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Short Review

RIFM fragrance ingredient safety assessment, 4-hydroxybutanoic acid lactone, CAS Registry Number 96-48-0

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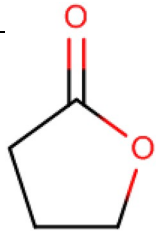
Version: 121918. This version replaces any previous versions.

Name: 4-Hydroxybutanoic acid lactone
CAS Registry Number: 96-48-0



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

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exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

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

4-Hydroxybutanoic acid lactone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-hydroxybutanoic acid lactone is not genotoxic. Data on 4-hydroxybutanoic acid lactone provide a calculated MOE > 100 for the repeated dose toxicity and developmental toxicity endpoints. The fertility and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to 4-hydroxybutanoic acid lactone is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). Data show that there are no safety concerns for 4-hydroxybutanoic acid lactone for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 4-hydroxybutanoic acid lactone is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-hydroxybutanoic acid lactone was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(NTP, 1981; ECHA REACH Dossier: γ-Butyrolactone; ECHA, 2011; Tsuchimoto and Matter, 1981; Salamone et al., 1981)
Repeated Dose Toxicity: NOAEL = 26.2 mg/kg/day.	NTP (1992)
Reproductive Toxicity:	
Developmental toxicity: NOAEL = 749 mg/kg/day. Fertility: No NOAEL available. Exposure is below the TTC.	(US EPA, 2002)
Skin Sensitization: Not a sensitization concern.	(ECHA REACH Dossier: γ-Butyrolactone; ECHA, 2011)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Screening-level: 77% in 14 days(OECD 301 C)	(ECHA REACH Dossier: γ-butyrolactone; ECHA, 2011)
Bioaccumulation:	
Screening-level: 3.162 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 11875 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	

Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 11875 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 11.875 μg/L	

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** 4-Hydroxybutanoic acid lactone
2. **CAS Registry Number:** 96-48-0
3. **Synonyms:** γ-Butyrolactone; c-Butyrolactone; 2(3H)-Furanone, dihydro-; BLO; Dihydro-2(3h)-furanone; Butyric acid lactone; Butyrolactone; γ - アルキルラクトン（Ｃ＝０～１４）; テトラヒドロフラノン - ２; γ-ﾌﾞﾁﾛﾗｸﾄﾝ; Dihydrofuran-2(3H)-one; 4-Hydroxybutanoic acid lactone
4. **Molecular Formula:** $C_4H_6O_2$
5. **Molecular Weight:** 86.09
6. **RIFM Number:** 6244
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 176.93 °C (EPI Suite)
2. **Flash Point:** 106 °C (GHS), > 200 °F; CC (FMA Database)
3. **Log K_{OW}:** -0.31 (EPI Suite)
4. **Melting Point:** -42.08 °C (EPI Suite)
5. **Water Solubility:** 447500 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.199 mm Hg @ 20 °C (EPI Suite v4.0), 0.6 mm Hg 20 °C (FMA Database), 0.295 mm Hg @ 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 colorless or very pale yellowish, oily liquid with a faint, sweet-aromatic odor; rather nondescript and sweet, slightly caramellic taste, overall weak

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** 1–10 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.000046% (RIFM, 2017)
3. **Inhalation Exposure*:** 0.0000002 mg/kg/day or 0.000017 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.00021 mg/kg/day (RIFM, 2017)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 4. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

4. Derivation of systemic absorption

1. **Dermal:** 7–11%

BG RCI, 2000; MAK, 2011 **(accessed 01/03/19):** In a percutaneous absorption study, male Sprague Dawley rats were administered 4-hydroxybutanoic acid lactone (undiluted) to both mechanically depilated (shaved) or mechanically and chemically depilated (depilating chemical: thioglycolic acid-based formulation) skin. Peak levels of 150 μg/mL were reached after 1.5–2 h for mechanical depilation, and 175 μg/mL was reached for the mechanically and chemically depilated skin within 10 min. The percentages of absorption through mechanically and mechanically and chemically depilated skin were 7% and 11%, respectively.

2. **Oral:** 98%

Lettieri and Fung, 1978; BG RCI, 2000: Male Sprague Dawley rats were orally administered 4-hydroxybutanoic acid lactone at doses of 136 and 546 mg/kg. Rapid and extensive absorption at 136 mg/kg resulted in peak levels of 350 μg/mL within 15–30 min. At a dose of 546 mg/kg, absorption was extremely rapid and virtually complete and yielded peak levels in the order of 1000 μg/mL for approximately 3 h. The total absorption was reported to be in the range of 85%–98%.

3. **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low* (Expert Judgment)

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

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

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

6. Metabolism

4-hydroxybutanoic acid lactone is rapidly metabolized to its primary metabolite, gamma-hydroxybutyrate (GHB) by the lactonase enzyme. Additionally, 4-hydroxybutanoic acid lactone can be degraded to CO_2 after oxidation to butanedioic acid (succinic acid or succinylic acid) in the citric acid cycle or through the β-oxidation pathway (BG RCI, 2000). The metabolic conversion of hydroxybutanoic acid lactone to GHB affects the central nervous system and induces weak narcotic-like effects (US EPA, 2006).

In a human study, 4 volunteers (2 men and 2 women) were administered 1 g of 4-hydroxybutanoic acid lactone. Urine samples were collected every hour post-dose up to 4 h and analyzed for metabolites. Increased excretion of the metabolites S-3,4-dihydroxybutyric acid, glycolic acid, γ-hydroxybutyric acid and the hydroxyepoxide tautomer of 4-hydroxy-3-oxobutyric were reported. These results indicated that 4-hydroxybutanoic acid lactone metabolism occurs primarily through the β-oxidation pathway in humans (MAK, 2011).

In an *in vivo* study performed on male Sprague Dawley rats, 4-hydroxybutanoic acid lactone was administered through oral gavage at doses of 1.92 and 5.77 mmol/kg. Peak plasma concentration (C_{max}) for 1.92 and 5.77 mmol/kg doses was determined to be 230 ± 5.7 and 972 ± 93 μg/mL, respectively. Times of peak plasma concentration (T_{max}) for 1.92 and 5.77 mmol/kg doses were 0.25 ± 0.12 and 1.13 ± 0.63 h, respectively. Total oral and renal clearance for 1.92 and 5.77 mmol/kg doses were 14.3 ± 4.9 and 0.197 ± 0.07 mL/kg/min, and 3.11 ± 0.26 and 0.545 ± 0.28 mL/kg/min, respectively (Morse and Morris, 2013).

In another *in vivo* study, 6 male Sprague Dawley rats were orally administered 4-hydroxybutanoic acid lactone at a dose of 500 mg/kg. Blood and brain were collected for metabolite identification and quantification after 60 min. At 15 min, blood concentrations of the primary metabolite GHB reached a peak level of 611 μg/mL. The absorption was reported to be in the range of 85%–98%. Post-dosing, the peak concentrations of 4-hydroxybutanoic acid lactone and GHB in the brain were 37 μg/g at 30 min and 98.9 μg/g at 60 min, respectively (BG RCI, 2000).

Additional References: None.

7. Natural occurrence (Discrete chemical) or composition (NCS)

4-Hydroxybutanoic acid lactone is reported to occur in the following food by the VCF*:

Apricot (*Prunus armeniaca* L.)
Beer.
Cashew nut (*Anacardium occidentale*)
Cider (apple wine)
Coffee.
Fish.
Honey.
Olive (*Olea europaea*)
Passion fruit (*Passiflora* species)
Wine.

*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.

8. IFRA standard

None.

9. REACH dossier

Available; accessed 12/19/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 4-hydroxybutanoic acid lactone does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. 4-Hydroxybutanoic acid lactone has been evaluated extensively for mutagenicity and clastogenicity both *in vitro* and *in vivo*. Overall, the results of these studies have been negative with the exception of 1 study. The weight of the evidence indicates that 4-hydroxybutanoic acid lactone is not a concern for genotoxic potential. The following summarized studies are representative of the array of studies conducted on the respective endpoints.

The mutagenic activity of 4-hydroxybutanoic acid lactone has been evaluated in a bacterial reverse mutation assay conducted in accordance with guidelines similar to OECD TG 471 by the National Toxicology Program (NTP) using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated with 4-hydroxybutanoic acid lactone in water at concentrations up to 10000 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, 1981). Under the conditions of the study, 4-hydroxybutanoic acid lactone was not mutagenic in the Ames test. Additionally, the mutagenic activity of 4-hydroxybutanoic acid lactone has also been evaluated in another bacterial reverse mutation assay conducted according to guidelines similar to OECD TG 471. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA102, TA104, *Escherichia coli* strain WP2uvrA, and *E. Coli* WP2uvrA\PKM101 were treated with 4-hydroxybutanoic acid lactone in water 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, 2011). Under the conditions of the study, 4-hydroxybutanoic acid lactone was not mutagenic in the Ames test.

4-hydroxybutanoic acid lactone has been studied extensively for clastogenicity. In one study, negative results were obtained with 4-hydroxybutanoic acid lactone for chromosomal aberration using a rat liver epithelial cell line without S9 (Dean, 1981). However, in another study, 4-hydroxybutanoic acid lactone caused induction of chromosomal aberrations at high concentrations (2500 μg/mL) in Chinese hamster ovary cells (NTP, 1992). Furthermore, the clastogenic activity of 4-hydroxybutanoic acid lactone was assessed in 2 *in vivo* micronucleus assays. Based on the weight of evidence of both assays, it was concluded that the material was negative as tested in mice (Tsuchimoto and Matter, 1981; Salamone et al., 1981). In the first test, doses of 0.11, 0.22, or 0.44 mg/kg body weight were administered via intraperitoneal injection to CD-1 mice twice, once at study start and 24 h later. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (Tsuchimoto and Matter, 1981). In the second test, doses equivalent to 100%, 80%, and 50% of the LD50 were administered via intraperitoneal injection to B6C3F1 mice twice, once at the study start and 24 h later. Then samples were taken at 48, 72, and 96 h. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (Salamone et al., 1981). Using both studies in a weight of evidence approach, 4-hydroxybutanoic acid lactone was considered to be not clastogenic in the *in vivo* micronucleus test.

As further weight of evidence, 2 year carcinogenicity studies in mice and rats exposed to 4-hydroxybutanoic acid lactone by oral gavage were conducted by the NTP and were concluded to be negative (NTP, 1992).

Based on the data available, 4-hydroxybutanoic acid lactone does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/26/19.

10.1.2. Repeated dose toxicity

The margin of exposure is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 4-hydroxybutanoic acid lactone. In a GLP-compliant repeated dose toxicity study, 10 F344 rats/sex/group were orally administered 4-hydroxybutanoic acid lactone via gavage at doses of 0 (vehicle control: corn oil), 56, 112, 225, 450, and 900 mg/kg/day for 13 weeks (5 days/week). In the 900 mg/kg/day group, all males and 1 female died by study week 8. Treatment-related average body weight and bodyweight gain was statistically significantly reduced in male rats at 450 mg/kg/day. No significant alterations were reported in gross pathology, histopathology, or absolute/relative organ weight at any dose level. However, increased incidences of nasal mucosa inflammation were reported in both treatment and control animals. The observed lesions were focal/multifocal consisting of neutrophils and macrophages accumulation in the lumen or mucosa were attributed to gavage-related effects based on historical data. Based on the reduced bodyweight gain at 450 mg/kg/day and reported mortality at 900 mg/kg/day in males, the NOAEL was considered to be 225 mg/kg/day. Subsequently, in a GLP-compliant carcinogenicity bioassay, 50

F344 rats/sex/dose were administered 4-hydroxybutanoic acid lactone through oral gavage at doses of 0 (vehicle control: corn oil), 112, and 225 mg/kg/day to male rats and 0 (vehicle control: corn oil), 225, and 450 mg/kg/day to female rats for 103 weeks (5 days/week). No treatment-related adverse effects were reported for survival rate, clinical signs, or histopathology at any dose level. The average body weight of high-dose females was significantly reduced 10%–20% from week 6 to the end of experiment. Various tumors were reported with significantly negative trend. Based on the reduced bodyweight gain at 450 mg/kg/day and mortality at 900 mg/kg/day, the NOAEL was considered to be 225 mg/kg/day (NTP, 1992).

In a GLP-compliant toxicity study, 10 B6C3F1 mice/sex/group were orally administered 4-hydroxybutanoic acid lactone through gavage at doses of 0 (vehicle control: corn oil), 65, 131, 262, 525, and 1050 mg/kg/day for a period of 13 weeks (5 days/week). Several animals from various dose group died due to improper gavage technique. At doses ≥525 mg/kg/day, recumbency was reported in animals that returned to normal after several hours. Similarly, lethargy was reported at ≥262 mg/kg/day but was reversed shortly. Statistically significant reduction in average body weight (11%) and bodyweight gain of males at 1050 mg/kg/day was reported. No treatment-related clinical signs were reported at any dose level. No significant macroscopic/microscopic alterations were found in any group, and absolute/relative organ weights were similar across treated and control groups. Based on the reported mortality and reduced body weight (males only) at 1050 mg/kg/day, the NOAEL was considered to be 525 mg/kg/day. Subsequently in a GLP-compliant carcinogenicity bioassay, 50 B6C3F1 mice/sex/dose were administered 4-hydroxybutanoic acid lactone through oral gavage at doses of 0 (vehicle control: corn oil), 262, and 525 mg/kg/day for a period of 103 weeks (5 days/week). No treatment-related clinical signs were reported at any dose level. Male mortality increased with dose while female survival rate was similar to the control. Incidences of adrenal medulla hyperplasia were significantly increased in low dose males. Average body weight and bodyweight gain were decreased from week 3 onwards in either sex at both doses. Various tumors were reported with a significantly negative trend and no dose dependency. Incidences of adrenal medulla hyperplasia were significantly increased in low-dose males compared to control and high-dose animals (2/48, 9/50, and 4/50 for 0, 262, and 525 mg/kg/day groups, respectively). In addition, increased benign or malignant pheochromocytomas were also reported in absence of a dose-response probably due to lower survival rate in the high-dose group. Based on the increased mortality in males at 525 mg/kg/day, decreased body weight at all doses, and significant increase in adrenal medulla hyperplasia accompanied with benign or malignant pheochromocytomas in males, a NOAEL could not be determined from the study. The lowest observed adverse effect level (LOAEL) was considered to be 262 mg/kg/day. (NTP, 1992).

From the available data, LOAEL of 262 mg/kg/day was considered as the most conservative point of departure. A default safety factor of 10 was used when deriving a NOAEL from the LOAEL. The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 262/10 or 26.2 mg/kg/day.

Therefore, the 4-hydroxybutanoic acid lactone MOE for the repeated dose toxicity endpoint can be calculated by dividing the NOAEL in mg/kg/day by the total systemic exposure to 4-hydroxybutanoic acid lactone, 26.2/0.0002131 or 122947.

In addition, the total systemic exposure to 4-hydroxybutanoic acid lactone (0.2131 μg/kg bw/day) is below the TTC (30 μg/kg bw/day) 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: ECHA, 2011 (accessed 01/03/19); US EPA, 2002; WHO, 1998 (accessed 01/03/19); WHO, 2014; US EPA, 2019; BG RCI, 2000; EFSA, 2011 (accessed 01/03/19); WHO, 1999; MAK, 2011 (accessed on 01/03/19)

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

10.1.3. Reproductive Toxicity

The margin of exposure for 4-hydroxybutanoic acid lactone is adequate for the developmental toxicity endpoint at the current level of use.

There are insufficient fertility data on 4-hydroxybutanoic acid lactone or any read-across materials. The total systemic exposure to 4-hydroxybutanoic acid lactone is below the TTC for the fertility endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are sufficient developmental toxicity data on 4-hydroxybutanoic acid lactone. An OECD 414/GLP inhalational prenatal developmental toxicity study was conducted in pregnant female Himalayan rabbits. Groups of 15 rabbits/dose were exposed to gamma-butyrolactone via whole-body inhalation exposure at concentrations of 0, 0.5, 1.4, or 5 mg/L (equivalent to 0, 75, 210, and 749 mg/kg/day, respectively, as per standard minute volume and body weight for rabbits) from days 7–19 post insemination (p.i.) for 6 h per day. The dams were necropsied on day 29 p.i. No treatment-related adverse effects were reported for the dams or on the development of pups. Thus, the NOAEL for maternal and developmental toxicity was considered to be 5 mg/L or 749 mg/kg/day, the highest dose tested (US EPA, 2002).

A teratogenicity study was conducted on pregnant female Sprague Dawley rats. Gamma-butyrolactone was administered via oral gavage to groups of rats (treatment groups: 10/dose and control: 9 females) at doses of 0, 10, 50, 125, 250, or 500 mg/kg/day in soybean oil from gestation day (GD) 6–15. Gross necropsy was performed on GD 21. Statistically significant reduced placental weight (7%–11%) was reported at all doses. Statistically significantly increased fetal weight (3%–5%) was reported at all doses (in the absence of a dose-response) except at 10 and 500 mg/kg/day. Both these effects were not attributed to treatment, as effects were marginal and not dose-dependent. No significant changes were reported in number of corpora lutea, total implantation sites, number of live and dead fetuses, number of resorptions, or pre- and post-implantation losses in any of the treated animals. No treatment-related external anomalies were reported at any dose. Visceral examination did not reveal any abnormalities related to treatment. Skeletal examination of fetuses from the 10 and 125 mg/kg/day dose groups were reported to have significantly increased incidences of unossified hyoid cartilage, and fetuses from the 500 mg/kg/day dose group were observed with significantly increased incidences of unossified bipartite thorax vertebrae compared to controls. Incidences of 7 sternum vertebrae and 15 ribs were significantly increased in 50 mg/kg/day treatment. The skeletal anomalies were not attributed to treatment in the absence of dose dependency and were considered to be spontaneous. The NOAEL for both maternal and developmental toxicity was considered to be 500 mg/kg/day, the highest dose tested (Kronevi et al., 1988).

Taken altogether, both developmental toxicity studies conducted in rabbits and rats via inhalation and oral gavage routes of exposure, respectively, did not indicate any adverse effects up to the highest dose level; the developmental toxicity NOAEL of 749 mg/kg/day from the rabbit study was selected for the developmental toxicity endpoint. **Therefore, the 4-hydroxybutanoic acid lactone MOE for the developmental toxicity endpoint can be calculated by dividing the 4-hydroxybutanoic acid lactone NOAEL in mg/kg/day by the total systemic exposure to 4-hydroxybutanoic acid lactone, 749/0.00021 or 3566667.**

In addition, the total systemic exposure to 2-hydroxyacetophenone (0.21 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental toxicity endpoint of a

Cramer Class I material at the current level of use.

There are insufficient fertility data on 4-hydroxybutanoic acid lactone or any read-across materials. The NTP has conducted 13-week toxicity studies on both mice and rats (NTP, 1992; see table below for study details) in which no adverse effects on the reproductive organs were reported up to the highest dose of 1050 mg/kg/day for mice and 900 mg/kg/day for rats. Since evaluation on spermatology or estrous cycles were not performed, a NOAEL for fertility could not be determined. The total systemic exposure to 4-hydroxybutanoic acid lactone (0.21 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the fertility endpoint of a Cramer Class I material at the current level of use.

Duration in detail	GLP/ Guideline	No. of animals/ dose (Species, strain, sex)	Route (vehicle)	Doses (in mg/kg/day; purity)	NOAEL/LOAEL/NOEL	Justification of NOAEL/ LOAEL/NOEL	Reference
13-Weeks, 5 days/week	GLP/NTP	10/sex/dose (B6C3F1 mice)	Oral (Corn Oil)	0, 65, 131, 262, 525, and 1050 mg/kg/day (purity-100.9%)	Fertility NOAEL = 1050 mg/kg/day	No treatment-related adverse changes reported in the reproductive organs	NTP (1992)
13-Weeks,5 days/week	GLP/NTP	10/sex/dose (F344/N rats)	Oral (Corn Oil)	0, 56, 112, 225, 450, and 900 mg/kg/day (purity-100.9%)	Fertility NOAEL = 900 mg/kg/day	No treatment-related adverse changes reported in the reproductive organs	NTP (1992)

Additional References: RIFM, 1961.

Literature Search and Risk Assessment Completed On: 01/23/19.

10.1.4. Skin sensitization

Based on the existing data, 4-hydroxybutanoic acid lactone does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the existing data, 4-hydroxybutanoic acid lactone is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.1). In a murine local lymph node assay (LLNA), 4-hydroxybutanoic acid lactone was not found to be sensitizing up to 100% (ECHA, 2011).

Based on weight of evidence (WoE) from structural analysis and an animal study, 4-hydroxybutanoic acid lactone does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/24/18.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 4-hydroxybutanoic acid lactone would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for 4-hydroxybutanoic acid lactone in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 4-hydroxybutanoic acid lactone does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/15/19.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 4-hydroxybutanoic acid lactone is below the Cramer Class I TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are insufficient inhalation data available on 4-hydroxybutanoic acid lactone. Based on the Creme RIFM Model, the inhalation exposure is 0.000017 mg/day. This exposure is 82,353 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: US EPA, 2002.

Literature Search and Risk Assessment Completed On: 01/29/19.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 4-hydroxybutanoic acid lactone was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/ Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class–specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 4-hydroxybutanoic acid lactone 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 4-hydroxybutanoic acid lactone as possibly being persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment

considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 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).

10.2.2. Risk assessment

Based on the current Volume of Use (2015), 4-hydroxybutanoic acid lactone presents no risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Key studies

10.2.2.1.1. Biodegradation. No data available.

10.2.2.1.2. Ecotoxicity. No data available.

10.2.2.1.3. Other available data. 4-Hydroxybutanoic acid lactone has been registered for REACH, and the following data is available:

The ready biodegradability test was conducted according to the OECD 301 C method (Modified MITI test [I]). Biodegradation of 77% was observed in 14 days.

A Fish (Bluegill Sunfish) acute toxicity study was conducted according to the OECD 203 method under static conditions. The 96-hour LC50 was reported to be 56 mg/L (95% confidence interval: 32–100 mg/L).

A *Daphnia* acute toxicity test was conducted according to the EU method C.2 under static conditions. The 48-hour EC50 was reported to be greater than 500 mg/L.

The acute toxicity of the test material to aquatic algae was tested according to DIN38412, part 9, under static conditions. The 72-hour EC50 value was reported to be greater than 1000 mg/L.

10.2.3. Risk assessment refinement

Since 4-hydroxybutanoic acid lactone 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*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>11875</u>			1,000,000	11.875	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	−0.31	−0.31
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 1187.5 μg/L. The revised PEC/PNECs for EU and North America are: not applicable. The material was cleared at the screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 01/29/19.

11. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as

appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 05/31/19.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix

Explanation of Cramer Classification

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

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? Yes
Q8. Lactone or cyclic diester? Yes
Q9. Lactone, fused to another ring, or 5- or 6-membered alpha,beta-unsaturated lactone? No
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 detailed explanation on list of categories) No, Low (Class I)

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