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Short Review

RIFM fragrance ingredient safety assessment, piperonal, CAS Registry Number 120-57-0

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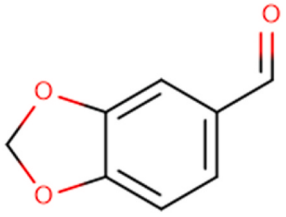

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Name: Piperonal
CAS Registry Number: 120-57-0

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
CAESAR - Computer-Assisted Evaluation of industrial chemical Substances According to Regulations

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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., 2015, 2017, 2024) 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; please note that the citation dates used for studies sourced from the ECHA website are the dates the dossiers were first published, not the dates that the studies were conducted
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
HESS - Hazard Evaluation Support System; a repeated dose profiler that is used to identify the toxicological profiler of chemicals
IFRA - The International Fragrance Association
IRB - Institutional Review Board
ISS - Istituto Superiore di Sanità (Italian National Institute of Health)
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OASIS - OASIS Laboratory of Mathematical Chemistry (LMC)
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
Toxtree - an *in silico* tool that can estimate toxic hazard by applying a decision tree approach
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 material has not been fully evaluated for photoallergenic potential.

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 who provide RIFM with guidance relevant to human health and environmental protection.

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Summary: The existing information supports the use of this material as described in this safety assessment. This material has not been fully evaluated for photoallergenic potential.

Piperonal was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that piperonal is not genotoxic. Data on piperonal provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints and a No Expected Sensitization Induction Level (NESIL) of 4100 μg/cm^2 for the skin sensitization endpoint. The photoirritation endpoint was evaluated based on data; piperonal is not photoirritating. Piperonal was not evaluated for photoallergenicity due to a lack of suitable data and validated *in vitro* tests. To address this data gap, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of piperonal. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material, and the exposure to piperonal is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; piperonal 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 (VoU) 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, 1983; RIFM, 1988)
Repeated Dose Toxicity: NOAEL = 300 mg/kg/day.	ECHA (2015)
Developmental and Reproductive Toxicity: NOAEL = 300 mg/kg/day.	ECHA (2015)
Skin Sensitization: NESIL = 4100 μg/cm^2.	RIFM (2023)
Photoirritation/Photoallergenicity: Not photoirritating. Not evaluated for photoallergy.	(RIFM, 1981; Tenenbaum et al., 1984)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:	
Persistence: Critical Measured Value: 99.3% (OECD 301 B)	RIFM (1993)
Bioaccumulation: Screening-level: 2.29 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Critical Measured Value: 96-h Fish LC50: 2.5 mg/L	ECHA (2015)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) > 1	(Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 96-h Fish LC50 (OECD 203): 2.5 mg/L	ECHA (2015)
RIFM PNEC is: 2.5 μg/L	
• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe <1	

1. Identification

1. **Chemical Name:** Piperonal
2. **CAS Registry Number:** 120-57-0
3. **Synonyms:** 1,3-Benzodioxole-5-carboxaldehyde; Dioxymethylene protocatechuic aldehyde; Heliotropine; Heliotropin; 3,4-Methylenedioxybenzaldehyde; Piperonyl aldehyde; Protocatechuic aldehyde methylene ether; Piperonylaldehyde; Piperpmaldehyde; 5-Formylbenzodioxole; 3,4-Dimethylenedioxybenzaldehyde; Helio-Tropin; ピペロナール; 1,3-Benzodioxole-5-carbaldehyde; Heliotropine Crystals; Piperonal
4. **Molecular Formula:** $C_8H_6O_3$
5. **Molecular Weight:** 150.13 g/mol
6. **RIFM Number:** 146
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 264 °C (Bowles and Juneja, 1998), 265 °C (Fragrance Materials Association [FMA]), 256.96 °C (EPI Suite v4.11)

2. **Flash Point:** >200 °F (closed cup) (FMA), >93 °C (Globally Harmonized System)
3. **Log K_{OW}:** 1.05 (Abraham and Rafols, 1995), 1.2 at 35 °C (RIFM, 1997), 1.77 (EPI Suite v4.11)
4. **Melting Point**: 35 °C (FMA), 35–37 °C (Dahl, 1982), 55.96 °C (EPI Suite v4.11)
5. **Water Solubility:** 9612 mg/L at 25 °C (EPI Suite v4.11)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.003 mm Hg at 20 °C (FMA), 0.0106 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm, with peak absorbance at 310 nm and returning to baseline by 350 nm; molar absorption coefficient (6370 L mol^{-1} • cm^{-1}) is above the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** White or colorless, lustrous crystals with heliotrope odor or very sweet, floral, warm, slightly spicy odor

3. Volume of use (worldwide band)

1. 100–1000 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v2.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.44% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00055 mg/kg/day or 0.041 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0089 mg/kg/day (RIFM, 2019)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017, 2024).

**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, 2017; Safford et al., 2015, 2017, 2024).

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)

Expert Judgment*	Toxtree v3.1	OECD QSAR Toolbox v4.5 (OECD, 2021b)
II	III	III

*See the Appendix below for details.

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

7. Metabolism

No relevant data available for inclusion in this safety assessment. **Additional References:** None.

8. Natural occurrence

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

Capers (Capparis spinoza)	*Mangifera* species
Chicken	Melon
Dill (*Anethum* species)	Pepper (*Piper nigrum* L.)
Sherry (non-categorized)	Vanilla
Vaccinium species	

*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 (ECHA, 2015); accessed on 10/09/24.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for piperonal are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.32
2	Products applied to the axillae	0.094
3	Products applied to the face/body using fingertips	1.9
4	Products related to fine fragrances	1.8
5 A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.45
5 B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.45
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.45
5D	Baby cream, oil, talc	0.15
6	Products with oral and lip exposure	1.0
7	Products applied to the hair with some hand contact	3.6
8	Products with significant ano-genital exposure (tampon)	0.15
9	Products with body and hand exposure, primarily rinse-off (bar soap)	3.4
10 A	Household care products with mostly hand contact (hand dishwashing detergent)	8.3
10 B	Aerosol air freshener	12
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.15
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 piperonal, the basis was the subchronic reference dose of 3.0 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 4100

μg/cm^2.
As a conservative approach, we assumed that 100% of the material exposed via the skin is bioavailable (see Section V), thereby deriving the most stringent MOE. Since the MOE is > 100 (see the repeated dose and reproductive toxicity sections), we then refined the exposure to 80% using an *in silico* Skin Absorption Model (SAM) to determine the Maximum Allowable Concentrations for each category listed in Section X.
[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA/Guidance-for-the-use-of-IFRA-Standards.pdf; December 2019).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.4.1.

11. Summary

11.1. Human Health Endpoint Summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk Assessment. The mutagenic activity of piperonal 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with piperonal in dimethyl sulfoxide (DMSO) at concentrations up to 10,000 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, 1983). Under the conditions of the study, piperonal was not mutagenic in the Ames test.

The clastogenicity of piperonal was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster ovary cells were treated with piperonal in DMSO at concentrations up to 5000 μg/mL in the dose range finding study; the main study was conducted at concentrations up to 5000 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (RIFM, 1988). Under the conditions of the study, piperonal was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

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

Additional References: Sekizawa and Shibamoto, 1982; White et al., 1977; Heck et al., 1989; RIFM, 1983; Kasamaki et al., 1982; Kasamaki and Urasawa, 1985; Oda et al., 1978; RIFM, 1982.

Literature Search and Risk Assessment Completed On: 10/06/23.

11.1.2. Repeated Dose Toxicity

The MOE for piperonal is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk Assessment. There is sufficient repeated dose toxicity data on piperonal. In a GLP- and OECD 422-compliant study, 10 Wistar Han rats/sex/dose were administered piperonal via gavage at doses of 0, 100, 300, and 1000 mg/kg/day for a minimum of 13 weeks. No mortality occurred up to the highest dose. Trabecular bone was significantly increased in females at the mid dose and in both sexes at the high dose, diminishing the space for the bone marrow. This primarily affected bone adjacent to the intervertebral discs for the sternum. Due to the nature and severity of this effect, it was considered to be adverse at the high dose but not at the mid dose. Serum levels of thyroxine (T4) were significantly decreased in males at all doses in a dose-dependent manner; however, there were no corresponding effects on thyroid stimulating hormone (TSH) values, thyroid weight, or histopathology, and thus, this effect was not considered adverse. Furthermore, this effect remained within historical control ranges at the low dose and the mid dose. Body weights and bodyweight gains were significantly decreased in males at the high dose, but there was no effect on food consumption. Alanine aminotransferase, alkaline phosphatase, bile acids, and inorganic phosphate were significantly increased, while platelets and cholesterol (including high-density lipoprotein and low-density lipoprotein) were significantly decreased in males at the high dose. Hematocrit and platelets were significantly decreased, while serum glucose was significantly increased in females at the high dose. However, in the absence of adverse anatomic pathology findings or functional impairments, these clinical pathology parameters were not considered adverse. Increased liver weight and hepatocellular hypertrophy incidence (minimal severity) were observed in males at the high dose but were not considered adverse due to the absence of correlated gross or histopathological findings. Lymphoid atrophy in the thymus and decreased thymus weight were observed in males at the high dose; however, due to their minimal severity, these effects were considered non-adverse. Incidences of cystic epithelial hyperplasia in the thymus were increased in females at the high dose. Based on adverse increases in trabecular bone in both sexes at 1000 mg/kg/day, the NOAEL for this study was considered to be 300 mg/kg/day (ECHA, 2015).

Therefore, the piperonal MOE for the repeated dose toxicity endpoint can be calculated by dividing the piperonal NOAEL in mg/kg/day by the total systemic exposure for piperonal, 300/0.0089 or 33,707.

In addition, the total systemic exposure to piperonal (8.9 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 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 Subchronic 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. (2020) and a subchronic RfD of 3.0 mg/kg/day.

The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The subchronic RfD for piperonal was calculated by dividing the lowest NOAEL (from the Repeated Dose or Reproductive Toxicity sections) of 300 mg/kg/day by the uncertainty factor, 100 = 3.0 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/04/23.

11.1.3. Reproductive Toxicity

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

11.1.3.1. Risk Assessment. There are sufficient reproductive toxicity data on piperonal. In a GLP- and OECD 422-compliant study, 10 Wistar Han rats/sex/dose were administered piperonal via oral gavage at doses of 0, 100, 300, and 1000 mg/kg/day for a minimum of 13 weeks. No treatment-related changes were detected in the mating index, estrous

cycle, precoital time, spermatogenic profiling, or histopathology of reproductive organs. However, a decreased number of implantation sites and a low fertility index were observed at the high dose – none of the pairs treated at the high dose could produce healthy offspring. Increased trabecular bone was observed in 2/10 females in the mid-dose group and in 9/10 males and 10/10 females in the high-dose group. Given the incidence and severity, this finding was considered adverse at 1000 mg/kg/day and non-adverse at 300 mg/kg/day. Furthermore, blood calcium levels were significantly increased in females at 1000 mg/kg/day. Pituitary weight was significantly lower in females in the mid-and high-dose groups and in males only in the high-dose group. Thus, based on the lack of healthy offspring and low fertility index at 1000 mg/kg/day, the developmental toxicity and fertility NOAEL for the study was considered to be 300 mg/kg/day (ECHA, 2015).

In a GLP- and OECD 414-compliant study, 22 female Wistar Han rats/dose were administered piperonal via gavage at doses of 0, 100, 300, and 1000 mg/kg/day from days 6–20 post-coitum. No mortality occurred throughout the study period. No treatment-related effects were observed for the number of pregnant females, corpora lutea, implantation sites, or pre-implantation loss. Body weight, food consumption, and gravid uterus weight were significantly reduced at the high dose. Piloerection and hunched posture were observed in most females at the high dose. Total triiodothyronine (T3) and total T4 levels were decreased in a dose-dependent manner at all doses but only attained statistical significance at the highest dose. TSH levels were increased in a dose-dependent manner at all doses, but this change was not statistically significant at any dose. A 100% early resorption rate was observed in 2 females at the high dose; based on the rarity and severity of this effect, it was considered adverse. Mean litter size was reduced at the high dose. Mean fetal body weights of males and females were significantly reduced at the high dose. Incidence of adverse fetal morphological findings was significantly increased at the high dose. These findings included vertebral anomaly with or without associated rib anomaly, rib anomaly, vertebral centra anomaly, sternoschisis, and costal cartilage anomaly. All were localized in the same thoracic body region. Incidence of various ossification parameters (reduced ossification of the skull, vertebral centra, arches, pubis, and ribs; unossified metacarpals and/or metatarsals and sternebrae) were also increased at the mid dose and the high dose; however, these changes were considered secondary to reduced fetal weights (indicating delayed skeletal ossification), and thus were not considered adverse. Incidences of dilated ureter, convoluted ureter, and absent or small renal papillae were increased at the mid dose and the high dose; these changes were considered to be reversible and secondary to lower fetal body weights and, thus, non-adverse. Incidence of 7th cervical full ribs, 14th full ribs, caudal shift of the pelvic girdle, and misaligned sternebrae were increased at the high dose, while the incidence of 7th cervical ossification sites and bent ribs were increased at the mid dose and the high dose; these changes were all considered non-adverse. Thus, based on clinical signs, lower body weight, and reduced food intake at 1000 mg/kg/day, the fertility NOAEL was considered to be 300 mg/kg/day. Based on reduced fetal weights, skeletal malformations, and 2 cases of 100% implantation loss, the developmental toxicity NOAEL was considered to be 300 mg/kg/day (ECHA, 2015).

The points of departure for the developmental toxicity endpoint were congruent between the OECD 422 and OECD 414 studies. Therefore, the piperonal MOE for the developmental toxicity endpoint can be calculated by dividing the piperonal NOAEL in mg/kg/day by the total systemic exposure for piperonal, 300/0.0089 or 33,708.

The points of departure for the fertility endpoint were congruent between the OECD 422 and OECD 414 studies. Therefore, the piperonal MOE for fertility can be calculated by dividing the piperonal NOAEL in mg/kg/day by the total systemic exposure for piperonal, 300/0.0089 or 33,708.

In addition, the total systemic exposure to piperonal (8.9 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/04/23.

11.1.4. Skin Sensitization

Based on the existing data, piperonal is considered a skin sensitizer with a defined NESIL of 4100 μg/cm^2, and the maximum acceptable concentrations in finished products are provided in Section X.

11.1.4.1. Risk Assessment. Based on the existing data, piperonal is considered a skin sensitizer (Table 1). This material is predicted *in silico* to be reactive with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). Piperonal was found to be positive in a direct peptide reactivity assay (DPRA) and human cell line activation test (h-CLAT), borderline in a KeratinoSens, and negative in a U-SENS test (RIFM, 2016; RIFM, 2020a; RIFM, 2018; RIFM, 2020b). The results were evaluated following the OECD Guideline No. 497: Defined Approaches on Skin Sensitization (OECD, 2021a), and based on the 2 out of 3 Defined Approach, piperonal is a sensitizer. In a murine local lymph node assay (LLNA), piperonal was found to be sensitizing with an EC3 value of 25% (6250 μg/cm^2) (RIFM, 2012). In a guinea pig maximization test, piperonal led to skin sensitization reactions (Klecak et al., 1977; RIFM, 1977a). In a human maximization test, no skin sensitization reactions were observed when tested at 4140 μg/cm^2 (Greif, 1967). Additionally, in a Confirmation of No Induction in Humans (CNIH) test with 2953 μg/cm^2 of piperonal in 1:3 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 112 volunteers (RIFM, 2011). Additionally, in a CNIH test with 4109 μg/cm^2 of piperonal in 1:3 EtOH:DEP, no reactions indicative of sensitization were observed in any of the 99 volunteers (RIFM, 2023).

Based on weight of evidence (WoE) from structural analysis, *in vitro* studies, animal studies, and human studies, piperonal is a sensitizer with a WoE NESIL of 4100 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (2020) and a subchronic RfD of 3.0 mg/kg/day.

Additional References: Klecak (1979); Ishihara et al., 1986; Klecak (1985); RIFM, 1977b; RIFM, 1964.

Literature Search and Risk Assessment Completed On: 09/07/23.

11.1.5. Photoirritation/Photoallergenicity

Based on the available *in vivo* and human study data, piperonal would not be expected to present a concern for photoirritation. Piperonal was not evaluated for photoallergy. However, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of piperonal.

11.1.5.1. Risk Assessment. UV/Vis absorption spectra for piperonal

Table 1
Summary of existing data on piperonal.

WoE Skin Sensitization Potency Category[1]	Human Data				Animal Data		
	NOEL-CNIH (induction) µg/cm²	NOEL-HMT (induction) µg/cm²	LOEL (induction) µg/cm²	WoE NESIL[2] µg/cm²	LLNA[3] Weighted Mean EC3 Value µg/cm²	GPMT[4]	Buehler
Weak	4109	4140	N/A	4100	6250 (25%)	Positive	N/A
	In vitro Data[5]				*In silico* protein binding alerts (OECD Toolbox v4.5)		
	KE 1	KE 2	KE 3		Target Material	Autoxidation simulator	Metabolism simulator
	Positive	Borderline	Positive (h-CLAT); Negative (U-Sens)		Schiff base formation	No alert found	Schiff base formation

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans; HMT = Human Maximization Test; LOEL = lowest observed effect level; EC3 = concentration of test chemical required to induce a 3-fold increase in lymph node cell proliferation; GPMT = Guinea Pig Maximization Test; KE = Key Event; N/A = Not Available.
[1]WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).
[2]WoE NESIL limited to 2 significant figures.
[3]Based on animal data using classification defined in the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Technical Report No. 87 (ECETOC, 2003).
[4]Studies conducted according to the OECD TG 406 are included in the table.
[5]Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

indicate that the material absorbs in the region of 290–700 nm, with peak absorbance at 310 nm and returning to baseline by 350 nm. The molar absorption coefficient is above the benchmark of concern for photoirritating effects (Henry et al., 2009). In a human photoirritation test, topical application of 0.1% piperonal in ethanol did not result in photoirritation (RIFM, 1981). In a guinea pig photoirritation study, there were no observed effects of topical application of 25% piperonal (Tenenbaum et al., 1984). Based on the available human and *in vivo* study data, piperonal does not present a concern for photoirritation. Piperonal was not evaluated for photoallergy. However, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of piperonal.

11.1.5.2. UV Spectra Analysis. UV/Vis absorption spectra (OECD TG 101) were generated for piperonal. The spectra demonstrate significant absorbance between 290 and 700 nm, with peak absorbance at 310 nm and returning to baseline by 350 nm. The peak molar absorption coefficient (6370 L mol^{-1} • cm^{-1}) within this range is above the benchmark of concern for photoirritating effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/28/23.

11.1.6. Local Respiratory Toxicity

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

11.1.6.1. Risk Assessment. There are insufficient inhalation data available on piperonal. Based on the Creme RIFM Model, the inhalation exposure is 0.041 mg/day. This exposure is 24.4 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

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

Additional References: Lorber (1972); UGCM, 1997; Piedelievre and Derobert, 1942

Literature Search and Risk Assessment Completed On: 10/03/23.

11.2. Environmental Endpoint Summary

11.2.1. Screening-level Assessment

A screening-level risk assessment of piperonal 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 of 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, piperonal was identified as a fragrance material with the 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 piperonal as possibly being 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 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, 2017). 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.

11.2.1.1. Risk Assessment. Based on the current VoU (IFRA, 2019), piperonal presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key Studies

11.2.1.2.1. Biodegradation. **RIFM, 1993:** The biodegradability was determined using the sealed bottle test based on the OECD 301 B guideline. The mean biodegradation at 28 days was 99.1%.

RIFM, 1998: The biodegradability was determined by the manometric respirometry test according to OECD Guidelines 301 F. Mineral medium inoculated with fresh activated sludge and 100 mg/L of piperonal was Incubated for 28 days. The biodegradation rate was 81% at the end of the 10-day window and was 82% after 28 days.

11.2.1.2.2. Ecotoxicity. No data available.

11.2.1.2.3. Other Available Data. Piperonal has been registered under REACH, and the following additional data is available (ECHA, 2015):

A 96-h fish (carp) acute toxicity study was conducted according to the OECD 203 method under static conditions, and the LC50 was reported to be 2.5 mg/L.

A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under static conditions, and the 48-h EC50 was reported to be 52 mg/L.

An algae inhibition study was conducted according to the OECD 201 method, and the 72-h ErC50 was reported to be 31 mg/L, and the EyC50 was 6.8 mg/L.

11.2.1.3. 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*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1005			1000000	1.005	
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	11.29	20.42	29.59			Aldehydes (mono)
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	448.3	0.168	1.475	10000	0.0168	Benzodioxoles
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	197.5	109.8	75.01			Neutral Organic

Tier 3: Measured Data (Including REACH Data)

	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	2.5			1000	2.5	
Daphnia		52				
Algae		6.8				

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	1.2	1.2
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional VoU Tonnage Band	100–1000	100–1000
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 2.5 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 10/03/23.

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
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine Technical Bulletin:** https://www.nlm.nih.gov/pubs/techbull/nd19/nd19_toxnet_new_locations.html
- **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 ChemView:** https://chemview.epa.gov/chemview/
- **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://pubchem.ncbi.nlm.nih.gov/source/ChemIDplus

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 11/21/24.

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

Explanation of Cramer Classification:

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).

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, divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? Yes
Q8. Lactone or cyclic diester? No
Q10. Three-membered heterocycle? No
Q11. Has a heterocyclic ring with complex substituents.? No
Q12. Heteroaromatic? No
Q22. A Common component of food? Yes, Class Intermediate (Class II)

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