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Short Review

RIFM fragrance ingredient safety assessment, dimethyl disulfide, CAS Registry Number 624-92-0

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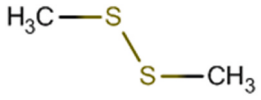

Name: Dimethyl disulfide CAS Registry Number: 624-92-0

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
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api, 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, 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.

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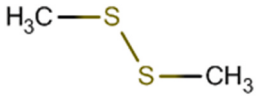
Version: 061020. This version replaces any previous versions.



Name: Dimethyl disulfide CAS Registry Number: 624-92-0

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

Dimethyl disulfide was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that dimethyl disulfide is not genotoxic. Data on dimethyl disulfide provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity, reproductive toxicity, and local respiratory toxicity endpoints. The skin sensitization endpoint was completed using the Dermal Sensitization Threshold (DST) for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; dimethyl disulfide is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; dimethyl disulfide 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.	(ECHA REACH Dossier: Dimethyl disulfide; ECHA, 2010)
Repeated Dose Toxicity: NOAEL = 5.5 mg/kg/day.	(ECHA REACH Dossier: Dimethyl disulfide; ECHA, 2010)
Reproductive Toxicity: Developmental toxicity: 20 mg/kg/day. Fertility: 80 mg/kg/day.	(ECHA REACH Dossier: Dimethyl disulfide; ECHA, 2010)
Skin Sensitization: Not a concern for skin sensitization under the declared use levels; exposure is below the DST.	
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra; RIFM Database)
Local Respiratory Toxicity: NOAEC = 38.5 mg/m^3.	(ECHA REACH Dossier: Dimethyl sulfide; ECHA, 2010)

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Critical Measured Value: 50%–60% (OECD 310)	(ECHA REACH Dossier: Dimethyl disulfide; ECHA, 2010)
Bioaccumulation:	
Screening-level: 0.3122 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 164.9 mg/L	(RIFM Framework; Salvito, 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 164.9 mg/L	(RIFM Framework; Salvito, 2002)
RIFM PNEC is: 0.1649 μg/L	

- **Revised PEC/PNECs (2015 IFRA VoU):** North America (No VoU) and Europe: Not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** Dimethyl disulfide
2. **CAS Registry Number:** 624-92-0
3. **Synonyms:** Disulfide, dimethyl; Methyl disulfide; Dimethyl disulfide
4. **Molecular Formula:** $C_2H_6S_2$
5. **Molecular Weight:** 94.19
6. **RIFM Number:** 6811
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 109 °C (Fragrance Materials Association [FMA]), 113.62 °C (EPI Suite)
2. **Flash Point:** 68 °F; CC (FMA)
3. **Log K_{OW}:** 1.87 (EPI Suite)
4. **Melting Point**: −69.69 °C (EPI Suite)
5. **Water Solubility:** 3739 mg/L (EPI Suite)
6. **Specific Gravity:** 1.046 (FMA)
7. **Vapor Pressure:** 18.5 mm Hg at 20 °C (EPI Suite v4.0), 22 mm Hg at 20 °C (FMA), 24.5 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** A pale yellowish mobile liquid

3. Volume of use (worldwide band)

1. <0.1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance**: 0.00000040% (RIFM, 2017)
2. **Inhalation Exposure***: <0.0001 mg/kg/day or <0.0001 mg/day (RIFM, 2017)
3. **Total Systemic Exposure****: 0.0000015 mg/kg/day (RIFM, 2017)

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

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

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

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

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
3. **Read-across Justification**: None

7. Metabolism

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

8. Natural occurrence

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

Allium species	Honey
Asafoetida oil	Hop (*Humulus lupulus*)
Beef	Loquat (*Eriobotrya japonica* Lindl.)
Cabbage (*Brassica oleracea*)	Mushroom
Cocoa category	Passion fruit (*Passiflora* species)
Coffee	Shrimps (prawn)
Grape brandy	Truffle

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

9. REACH dossier

Available; accessed 03/26/20 (ECHA, 2010).

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, dimethyl disulfide does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of dimethyl disulfide 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, and TA1537, and *Escherichia coli* strain WP2uvrA were treated with dimethyl disulfide in dimethyl sulfoxide (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 (ECHA, 2010). Under the conditions of the study, dimethyl disulfide was not mutagenic in the Ames test.

The clastogenic activity of dimethyl disulfide was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in the air via inhalation to groups of male and female Sprague Dawley rats. Doses of 175, 350, and 700 ppm (mg/kg) were administered. Mice from each dose level were euthanized at 72 h and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (ECHA, 2010). Under the conditions of the study, dimethyl disulfide was considered to be not clastogenic in the *in vivo* micronucleus test.

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

11.1.1.2. Additional references. None.

11.1.1.3. Literature search and risk assessment completed on. 05/22/20.

11.1.2. Repeated dose toxicity

The MOE for dimethyl disulfide 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 dimethyl disulfide. In an OECD 413/GLP-compliant subchronic inhalation toxicity study, 20 Sprague Dawley rats/sex/group were exposed to dimethyl disulfide through whole-body inhalation at concentrations of 0, 10, 50, 150, and 250 ppm (equivalent to 0, 10, 50, 150, and 250 mg/kg/day) for 6 h/day, 5 days/week, for 13 weeks. No treatment-related mortality was observed during the study. Initially, treatment-related salivation, lacrimation or reduced activity, and dyspnea were observed at the 150 and 250 ppm doses. At 10, 50, and 250 ppm concentrations, a treatment-related effect on nasal mucosa characterized by squamous metaplasia was observed. Additionally, in groups receiving 50 and 250 ppm doses, squamous metaplasia was accompanied by atrophy and micro cavitation in the anterior olfactory epithelium. At the end of the recovery period, metaplasia was reversed in the 10 ppm group but not in groups treated with higher doses. Hence, the no observed adverse effect level (NOAEL) was considered to be 10 mg/kg/day (ECHA, 2010).

In an OECD-413/GLP-compliant subchronic inhalation toxicity study, Fischer 344 rats (10 animals/sex/group) were exposed to dimethyl disulfide (purity: 99%) through whole-body inhalation at concentrations of 0 (control: dilution air), 5, 25, and 125 ppm (equivalent to 5.5, 27.4, and 137 mg/kg/day) for 6 h/day, 5 days/week, for 13 weeks (65 exposures). Based on decreased bodyweight gain and food intake in males at 25 ppm and decreased bodyweight gain and food intake at 125 ppm in both sexes, the NOAEL was considered to be 5.5 mg/kg/day (ECHA, 2010).

Therefore, the dimethyl disulfide MOE for the repeated dose toxicity endpoint can be calculated by dividing the dimethyl disulfide NOAEL in mg/kg/day by the total systemic exposure to dimethyl disulfide, 5.5/0.0000015 or 3666667.

In addition, the total systemic exposure to dimethyl disulfide (0.0015 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.2.2. Additional references. None.

11.1.2.3. Literature search and risk assessment completed on. 04/02/20.

11.1.3. Reproductive toxicity

The MOE for dimethyl disulfide 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 dimethyl disulfide. In an OECD 414/GLP prenatal developmental toxicity study, female Sprague Dawley rats (27/group) were exposed to dimethyl disulfide via whole-body inhalation at concentrations of 0, 5, 20, or 80 ppm (equivalent to 0, 5, 20, 82 mg/kg/day, respectively, using standard minute volume and body weight values for female Sprague Dawley rats) 6 h/day for gestation days (GDs) 6–19. At 80 ppm, maternal food consumption was statistically significantly lower than the control group throughout the exposure period, which corresponded to bodyweight loss and lower bodyweight gains reported for this group. The bodyweight loss was associated with a statistically significant decrease in gravid uterine weight in the 80 ppm dose group dams. The mean fetal weight in the 80 ppm group was statistically significantly lower than the control group and was outside the historical control data. Skeletal malformations/variations were noted in the 80 ppm group fetuses, which were considered to be treatment-related since they corresponded to the reduced fetal body weight at 80 ppm, indicating developmental delay. Therefore, the NOAEC for maternal toxicity was considered to be 20 ppm or 20 mg/kg/day, based on lower maternal bodyweight gains and food consumption observed at 80 ppm. The NOAEC for developmental toxicity was considered to be 20 ppm or 20 mg/kg/day, based on decreased fetal weight and increased incidences of skeletal variations reported at 80 ppm (ECHA, 2010; also available in Kirkpatrick, 2007).

In another OECD 414/GLP prenatal developmental toxicity study, female Sprague Dawley rat (30/group) were exposed to dimethyl disulfide via whole-body inhalation at concentrations of 0, 5, 15, or 50 ppm (equivalent to 0, 5, 15, and 51 mg/kg/day), in which adverse effects were observed at the highest dose of 51 mg/kg/day. The developmental toxicity NOAEL was considered to be 15 mg/kg/day (ECHA, 2010).

In another OECD 414/GLP prenatal developmental toxicity study, female New Zealand White rabbits (24/group) were exposed to dimethyl disulfide, and the developmental toxicity NOAEL was determined to be

Table 1
Maximum acceptable concentrations for dimethyl disulfide that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	NRU[b]
2	Products applied to the axillae	0.0015%	1.3×10^{-7}%
3	Products applied to the face using fingertips	0.029%	NRU[b]
4	Fine fragrance products	0.027%	4.0×10^{-7}%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	NRU[b]
6	Products with oral and lip exposure	0.016%	1.4×10^{-4}%
7	Products applied to the hair with some hand contact	0.056%	NRU[b]
8	Products with significant ano-genital exposure	0.0029%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054%	3.0×10^{-7}%
10	Household care products with mostly hand contact	0.19%	NRU[b]
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	NRU[b]

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

71 mg/kg/day, the highest dose tested (ECHA, 2010; details of the study are included in Table 1 below).

Since there were no effects observed in the mid-dose level for both rat studies, the higher NOAEL of 20 mg/kg/day was selected for the developmental toxicity endpoint. **Therefore, the dimethyl disulfide MOE for the developmental toxicity endpoint can be calculated by dividing the dimethyl disulfide NOAEL in mg/kg/day by the total systemic exposure to dimethyl disulfide, 20/0.0000015, or 13333333.**

In an OECD 416/GLP 2-generation reproduction toxicity study, Sprague Dawley Rats (30 animals/sex/group) were exposed to dimethyl disulfide via whole-body inhalation at concentrations of 0, 5, 20, or 80 ppm (equivalent to 0, 5, 20, and 80 mg/kg/day, respectively, using standard minute volume and body weight values for male and female Sprague Dawley rats) for 6 h/day, 7 days/week, for at least 70 consecutive days prior to mating for the F0 and F1 generations. The offspring selected to become the F1 parental generation were exposed following weaning (beginning on postnatal day [PND] 28). General systemic toxicity was evident in the ≥20 ppm dose group of F0 and F1 parental males and females with persistent statistically significant decreases in body weights, bodyweight gains, and/or food consumption. Potential treatment-related effects on the adrenal glands (an increase in the incidence of vacuolization of the adrenal cortex or increased relative adrenal gland weights) were reported in the F0 and F1 parental animals in the 80 ppm group. Therefore, the NOAEC for parental systemic toxicity was considered to be 5 ppm or 5 mg/kg/day, based on decreases in body weights, bodyweight gains, and/or food consumption at ≥20 ppm and increased incidence of vacuolization of the adrenal cortex or increased adrenal gland weights in the 80 ppm dose group animals. There were no effects on reproduction (e.g., estrous cycles, mating and fertility indices, number of days between pairing and coitus, gestation length, spermatogenetic parameters, primordial ovarian follicles) in any treatment group for both F0 and F1 generations. There were no adverse effects observed on pups born to exposed dams (F1 and F2 generation) and no effect on postnatal growth prior to weaning with exposure of the lactating dams in any treatment groups. Thus, the NOAEC for effects on fertility and the development of pups was considered to be 80 ppm or 80 mg/kg/day, the highest dose tested. (ECHA, 2010). In an OECD/GLP 421 reproduction and developmental toxicity screening test, Sprague Dawley rats were exposed to dimethyl disulfide, and the fertility NOAEL was considered to be 153 mg/kg/day, the highest dose tested (ECHA, 2010; details of the study are included in Table 1 above).

The NOAEL of 80 mg/kg/day was selected from the more robust OECD 416 study for the fertility endpoint. **Therefore, the dimethyl disulfide MOE for the fertility endpoint can be calculated by dividing the dimethyl disulfide NOAEL in mg/kg/day by the total systemic exposure to dimethyl disulfide, 80/0.0000015, or 53333333.**

In addition, the total systemic exposure to dimethyl disulfide (0.0015 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.2. Additional references. None.

11.1.3.3. Literature search and risk assessment completed on. 05/09/20.

11.1.4. Skin sensitization

Based on existing data, dimethyl disulfide is a sensitizer. However, based on the application of DST, it does not present a safety concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for dimethyl disulfide. The chemical structure of this material indicates that it would be expected to react with skin proteins directly (Roberts, 2007; OECD Toolbox v4.2; TIMES-SS v2.28.1). Dimethyl disulfide was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA) and U-SENS, and negative in KeratinoSens (ECHA, 2010 (a); ECHA, 2010 (b); ECHA, 2010 (c)). In a murine local lymph node assay (LLNA), dimethyl disulfide was found to be sensitizing with an EC3 value of 2.5% (625 μg/cm^2) (ECHA, 2010). Acting conservatively due to the limited data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008, 2011, 2015b; Roberts, 2015). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for dimethyl disulfide that present no appreciable risk for skin sensitization based on the reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

11.1.4.2. Additional references. ECHA, 2010: 003 Key study; 004 Key study.

11.1.4.3. Literature search and risk assessment completed on. 05/06/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, dimethyl disulfide 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 dimethyl disulfide in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, dimethyl disulfide does not present a concern for phototoxicity or photoallergenicity.

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

11.1.5.3. Additional references. None.

11.1.5.4. Literature search and risk assessment completed on. 05/06/20.

11.1.6. Local respiratory toxicity

The MOE for dimethyl disulfide is adequate for the local respiratory toxicity endpoint at the current level of use.

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In an OECD 413/GLP-compliant inhalation toxicity study, 10 Sprague Dawley rats/sex/group were exposed to dimethyl disulfide vapor through whole-body inhalation at concentrations of 0, 10, 50, 150, and 250 ppm (equivalent to 0, 38.5, 192.6, 577.9, 963.1 mg/m^3) for 13 weeks (6 h/day, 5 days/week). An additional 10 Sprague Dawley rats/sex/group were maintained as recovery groups for 2 weeks after the treatment period. Squamous metaplasia of the nasal mucosa of the respiratory epithelium was reported in all treatment groups in a dose-dependent manner; at concentrations ≥50 ppm, this effect was accompanied by atrophy and microcavitation in the anterior olfactory epithelium. However, at 10 ppm, this effect was limited to a local, minor degree of squamous metaplasia of the anterior nasal cavity. During the recovery period, changes in the nasal cavity persisted at concentrations ≥50 ppm but were reversed at 10 ppm. Based on squamous metaplasia of the nasal mucosa of the respiratory tract, the NOAEC

for this study was considered to be 10 ppm or 38.5 mg/m^3 (ECHA, 2010).

In another GLP and OECD 413-compliant inhalation study, 10 Fischer 344 rats/sex/group were exposed to dimethyl disulfide vapor through whole-body inhalation at concentrations of 0, 5, 25, 125 ppm (equivalent to 0, 19.3, 96.3, and 481.5 mg/m^3) for 13 weeks (6 h/day, 5 days/week) for 13 weeks (6 h/day, 5 days/week). Based on no toxicologically relevant respiratory effects seen up to the highest concentration, the NOAEC for this study was considered to be 125 ppm or 481.5 mg/m^3 (ECHA, 2010).

The most conservative NOAEC was derived from the first study (38.5 mg/m^3).

This NOAEC expressed in mg/kg lung weight/day is:

- (38.5 mg/m^3) × (1 m^3/1000 L) = 0.0385 mg/L
- MV of 0.17 L/min for a Sprague Dawley rat × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 61.2 L/day
- (0.0385 mg/L) × (61.2 L/d) = 2.36 mg/day
- (2.36 mg/day)/(0.0016 kg lung weight of rat*) = 1472 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.0001 mg/day—this value was derived from the concentration survey data in the Creme RIFM exposure model (Comiskey, 2015; Safford, 2015a). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew, 2009) to give 0.00015 mg/kg lung weight/day resulting in a MOE of 9813333 (i.e., [1472 mg/kg lung weight/day]/[0.00015 mg/kg lung weight/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to inter-species and intra-species variation, the material exposure by inhalation at 0.0001 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: "Comparative Physiology and Anatomy," subsection, "Comparative Airway Anatomy."

11.1.6.2. Additional references. None.

11.1.6.3. Literature search and risk assessment completed on. 05/05/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of dimethyl disulfide was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, dimethyl disulfide 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 dimethyl disulfide 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), dimethyl disulfide presents no risk to the aquatic compartment in the screening-level assessment.

11.2.3. Key studies

11.2.3.1. Biodegradation. No data available.

11.2.3.2. Ecotoxicity. No data available.

11.2.4. Other available data

Dimethyl disulfide has been registered for REACH, with the following additional data available at this time (ECHA, 2010):

The ready biodegradability of the test material was evaluated using the Headspace test, according to the OECD 310 guideline. Biodegradation of 50%–60% (CO_2 evolution) was observed after 28 days.

The ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D guideline. Biodegradation of <10% was observed after 28 days.

Acute toxicity to fish (*Oncorhynchus mykiss*) was investigated according to the EPA OPPTS Guideline 850.1075 (1996), under semi-static conditions. The 96-h LC50 based on nominal concentration was reported to be 0.97 mg/L, and the NOEC value was reported to be 0.541 mg/L.

Early-Life Stage Toxicity to fish (*Pimephales promelas*) was investigated according to the OECD 210 guidelines under flow-through conditions. The NOEC and LOEC values based on mean measured concentrations for egg hatchability, total length, and blotted wet weight were reported to be 1.87 and > 1.87 mg a.s./L, respectively. Based on the mean measured concentrations of DMDS, the NOEC, LOEC, and MATC values for fry survival were 0.936, 1.87, and 1.32 mg a.s./L, respectively.

The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions. The 48-h EC50 value based on mean measured concentrations was reported to be 1.82 mg/L (95% CI: 1.78–1.86 mg/L).

Daphnia magna Reproduction Test was conducted according to the OECD 211 guideline under semi-static conditions. The 21-day NOEC value based on nominal test concentrations was reported to be 0.003 mg/L.

The algae growth inhibition test was conducted according to the

OECD 201 guidelines under static conditions. The 96-h EC50 values based on mean measured concentrations for growth rate and biomass were reported to be 6.7 mg/L and 0.55 mg/L, respectively.

11.2.4.1. Risk assessment refinement. Since dimethyl disulfide 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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	164.9			1000000	0.1649	

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	1.87	1.87
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	No VoU
Risk Characterization: PEC/PNEC	**<1**	**NA**

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

The RIFM PNEC is 0.1649 μg/L. The revised PEC/PNECs for EU and NA (No VoU) 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.

11.2.4.2. Literature search and risk assessment completed on. 05/06/20.

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 09/30/20.

Declaration of competing interest

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

Appendix

1N,2N,3N,5N,6N,7N,16N,17N, 19Y, 20Y,21N,18N, I.

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. 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.
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No.
Q6. Benzene derivative with certain substituents? No.
Q7. Heterocyclic? No.
Q16. Common terpene? (see Cramer et al., 1978 for detailed explanation). No.
Q17. Readily hydrolyzed to a common terpene? No.
Q19. Open chain? Yes.
Q20. Aliphatic with some functional groups (see Cramer et al., 1978 for detailed explanation)? Yes.

Q21.3 or more different functional groups? No.
Q18. One of the list? (see Cramer et al., 1978 for a detailed explanation on list of categories). No. Class Low (Class I)

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of cramer classification between toxtree, the OECD QSAR Toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

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

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S. H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food Chem. Toxicol. 16 (3), 255–276.

ECHA, 2010. Dimethyl Disulfide Registration Dossier. https://echa.europa.eu/registration-dossier/-/registered-dossier/13671.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment, November 2012 v2.1. http://echa.europa.eu/.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.

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

Kirkpatrick, D., Thullen, T., Farr, C., Nemec, M., Sloter, E., Weedman, K., Davis, S., Sherman, J., Foster, K., 2007. The effects of inhaled vapors of dimethyl disulfide on embryo/fetal development in rats and rabbits. Toxicologist 96 (1), 92.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.

RIFM (Research Institute for Fragrance Materials, Inc), 2017. Exposure Survey 16, May 2017.

Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of high potency category chemicals for which the dermal sensitization threshold (DST) approach should not be applied. Regul. Toxicol. Pharmacol. 72 (3), 683–693.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C. A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.

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

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

Safford, R.J., 2008. The dermal sensitisation threshold–A TTC approach for allergic contact dermatitis. Regul. Toxicol. Pharmacol. 51 (2), 195–200.

Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015a. Extension of the dermal sensitization threshold (DST) approach to incorporate chemicals classified as reactive. Regul. Toxicol. Pharmacol. 72 (3), 694–701.

Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regul. Toxicol. Pharmacol. 60 (2), 218–224.

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

US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.