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Short Review

RIFM fragrance ingredient safety assessment, *o*-methoxybenzaldehyde, CAS Registry Number 135-02-4

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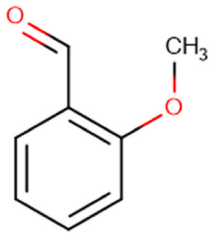


Name: *o*-Methoxybenzaldehyde
CAS Registry Number: 135-02-4

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., 2015, 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; 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
IFRA - The International Fragrance Association
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
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

(*continued on next column*)

(*continued*)

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 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. This material has not been fully evaluated for photoallergenic potential.

o-Methoxybenzaldehyde was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog *p*-methoxybenzaldehyde (CAS # 123-11-5) show that *o*-methoxybenzaldehyde is not expected to be genotoxic and 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 3500 μg/cm^2 for the skin sensitization endpoint. The photoirritation endpoint was evaluated based on data from read-across analog *p*-methoxybenzaldehyde (CAS # 123-11-5); *o*-methoxybenzaldehyde is not expected to be photoirritating. *o*-Methoxybenzaldehyde has not been fully evaluated for photoallergenic potential. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to *o*-methoxybenzaldehyde is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; *o*-methoxybenzaldehyde 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 expected to be genotoxic.	(NTP, 2002; ECHA, 2013; Ishidate et al., 1984)
Repeated Dose Toxicity: NOAEL = 100 mg/kg/day.	RIFM (2018)
Reproductive Toxicity: Developmental Toxicity NOAEL = 20 mg/kg/day. Fertility NOAEL = 100 mg/kg/day.	(RIFM, 2018; JEHB, 2010)
Skin Sensitization: NESIL = 3500 μg/cm^2.	RIFM (2016a)
Photoirritation/Photoallergenicity: Not photoirritating/not evaluated for photoallergy.	RIFM (2002)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence:	
Screening-level: 2.8625 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 6.337 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: LC50: 279.7 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: LC50: 279.7 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.2797 μg/L

- **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at the screening-level

1. Identification

1. **Chemical Name:** *o*-Methoxybenzaldehyde
2. **CAS Registry Number:** 135-02-4
3. **Synonyms:** *o*-Anisaldehyde; Benzaldehyde, 2-methoxy-; Benzaldehyde, 2-methoxy-; 2-Anisaldehyde; 2-Methoxybenzaldehyde; 2-Methoxybenzenecarboxaldehyde; 2-Methoxyphenylformaldehyde; *o*-Formylanisole; Salicylaldehyde methyl ether; ﾒﾄｷｼﾍﾞﾝｽﾞｱﾙﾃﾞﾋﾄﾞ; *o*-Methoxybenzaldehyde
4. **Molecular Formula:** $C_8H_8O_2$
5. **Molecular Weight:** 136.15 g/mol
6. **RIFM Number:** 912
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 238 °C (Private communication to FEMA), 238 °C (Fragrance Materials Association [FMA]), 221.63 °C (EPI Suite v4.11)
2. **Flash Point:** >200 °F; closed cup (FMA), 118 °C (Private communication to FEMA)
3. **Log K_{OW}:** 1.79 (EPI Suite v4.11)
4. **Melting Point**: 37 °C (FMA), 35 °C (Private communication to FEMA), 12.84 °C (EPI Suite v4.11)
5. **Water Solubility:** 2951 mg/L (EPI Suite v4.11)
6. **Specific Gravity:** 1.127 (Private communication to FEMA)
7. **Vapor Pressure:** 0.0166 mm Hg at 20 °C (EPI Suite v4.0), 0.0286 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm, with a peak at 330 nm and returning to baseline by approximately 360 nm. Molar absorbance coefficients (1168, 2589, 2329 L $mol^{-1} \bullet cm^{-1}$ under neutral, acidic, and basic conditions, respectively) are above the benchmark of concern (1000 L $mol^{-1} \bullet cm^{-1}$)
9. **Appearance/Organoleptic:** Colorless or creme-colored crystals or translucent crystalline mass. Faint sweet balsamic and floral odor of good tenacity (Arctander, 1969)

3. Volume of use (worldwide band)

1. <0.1 metric ton per year (IFRA, 2019)

4. Exposure to fragrance ingredient (creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.0018% (RIFM, 2020b)
2. **Inhalation Exposure*:** 0.0000043 mg/kg/day or 0.00035 mg/day (RIFM, 2020b)
3. **Total Systemic Exposure**:** 0.000076 mg/kg/day (RIFM, 2020b)

*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, 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, 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.5
I	I	I

2. **Analogs Selected**:
a. **Genotoxicity:** *p*-Methoxybenzaldehyde (CAS # 123-11-5)
b. **Repeated Dose Toxicity:** *p*-Methoxybenzaldehyde (CAS # 123-11-5)
c. **Reproductive Toxicity:** *p*-Methoxybenzaldehyde (CAS # 123-11-5)
d. **Skin Sensitization:** *p*-Methoxybenzaldehyde (CAS # 123-11-5)
e. **Photoirritation/Photoallergenicity:** *p*-methoxybenzaldehyde (CAS # 123-11-5)
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None
3. **Read-across Justification**: See Appendix below

7. Metabolism

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

8. Natural occurrence

o-Methoxybenzaldehyde is reported to occur in the following foods by the VCF*:
Cinnamomum species.
Honey.
Vanilla.
*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

o-Methoxybenzaldehyde has been pre-registered for 2010; no dossier available as of 06/06/23.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for *o*-methoxybenzaldehyde 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)	0.018
2	Products applied to the axillae	0.046
3	Products applied to the face/body using fingertips	0.0092
4	Products related to fine fragrances	0.43
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.38
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.092

(*continued on next page*)

(*continued*)

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0092
5D	Baby cream, oil, talc	0.0031
6	Products with oral and lip exposure	0.29
7	Products applied to the hair with some hand contact	0.018
8	Products with significant anogenital exposure (tampon)	0.0031
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.17
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.046
10B	Aerosol air freshener	0.073
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.0031
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	3.3

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 *o*-methoxybenzaldehyde, the basis was the reference dose of 0.20 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 3500 μg/cm^2.

[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf; December 2019).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.2.10.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, *o*-methoxybenzaldehyde does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Although there were 2 Ames studies conducted on *o*-methoxybenzaldehyde, using *Salmonella typhimurium* strains TA97 and TA102 (Marcus and Lichtenstein, 1982) and TA100 and TA98 (Fujita and Sasaki, 1987) with negative results, the tests were not conducted according to OECD guidelines and GLP conditions; hence read-across data on *p*-methoxybenzaldehyde (CAS # 123-11-5; see Section VI) was considered to assess genotoxicity potential of *o*-methoxybenzaldehyde.

The mutagenic activity of read-across material *p*-methoxybenzaldehyde was assessed in an Ames study conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the modified preincubation method. *Salmonella typhimurium* strains TA 98, TA100, TA1535, and TA97 were treated with *p*-methoxybenzaldehyde in dimethyl sulfoxide (DMSO) at concentrations up to 3333 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (NTP, 2002). Under the conditions of the study, p-methoxybenzaldehyde was considered not mutagenic in the Ames test.

The potential clastogenicity of *o*-methoxybenzaldehyde was assessed using read-across analog p-methoxybenzaldehyde. An *in vitro* chromosomal aberration test using Chinese hamster fibroblasts (CHL) was performed on the analog. CHL cells were treated with *p*-methoxybenzaldehyde in DMSO at 3 different concentrations up to 0.5 μg/mL for 48 h in the absence of metabolic activation. There were no polyploid erythrocytes detected at 48 h, and no significant increases in chromosomal aberrations were observed (Ishidate et al., 1984). Similar negative results were obtained in a GLP *in vitro* chromosome aberration study conducted by the Japanese National Institute of Health Sciences at concentrations up to 1362 μg/mg (JECDB, 2000). Conflicting data do exist in multiple studies where p-methoxybenzaldehyde was shown to induce chromosomal aberrations in non-GLP-compliant studies conducted in accordance with OECD TG 473. However, it has been argued that the established rodent cell lines, which are used in studies such as the *in vitro* SCE, as well as other genotoxicity testing, are p53 deficient and may give rise to false-positive results compared to human-derived cell cultures or primary cells (Fowler et al., 2012; Chuang et al., 1997). Further *in vivo* testing in mice has shown that *p*-methoxybenzaldehyde did not result in clastogenic effects in the bone marrow (ECHA, 2013; Exp WoE Genetic toxicity *in vivo*.001). Briefly, male ddY mice were orally administered a single dose of *p*-methoxybenzaldehyde in olive oil at concentrations of 250, 313, and 500 mg/kg. After 24 h, animals were euthanized, and smears were prepared. Chromosome aberrations were monitored by the occurrence of polychromatic erythrocytes with micronuclei in bone marrow cells. It should be noted that the study was an evaluation of X-ray-(200 rad) induced chromosome aberrations after *p*-methoxybenzaldehyde was given orally to mice. Control animals included those that were given the test material without radiation. At the end of the study, there were no effects on the frequency of PCEs in any of the test groups. Under the conditions of the study, *p*-methoxybenzaldehyde did not cause chromosome damage in the test system.

Based on the available data, *p*-methoxybenzaldehyde does not present a concern for genotoxic potential, and this can be extended to *o*-methoxybenzaldehyde.

Additional References: Rapson et al., 1980; Florin et al., 1980; Kasamaki et al., 1982; Muller et al., 1993.

Literature Search and Risk Assessment Completed On: 05/20/22.

11.1.2. Repeated dose toxicity

The MOE for *o*-methoxybenzaldehyde is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on *o*-methoxybenzaldehyde. Read-across material *p*-methoxybenzaldehyde (CAS # 123-11-5; see Section VI) has sufficient data to support the repeated dose toxicity endpoint.

In an OECD 408 and GLP-compliant study, 10 Wistar rats/sex/dose were administered *p*-methoxybenzaldehyde via gavage (vehicle: corn oil) at doses of 0, 20, 100, and 500 mg/kg/day for 90 days. No mortality occurred throughout the study period. No treatment-related effects were seen in clinical signs, food consumption, water consumption, body weights, functional observation battery, motor activity, ophthalmology, organ weights, or gross lesions. Absolute and relative eosinophil counts were reduced in both sexes at the high dose. Total protein levels were reduced in both sexes at the high dose. Glucose and inorganic phosphate levels were reduced in males at the high dose. Urine pH values were decreased in both sexes at the high dose. Specific gravity and incidences of crystals of unknown origin were increased in females at the high dose. Based on adverse effects detected by hematology, clinical chemistry, and urinalysis seen in both sexes at 500 mg/kg/day, the repeated dose toxicity NOAEL for this study was considered to be 100 mg/kg/day (RIFM, 2018).

In a GLP/OECD 422-compliant study, groups of 13 Sprague Dawley (Crj:CD(SD)IGS) rats/sex/dose were administered *p*-methoxybenzaldehyde via gavage (vehicle: corn oil) at doses of 0, 20, 100, or 500 mg/kg/day. Males were dosed for 2 weeks of premating, 2 weeks of mating, and 2 weeks after mating; females were dosed for 2 weeks of premating, 2 weeks of mating, and throughout pregnancy to day 4 of

lactation. At the high dose, there was a significant increase in the relative liver weights of male rats, while female rats exhibited a significant increase in the absolute liver weights. Histopathological examinations revealed centrilobular hypertrophy of hepatocytes in these animals. Thus, based on liver effects observed at 500 mg/kg/day, the repeated dose toxicity NOAEL for this study was considered to be 100 mg/kg/day (JEHB, 2010).

The NOAEL for the repeated dose toxicity endpoint was taken from the OECD 408-compliant study and determined to be 100 mg/kg/day.

Therefore, the *o*-methoxybenzaldehyde MOE for the repeated dose toxicity endpoint can be calculated by dividing the *p*-methoxybenzaldehyde NOAEL in mg/kg/day by the total systemic exposure to *o*-methoxybenzaldehyde, 100/0.000076 or 1315789.

In addition, the total systemic exposure to *p*-methoxybenzaldehyde (0.076 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.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/12/22.

11.1.3. Reproductive toxicity

The MOE for *o*-methoxybenzaldehyde is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on *o*-methoxybenzaldehyde. Read-across material *p*-methoxybenzaldehyde (CAS # 123-11-5; see Section VI) has sufficient data to support the repeated dose toxicity endpoint.

In an OECD 408 and GLP-compliant study, 10 Wistar rats/sex/dose were administered *p*-methoxybenzaldehyde via gavage (vehicle: corn oil) at doses of 0, 20, 100, and 500 mg/kg/day for 90 days. No mortality occurred throughout the study period. No treatment-related effects were seen on estrous cycle length or the number of cycles. Sperm motility and total sperm headcounts in the cauda epididymides were reduced in males at the high dose. Incidences of abnormal sperms in the cauda epididymides were increased in males at the high dose. Mean absolute epididymis weights were significantly decreased (−17%) in males at the high dose. Mean cauda epididymis weights were significantly reduced in males at the high dose (−29% absolute and −23% relative). Ductal atrophy at the epididymides' distal corpus and the caudal junction was seen in all males at the high dose (minimal to moderate). Oligospermia in epididymides' distal corpus and caudal junction were seen in all males at the high dose (minimal to slight). Based on adverse effects in sperm parameters seen at the high dose, the fertility NOAEL for this study was determined to be 100 mg/kg/day (RIFM, 2018).

An OECD 422-compliant gavage study was conducted in Sprague Dawley (Crj:CD(SD)IGS) rats. Groups of 13 rats/sex/dose were administered via gavage the test material, p-methoxybenzaldehyde, at doses of 0, 20, 100, or 500 mg/kg/day in corn oil. Males were dosed from 2 weeks premating, mating (2 weeks), and 2 weeks after the completion of the mating period, and females were dosed from 2 weeks premating, mating (2 weeks), and throughout pregnancy to day 4 of lactation. In addition to the systemic toxicity parameters, the effects on fertility and growth/development of pups were evaluated. At 500 mg/kg/day, the number of non-pregnant females increased, although all pairs copulated, and thus, the conception rate significantly decreased at this dose level. There were no treatment-related effects observed in the female estrous cycles. No pathological abnormalities were seen in the testes, seminal vesicle, prostate, and uterus. A significant decrease in epididymides weight and one case of epididymal nodule were observed among the high-dose group. One case of cystic ovarian bursa was documented in the high-dose group. No abnormalities were observed in the parturition and lactation state, and there were no significant differences in the birth rate, gestation period, number of corpora lutea, number of implantation sites, and implantation rate between the control and treatment groups. There were no effects of the test material on pup body weight, morphology, sex ratio, and viability on day 4. However, at the highest dose, the number of pups born, delivery index, and the number of live pups on lactation days 0 and 4 decreased significantly as compared to the control group, and at the mid-dose, it showed a declining trend in the live-birth rate ((Japanese Environmental Health BureauMinistry of Health and Welfare, 2010)). In addition, using dose-response modeling, a BMD lower confidence limit for a benchmark response of 5% (BMDL05) for live-birth rate was calculated as being 36 mg/kg/day.

The Expert Panel for Fragrance Safety* and the Reproductive Adjunct Advisory Group reviewed the report and conservatively determined the NOAEL for developmental toxicity to be 20 mg/kg/day, based on a non-significant but clear trend towards a decreased number of pups born (litter size) at the 100 mg/kg/day group. In addition, A new OECD 443 study is in progress for REACH, and the safety assessment will be updated when the new data become available.

The NOAEL for the fertility endpoint was taken from the more robust OECD 408-compliant study and determined to be 100 mg/kg/day. The NOAEL for the developmental endpoint was taken from the OECD 422-compliant study and determined to be 20 mg/kg/day.

Therefore, the *o*-methoxybenzaldehyde MOE for the fertility endpoint can be calculated by dividing the *p*-methoxybenzaldehyde NOAEL in mg/kg/day by the total systemic exposure to *o*-methoxybenzaldehyde, 100/0.000076, or 1315789.

The *o*-methoxybenzaldehyde MOE for the developmental toxicity endpoint can be calculated by dividing the *p*-methoxybenzaldehyde NOAEL in mg/kg/day by the total systemic exposure to *o*-methoxybenzaldehyde, 20/0.000076, or 263157.

In addition, the total systemic exposure to *o*-methoxybenzaldehyde (0.076 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1.1. Derivation of Reference Dose (RfD). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (2020) and an RfD of 0.20 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 RfD for *o*-methoxybenzaldehyde was calculated by dividing the lowest NOAEL (from the Repeated Dose or Reproductive Toxicity sections) of 20 mg/kg/day by the uncertainty factor, 100 = 0.20 mg/kg/day.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/12/22.

11.1.4. Skin sensitization

Based on the material-specific data and read-across to *p*-methoxybenzaldehyde (CAS # 123-11-5), *o*-methoxybenzaldehyde is a skin sensitizer with a defined NESIL of 3500 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for *o*-methoxybenzaldehyde. Therefore, read-across material *p*-methoxybenzaldehyde (CAS # 123-11-5; see Section VI) was used for the risk assessment of *o*-methoxybenzaldehyde. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, *o*-methoxybenzaldehyde is a skin sensitizer. The

Table 1
Summary of existing data on *p*-methoxybenzaldehyde as a read-across for *o*-methoxybenzaldehyde.

WoE Skin Sensitization Potency Category[1]	Human Data				Animal Data		
	NOEL-CNIH (induction) µg/cm²	NOEL-HMT (induction) µg/cm²	LOEL[2] (induction) µg/cm²	WoE NESIL[3] µg/cm²	LLNA Weighted Mean EC3 Value µg/cm²	GPMT[4]	Buehler[4]
Weak	3543	6900	4724	3500	>6250	NA	NA
	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	Negative (Keratinosens) Weak (SENS-IS)	Positive (h-CLAT and U-SENS)		No alert found	No alert found	Schiff base formation

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; GPMT = Guinea Pig Maximization Test; LOEL = lowest observed effect level; KE = Key Event; NA = 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]Data derived from CNIH or HMT.
[3]WoE NESIL limited to 2 significant figures.
[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..

chemical structure of these materials indicate that they would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0). Read-across material *p*-methoxybenzaldehyde was found to be positive in a DPRA and human cell line activation test (h-CLAT) (RIFM, 2016b; RIFM, 2016c). It was not found to be sensitizing in a KeratinoSens assay (RIFM, 2020c). *p*-Methoxybenzaldehyde was also found to be positive in the SENS-IS and U-SENS assays (RIFM, 2017; RIFM, 2020a). In a murine LLNA, read-across material *p*-methoxybenzaldehyde was not found to be sensitizing when tested up to 25% (6250 µg/cm^2) (RIFM, 2007). In 2 human maximization tests with 25 subjects each, no skin sensitization reactions were observed when read-across material *p*-methoxybenzaldehyde was tested at 6900 µg/cm^2 (RIFM, 1975; RIFM, 1973). Moreover, a human maximization study on *o*-methoxybenzaldehyde with 25 subjects showed no sensitization reactions at 2760 µg/cm^2 (RIFM, 1976). In 2 Confirmation of No Induction in Humans tests (CNIHs) with 4724 µg/cm^2 and 6496 µg/cm^2 of read-across material *p*-methoxybenzaldehyde in 1:3 ethanol:diethyl phthalate (EtOH:DEP), reactions indicative of sensitization were observed in 1/111 and 1/109 volunteers, respectively (RIFM, 2016a; RIFM, 2009b). However, in 2 additional CNIHs with 3543 µg/cm^2 and 2363 µg/cm^2 of read-across material *p*-methoxybenzaldehyde in 1:3 EtOH:DEP, no reactions indicative of sensitization were observed in any of the 102 and 109 volunteers, respectively (RIFM, 2009a; RIFM, 2008).

Based on weight of evidence (WoE) from structural analysis and *in vitro*, animal, and human studies on the read-across material and the target material, *o*-methoxybenzaldehyde is a sensitizer with a WoE NESIL of 3500 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 an RfD of 0.20 mg/kg/day.

Additional References: Klecak (1979); Ishihara et al., 1986; Watanabe et al., 2001; Klecak (1985); RIFM, 1972; RIFM, 1961.

Literature Search and Risk Assessment Completed On: 05/22/22.

11.1.5. Photoirritation/photoallergenicity

Based on the available *in vitro* study data for the read-across material, *p*-methoxybenzaldehyde (CAS # 123-11-5), *o*-methoxybenzaldehyde does not present a concern for photoirritation. *o*-Methoxybenzaldehyde was not evaluated for photoallergy. However, RIFM is sponsoring an *in*

vitro photoallergy research program to evaluate the photoallergy potential of *o*-methoxybenzaldehyde.

11.1.5.1. Risk assessment. The UV spectra for *o*-methoxybenzaldehyde indicate significant absorption in the region of 290–700 nm; molar absorption coefficients within that range are above the benchmark of concern for photoirritating effects (Henry et al., 2009). There are no photoirritation studies available for *o*-methoxybenzaldehyde in experimental models. The structural analog, *p*-methoxybenzaldehyde (CAS # 123-11-5; see Section VI), demonstrates an even greater degree of UV absorbance than the target material and has sufficient study data to address photoirritation. In an *in vitro* 3T3-Neutral Red uptake photoirritation assay (OECD 432), *p*-methoxybenzaldehyde was not photoirritating (RIFM, 2002). Based on the available *in vitro* study data for the read-across material, p-methoxybenzaldehyde (CAS # 123-11-5), *o*-methoxybenzaldehyde does not present a concern for photoirritation. *o*-Methoxybenzaldehyde was not evaluated for photoallergy. However, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of *o*-methoxybenzaldehyde.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The UV spectra for *o*-methoxybenzaldehyde indicate significant absorbance between 290 and 700 nm, with peak absorbance at 330 nm and returning to baseline by approximately 360 nm. The molar absorbance coefficients (1168, 2589, 2329 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are 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: 04/29/22.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for *o*-methoxybenzaldehyde 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 *o*-methoxybenzaldehyde. Based on the Creme RIFM Model, the inhalation exposure is 0.00035 mg/day. This exposure is 4000 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: 05/19/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of *o*-methoxybenzaldehyde 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 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, *o*-methoxybenzaldehyde 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 *o*-methoxybenzaldehyde as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative, as defined in the Criteria Document (Api 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, 2017a). 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).

11.2.1.1. Risk assessment. Based on the current VoU (2019), *o*-methoxybenzaldehyde does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies. ***Biodegradation***:

No data available.

Ecotoxicity:

No data available.

11.2.1.3. Other available data. o-Methoxybenzaldehyde has been preregistered with REACH and has no other data at this time.

11.2.2. 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	1.79	1.79
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	<1	<1
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 0.2797 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 05/18/22.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	279.7			1000000	0.28	

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 06/06/23.

CRediT authorship contribution statement

G. Sullivan: Writing – review & editing.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.114417.

Appendix

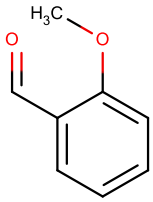
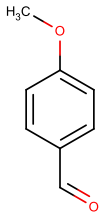
Read-across Justification:

Methods

The read-across analog was identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2017b).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.

- Protein binding was predicted using OECD QSAR Toolbox v4.5 (OECD, 2021).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.5 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	*o*-Methoxybenzaldehyde	*p*-Methoxybenzaldehyde
CAS No.	135-02-4	123-11-5
Structure		
Similarity (Tanimoto Score)		0.74
SMILES	COc1ccccc1C═O	COc1ccc(C═O)cc1
Endpoint		Genotoxicity Skin sensitization Repeated dose toxicity Reproductive toxicity Photoirritation
Molecular Formula	$C_8H_8O_2$	$C_8H_8O_2$
Molecular Weight (g/mol)	136.15	136.15
Melting Point (°C, EPI Suite)	37.50	12.84
Boiling Point (°C, EPI Suite)	243.50	248.00
Vapor Pressure (Pa @ 25°C, EPI Suite)	3.81E+00	4.39E+00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	2.95E+03	4.29E+03
Log KOW	1.72	1.76
J_{max} (μg/cm^2/h, SAM)	59.15	91.62
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	8.05E-02	8.05E-02
Genotoxicity		
DNA Binding (OASIS v1.4, QSAR Toolbox v4.5)	No alert found	No alert found
DNA Binding (OECD QSAR Toolbox v4.5)	No alert found	No alert found
Carcinogenicity (ISS)	Simple aldehyde (Genotox)|Structural alert for genotoxic carcinogenicity	Simple aldehyde (Genotox)|Structural alert for genotoxic carcinogenicity
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found
***In Vitro* Mutagenicity (Ames, ISS)**	Simple aldehyde	Simple aldehyde
***In Vivo* Mutagenicity (Micronucleus, ISS)**	H-acceptor-path3-H-acceptor|Simple aldehyde	Simple aldehyde
Oncologic Classification	Aldehyde-type Compounds	Aldehyde-type Compounds
Repeated Dose Toxicity		
Repeated Dose (HESS)	Coumarin (Hepatotoxicity) Alert	Not categorized
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.5)	Non-binder, without OH or NH2 group	Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	Non-toxicant (low reliability)	Non-toxicant (low reliability)
Skin Sensitization		
Protein Binding (OASIS v1.1)	Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes	No alert found
Protein Binding (OECD)	Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Schiff base formation identified.	Alert for Schiff base formation identified.
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.5)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on *o*-methoxybenzaldehyde (CAS # 135-02-4). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, *p*-methoxybenzaldehyde (CAS # 123-11-5) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- *p*-Methoxybenzaldehyde (CAS # 123-11-5) was used as a read-across analog for the target material, *o*-methoxybenzaldehyde (CAS # 135-02-4), for the genotoxicity, skin sensitization, repeated dose toxicity, reproductive toxicity, and photoirritation endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the benzaldehyde group.
  - o The key difference between the target material and the read-across analog is the methoxy is in the para-position in the target material and the ortho-position in the read-across analog. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.5, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o Both the target material and the read-across analog have simple aldehyde alerts for genotoxicity and Schiff base formation alerts for skin sensitization. The target material has a hepatotoxicity alert for repeated dose toxicity. The predictions are superseded by the data.
  - o The target material and the read-across analog do not have a chromophore that is expected to absorb in the UV/Vis range of the electromagnetic spectrum and that is of interest to human health toxicity. The data on the read-across analog confirm that the substance does not absorb in the UV/Vis range. Therefore, the structural difference between the target material and the read-across analog is toxicologically insignificant for the photoirritation endpoint, and the target material can be predicted to not absorb in the UV/Vis range.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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