., 1978 for a detailed explanation on the list of categories)? No, Low (Class I)

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