Safety Assessment of *Lactobacillus* Ferment Ingredients as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e.**, by August 17, 2025)**to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ARE	antioxidant/electrophile response element
BoSS	Burden of Sensitive Skin
CAS	Chemical Abstracts Service
CFU	colony-forming units
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DPRA	direct peptide reactivity assay
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FOU	frequency of use
HRIPT	human repeated-insult patch test
INCI	International Nomenclature of Cosmetic Ingredients
l.o.	leave-on
MoCRA	Modernization of Cosmetics Regulation Act
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Nrf2	nuclear factor erythroid 2-related factor 2
OECD	Organisation for Economic Co-operation and Development
OTC	over-the-counter
QPS	qualified presumption of safety
Panel	Expert Panel for Cosmetic Ingredient Safety
RLD	Registration and Listing Data
r.o.	rinse-off
SLS	sodium lauryl sulfate
TEWL	transepidermal water loss
TG	test guideline
US	United States
UVA	ultraviolet A
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 4 *Lactobacillus*-ferment derived ingredients, which are predominantly reported to function as skin-conditioning agents – miscellaneous in cosmetic products. The Panel reviewed the available data and determined that these ingredients are safe in cosmetics in the present practices of use and concentration as described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of the following 4 ingredients as used in cosmetic formulations:

Lactobacillus Ferment
Lactobacillus Ferment Filtrate
Lactobacillus Ferment Lysate
Lactobacillus Ferment Lysate Filtrate

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), all of these *Lactobacillus* ferment ingredients are reported to function in cosmetics as skin-conditioning agents – miscellaneous; Lactobacillus Ferment is reported to have numerous other functions in cosmetics (Table 1).[1] These ingredients were grouped together as they are products of the fermentation of bacteria, predominantly from the genus *Lactobacillus.*

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted April 2025. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics and without abbreviations. When referring to the bacterial species from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Lactobacillus acidophilus*). If it is not known whether the substance being discussed is equivalent to the cosmetic ingredient, the test substance will be identified by the name used in the publication (e.g., *Lactobacillus* ferment lysate or *Lactobacillus acidophilus* ferment lysate) that is being cited. However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., Lactobacillus Ferment or Lactobacillus Ferment derived from *Lactobacillus acidophilus*) will be used.

A search was performed on the ingredients and relevant bacterial species included in this grouping. Abundant data were found on *Lactobacilli* as live bacteria (e.g., as probiotics in clinical studies, safety assessments, and case reports); however, these data were not included herein as they are not relevant to the cosmetic ingredients reviewed. The main components of the ingredients reviewed in this report are not the live bacteria themselves, but byproducts of the fermentation of the bacteria (e.g., intracellular and extracellular metabolites, enzymes, peptides, teichoic acids, polysaccharides, organic acids (e.g., lactic acid), fragments of the dead bacteria).[2,3] As there are many potential byproducts, and the composition of these ingredients is unknown, clarification and information on composition is requested from industry.

In addition, a sampling of relevant studies found on heat-killed bacteria have been briefly summarized in the "Other Relevant Studies" section of this report. Details on these studies were omitted as these dead bacteria are assumed to be only a potential component of the finished ingredient (the amount of dead bacteria/bacterial fragments in finished ingredients is unknown).

CHEMISTRY

Definition

Lactobacillus Ferment is the product obtained through fermentation by the organism, *Lactobacillus*.[1] The definitions of the ingredients included in this review are provided in Table 1.

Chemical Properties

Lactobacillus Ferment and Lactobacillus Ferment Lysate Filtrate are liquids that is clear to yellow in color.[4] Other chemical properties on these two ingredients may be found in Table 2.

Bacteria Identification

According to personal communication with Personal Care Products Council and data from industry, several species of bacteria have been reported to be used in the manufacture of these *Lactobacillus* ferment ingredients. A listing of these species may be found in Table 3. The majority of the species of bacteria used in the preparation of these ingredients belong to the genus *Lactobacillus*.[5] Several of these species previously belonged to the *Lactobacillus* genus, but have recently been renamed. All listed species are non-spore-forming, gram-positive, lactic-acid producing bacteria, that are a normal part of the

intestinal microbiota of various mammalian species, including humans.[6] The taxonomy of all of the species that have been reported to be used in the manufacturing of these ingredients may be found in Table 4.

Method of Manufacture

To manufacture *Lactobacillus* ferment-derived cosmetic ingredients, an appropriate growth medium is chosen and sterilized to ensure no unexpected microorganisms are present.[2] Bacteria are typically grown in modified media supplemented with determined substances (e.g., cysteine).[3] The *Lactobacillus* species is then inoculated into the media and incubated at an appropriate temperature for optimal growth. When the number of bacteria reaches a critical concentration, the culture is harvested. If only intracellular content is desired, cells are harvested by centrifugation, and cell pellets are washed and then disrupted; washing and disruption are not performed if extracellular contents are desired.[3] Medium centrifugation is performed to remove intact cells and cellular debris. The products taken during this process (e.g., growth media, cellular content) to compose these ingredients is dependent upon ingredient type (described below). It should be noted that growth conditions, substrates, and manufacturing specifics may vary depending on the postbiotics (non-viable bacterial products or metabolic byproducts produced by probiotic microorganisms)[7] of interest.

It should be noted that according to International Nomenclature of Cosmetic Ingredients (INCI) naming conventions for ferments, if fermentation is completed with an atypical substrate, the atypical substrate would be included in the INCI name.[8] Because no substrates are specifically listed in the INCI names under review of this report, the fermentations for the preparation of these ingredients are completed with standard substrates.

Manufacturing data on several trade name mixtures containing Lactobacillus Ferment, Lactobacillus Ferment Filtrate, and Lactobacillus Ferment Lysate were submitted by industry. These data may be found in Table 5.

Lactobacillus Ferment

According to a manufacturer, the fermentation process for Lactobacillus Ferment occurs in the presence of both standard growth media components and undecylenic acid derived from castor beans.[9] Secondary metabolites (e.g., water-soluble undecylenates) are formed as a stress response from the change in pH and oxygen levels for the bacteria. After fermentation, lysozyme is added to the culture to facilitate a controlled cell lysis to ensure the release of the antimicrobial peptides for maximized activity. Any existing lactic acid bacteria is removed via filtration.

Lactobacillus Ferment derived from *Lactobacillus acidophilus* is prepared by first testing for acceptance of the materials.[10,11] Following acceptance, fermentation of *Lactobacillus acidophilus* is performed in a defined media, under controlled conditions (pH, temperature, and time). Refiltration is then performed, followed by quality control, packing, sampling for micro, and shipping.

In the production of Lactobacillus Ferment, the whole culture is taken, and therefore the ingredient comprises both the microorganism and growth media.[2] The ferment is typically sterilized to kill the active bacteria.

Lactobacillus Ferment Filtrate

When preparing Lactobacillus Ferment Filtrate, following fermentation, the growth media and bacteria are filtered to separate the spent media from the bacterial cells.[2] The ingredient is composed of the conditioned media which contains the products that were excreted by the bacteria into the culture media.

Lactobacillus Ferment Lysate

In the preparation of Lactobacillus Ferment Lysate, the bacteria are broken open into the culture media.[2] The bacteria are lysed (and thus, killed), and the cellular contents are spilled into the media. The final ingredient contains the media containing these cellular contents, along with bacterial cell remainders (e.g., cell membranes, cell wall components, exopolysaccharides).

Lactobacillus Ferment Lysate Filtrate

Lactobacillus Ferment Lysate Filtrate is prepared by breaking open the bacteria into cultured media.[2] The resulting spilled cellular contents in the media, as well as the remainders of the lysed bacteria are then filtered and clarified.

Composition and Impurities

The *Lactobacillus* fermentation process produces postbiotics of interest including intracellular and extracellular metabolites, bacterial components, lactic acid, lipotechoic acid, and other organic acids through conversion of the sugars in the growth media, enzymes, and peptides.[2,3] Finished ingredients may contain all these components, including residual dead bacteria fragments, at varying amounts. It should be noted that these ingredients are often sold as complex mixtures with other ingredients.

Lactobacillus Ferment

According to a manufacturer's specifications, Lactobacillus Ferment contains < 20 ppm heavy metals, < 10 ppm lead, < 2 ppm arsenic, and < 1 ppm cadmium, and may contain up to 10% water-soluble undecylenates.[12] In addition, none of the potential fragrance allergens listed in Annex III of European Union (EU) Cosmetic Regulation (EC) No. 1223/2009 or pesticides are present in this ingredient or in several trade name mixtures containing this ingredient.[13]

Lactobacillus Ferment derived from *Lactobacillus acidophilus* was determined to contain < 20 ppm heavy metals.[14] This ingredient was also reported to contain < 20 ppm chromium;, < 10 ppm lead, < 10 ppm nickel, < 10 ppm cobalt, < 5 ppm antimony, < 2 ppm arsenic, < 1 ppm mercury, and < 1 ppm cadmium. This ingredient may contain 1 – 10% bacteriocins.

Lactobacillus Ferment Lysate Filtrate

According to a manufacturer, Lactobacillus Ferment Lysate Filtrate is derived from *Lactobacillus bulgaricus* isolated from a yogurt starter culture and propagated on animal product-free media.[15,16] In addition, specifications indicate that this ingredient is 10 – 16% solids and contains < 20 ppm heavy metals, < 10 ppm lead, < 2 ppm arsenic, and < 1 ppm cadmium. The microbial content is reported to be < 100 colony-forming units (CFU)/g aerobic mesophiles, < 10 CFU/g anaerobic mesophiles, < 100 CFU/g mold and yeast, and no pathogens. None of the potential fragrance allergens listed in Annex III of EU/Cosmetic Regulation (EC) No. 1223/2009 or pesticides are present in this ingredient.[17]

Lactobacillus Ferment, Lactobacillus Ferment Filtrate, Lactobacillus Ferment Lysate

Composition information regarding trade name mixtures containing Lactobacillus Ferment, Lactobacillus Ferment Filtrate, and Lactobacillus Ferment Lysate were received. The majority of these mixtures contain Lactobacillus Ferment (97 – 99%) and 1,2-hexanediol (2 – 3%).[18] These data may be found in Table 6.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of *Lactobacillus* ferment ingredients in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Council, and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was discontinued in 2023 and, as of 2024, manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-year period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. However, to utilize the exemption, the small business must not sell eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[19] Please note, at this time, it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting). Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to 2023 VCRP data and 2024 RLD, Lactobacillus Ferment is reported to have the highest number of uses (it is reported be used in 266 and 2106 formulations, respectively; Table 7).[20,21] All other ingredients are reported to be used in 876 formulations or less (according to 2024 RLD). The results of the concentration of use survey conducted by the Council indicate Lactobacillus Ferment also has the highest concentration of use in a leave-on formulation; it is used at up to 5.6% in face and neck products (not spray) and other skin care preparations.[22]

These ingredients are used in formulations that may result in incidental ingestion and ocular exposure as they are reported to be used in products applied near the mouth (e.g., Lactobacillus Ferment is used in lipsticks and lip glosses; concentration not reported) and near the eyes (e.g., Lactobacillus Ferment Lysate is used in eye lotions at up to 4.6%).[20-22] In addition, these ingredients are reported to be used in baby products (e.g., Lactobacillus Ferment is used in several baby products categories).

Additionally, some of the *Lactobacillus* ferment-derived ingredients are used in cosmetic powders and sprays and could possibly be inhaled; for example, Lactobacillus Ferment is reported to be used in cologne and toilet waters and face powders (concentrations not stated). In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Some products containing these ingredients are marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available in some instances. Some of the reported product categories for this ingredient as listed in the RLD do require designation if airbrush application is used, and

this type of application was reported (e.g., Lactobacillus Ferment is reported to be used in foundations, makeup bases, and indoor tanning products with airbrush application). Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available when submitted. Please note that no concentration of use data were provided indicating airbrush application. Nevertheless, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

All of the *Lactobacillus* ferment ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[23]

Non-Cosmetic

The following information is on live bacterial strains; therefore, the relevance to the ingredients in this report is unknown.

According to 21CFR131.200, yogurt may be produced by culturing dairy ingredients with the lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus*. Prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods.[24] These bacteria are permitted for use in cultured milk (which includes buttermilk) (21CFR131.112), sour cream (21CFR131.160), cottage cheese (21CFR133.128), and yogurt (21CFR131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* are also used in the yogurt. All of the bacterial species reported to be used in the production of the *Lactobacillus* ferment ingredients derived herein have a qualified presumption of safety (QPS) status, excluding *Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus kunkeei,* and *Lactobacillus gasseri*, as designated by the European Food Safety Authority (EFSA).[25]

In addition, all of the bacterial species (as listed in Table 3 and Table 4) are also found/used in common food products.[26-41] These products include, but are not limited to, yogurt, dairy products, fermented foods, and dietary supplements.

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on the *Lactobacillus* ferment-derived ingredients were found in the published literature, and unpublished data were not submitted.

TOXICOLOGICAL STUDIES

Acute and repeated-dose toxicity studies were not found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies were not found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Details regarding the Ames assays summarized below may be found in Table 8.

Ames assays were performed with Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate, either as pure ingredients or as part of a trade name mixture.[42-49] The assays were performed in various strains for *Salmonella typhimurium* and with *Escherichia coli*, with and without metabolic activation, at concentrations up to 5000 µg/plate, and yielded negative results.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Endocrine Effects

Lactobacillus Ferment

An in vitro H295R steroidogenesis assay was performed in order to determine the potential of Lactobacillus Ferment derived from *Lactobacillus acidophilus* to induce or inhibit the production of 17β-estradiol and testosterone.[50] Human adrenocarcinoma H295R cells were exposed for 48 h to the test substance, diluted in dimethyl sulfoxide, at concentrations of

0.01, 0.1, 1, 10, 100, 1000, and 10,000 μM (each concentration evaluated in triplicate). Solvent and appropriate negative and positive controls were used. Following incubation, medium was removed, and cellular viability was analyzed. Concentrations of 17β-estradiol and testosterone were then measured, and absorbance values evaluated. The test substance was determined to be non-cytotoxic, and did not inhibit or induce the production of 17β-estradiol or testosterone. Control substances performed as expected.

Lactobacillus Ferment Lysate

An in vitro H295R steroidogenesis assay was performed according to the same procedure and concentrations stated above using a trade name mixture consisting of 2% Lactobacillus Ferment Lysate derived from *Lactobacillus acidophilus* and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract.[51] The test substance was determined to be non-cytotoxic, and did not inhibit or induce the production of 17β-estradiol or testosterone. Control substances performed as expected.

Effects on Pigmentation

Postbiotics obtained via fermentative processes of *Lactobacillus* bacteria, such as lactic acid, have been reported to result in skin depigmentation.[52] Other tyrosinase-inhibitors that may be present in these ingredients include cyclotetrapeptide, lipotechoic acid, daidzein, equol, uracil, and exo-polysaccharides (derived from *Lactobacillus sakei)*. These acids may be a component of the finished cosmetic ingredients reviewed in this report.

Lactobacillus Ferment Filtrate

A *Lactobacillus rhamnosus* spent-culture supernatant (heated for 30 min at 100°C) was evaluated for tyrosinase inhibitory activity in vitro.[53] An aqueous solution of mushroom tyrosinase was incubated with the test substance for 30 min. The amount of dopachrome produced in the reaction mixture was determined via spectrophotometric analysis, and percent inhibition of tyrosinase activity was calculated. Kojic acid (2 and 10 mM) was used as a standard. The test substance resulted in a 72.1 ± 1.2% inhibition of tyrosinase activity. (Inhibitory activities for 2 and 10 mM kojic acid were 43.6 ± 2.5 and 83.6 ± 1.0%, respectively.)

Effect on Damaged Skin

Lactobacillus Ferment Lysate

The effect of a cream containing *Lacticaseibacillus rhamnosus* ferment lysate on damaged skin was evaluated in 24 healthy female subjects.[54] Two sites of the skin were chosen on the forearms. Sites were treated with a 1% sodium lauryl sulfate (SLS) solution under occlusive conditions to induce skin damage. After a 24-h patch treatment with SLS, sites were evaluated, and subjects began application of the test substance to one site (application amount not stated), 2x/d, for 5 d. (The second site was left untreated for comparison.) After 5-d, redness and drying by the SLS treatment were reduced in cream-treated sites compared to non-treated sites.

Heat-Killed Lactobacillus

Tyndallized *Lactobacillus acidophilus* has been observed to induce anti-melanogenesis via the reduction of mRNA expression of melanogenesis-related genes such as tyrosinase, tyrosinase-related protein 1, and tyrosinase-related protein 2.[55] Oral administration of heat-killed *Lactobacillus brevis* (0.05 or 0.5%) in mice via diet for 4 wk resulted in an inhibition of immunoglobin E production.[56] Heat-killed *Lacticaseibacillus paracasei* is reported to induce high levels of interleukin-12, resulting in immunomodulation, in studies performed in mice.[26] Oral administration (via diet; 55-d treatment) of heat-treated *Lactobacillus delbrueckii* subsp. *lactis* (0.08 and 0.33%) resulted in the prevention and alleviation of atopic dermatitis symptoms in mice.[57] In clinical studies, heat-killed *Lacticaseibacillus paracasei* was reported to potentiate immunity and prevent infection when orally administered to subjects (n = 45 – 241).[26,58] No adverse effects were reported in these studies. No adverse effects caused by the test substance were observed in a study in which 42 subjects were orally administered heat-killed *Lactobacillus brevis*.[59] Subjects were instructed to take either 25 or 50 mg of the test substance for 12 wk. Similarly, no adverse effects were observed in an assay in which subjects (n = 29) were orally given heat-killed *Lactobacillus helveticus* powder 1x/d for 4 wk.[60]

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details regarding the dermal irritation, sensitization, and phototoxicity studies summarized below may be found in Table 9.

EpiDerm™ assays were performed using Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate (either as a pure ingredient or as part of a trade name mixture).[61-67] All test substances were tested undiluted and were predicted to be non-irritating. No irritation was reported in a use assay in which 21 females applied cream containing *Lacticaseibacillus rhamnosus* lysate filtrate to the face, 2x/d, for 4 wk.[54]

The majority of the test substances listed above were evaluated in direct peptide reactivity assays (DPRAs; at 100 mM) and antioxidant/electrophile response element (ARE)-nuclear factor erythroid 2-related factor (Nrf2) luciferase assays (at up to 2000 μM).[68-81] All test substances were predicted to be non-sensitizing. Human repeated-insult patch tests (HRIPTs) were performed using Lactobacillus Ferment in 2 assays (tested at 4 - 10%; 50 - 51 subjects) and using a product containing 5%

Lactobacillus Ferment Lysate Filtrate (tested at 100%; 50 subjects).[82-84] All test substances were considered to be non-irritating and non-sensitizing.

Phototoxicity

In vitro phototoxicity assays were performed using Lactobacillus Ferment and Lactobacillus Ferment Lysate (either as pure ingredients or part of a trade name mixture).[85-88] The test substances were considered to be non-phototoxic. In vitro phototoxicity assays were also performed on Lactobacillus Ferment derived from *Lactobacillus acidophilus* (tested at 0.4, 1.3, 3.8, and 12%), Lactobacillus Ferment derived from *Lactobacillus acidophilus* (tested at 0.5, 1.5, 5, and 10%), and Lactobacillus Ferment (tested at 0.4, 1.2, 3.7, and 11%).[9] In these assays, no phototoxicity was observed at lower concentrations; however, at the highest concentrations tested, a decrease in cell viability was observed in non-irradiated and irradiated tissues (compared to 100% viability in non-irradiated and irradiated tissues in the negative control groups).

OCULAR IRRITATION STUDIES

Details regarding the in vitro ocular irritation studies summarized below may be found in Table 10.

EpiOcular™ assays were performed using Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate (either as pure ingredients or as part of a trade name mixture).[61-67] All test substances were tested at 100% and considered to be non-irritating.

CLINICAL STUDIES

Single Center Study

Lactobacillus Ferment

A redness assessment (evaluated using melanin measurement probes; redness profiles created using facial imaging system) and sensitivity self-assessment (Burden of Sensitive Skin (BoSS) questionnaire) was performed in 52 subjects with confirmed sensitive skin following use of a lotion containing a trade mixture consisting of fermented cultures of both *Lacticaseibacillus rhamnosus* and *Lactobacillus paracasei* (this mixture comprised 3% of the total lotion composition).[89] Lotion (0.6 – 0.8 g) was applied 2x/d, for 30 d. The mean value of skin redness decreased from 33.98 at day 0 to 29.3 at day 30 wasdetermined to be statistically significant compared to baseline ($p < 0.01$). The median skin redness profile values decreased from 12.79 to 11.08 after 30 d of treatment (compared to baseline; $p < 0.01$). Mean total BoSS questionnaire scores decreased from 23.29 to 17.63 after 30 d of treatment (compared to baseline; $p < 0.01$). Users reported decreased inflammation, scaling, itching, tingling, and pain following use of lotion.

The effect of topical application of postbiotic Himalaya-derived *Saccharomyces* and *Lactobacillus* ferment complex (details regarding composition of complex not provided) on the alleviation of sensitive scalp syndrome was evaluated in female volunteers ($n = 30$).[90] Subjects were treated with the ferment complex, 2x/d, for 4 wk. Symptoms were evaluated using a self-assessment questionnaire on day 0, 14, and 28. The self-assessment questionnaire revealed an overall syndrome alleviation effect of 100% after 14 and 28 d of treatment. The self-assessment demonstrated that all subjects felt alleviation of itching symptoms, 96% felt alleviation of prickling, tightness, pain, and dandruff symptoms, and 92% felt alleviation of burning symptoms.

SUMMARY

The safety of Lactobacillus Ferment, Lactobacillus Ferment Filtrate, Lactobacillus Ferment Lysate and Lactobacillus Ferment Lysate Filtrate is reviewed in this safety assessment. According to the *Dictionary*, all of these ingredients are mainly reported to function in cosmetics as skin-conditioning agents – miscellaneous. It should be noted that the main components of the ingredients reviewed in this report are not the live bacteria themselves, but byproducts of the fermentation of the bacteria (e.g., intracellular and extracellular metabolites, enzymes, peptides, teichoic acids, polysaccharides, organic acids (e.g., lactic acid), fragments of the dead bacteria).

According to 2023 FDA VCRP data and 2024 RLD, Lactobacillus Ferment is reported to have the highest number of uses (266 and 2106 formulations, respectively). Lactobacillus Ferment also has the highest concentration of use (it is used at up to 5.6% in face and neck products (not spray) and other skin care preparations).

No genotoxicity was observed in Ames assays performed with Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate (either as a pure ingredient or as part of a trade name mixture). All test substances were evaluated with and without metabolic activation, at up to 5000 µg/plate.

In vitro H295R steroidogenesis assays were performed using a Lactobacillus Ferment derived from *Lactobacillus acidophilus* and a trade name mixture consisting of 2% Lactobacillus Ferment Lysate derived from *Lactobacillus acidophilus* and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract. Both test substances were tested at up to 10,000 µM, and neither test substance inhibited or induced the production of 17β-estradiol or testosterone.

Postbiotics obtained via a fermentative process of *Lactobacillus* bacteria (e.g., lactic acid, lipotechoic acid) are tyrosinase-inhibitors, and thus, may result in skin depigmentation. A heated *Lactobacillus rhamnosus* spent-culture

supernatant resulted in a 72.1 ± 1.2% inhibition of tyrosinase activity in in vitro assay using mushroom tyrosinase. (The standard, 2 and 10 mM kojic acid, resulted in 43.6 ± 2.5 and 83.6 ± 1.0% tyrosinase inhibition, respectively).

A cream containing *Lacticaseibacillus rhamnosus* ferment lysate resulted in an alleviation of redness and drying induced by SLS, compared to sites untreated with cream. The cream was applied to the forearms of 24 female subjects 2x/d, for 5 d, after treatment with SLS.

Heat-killed *Lactobacillus* species have been reported to induce anti-melanogenesis in vitro, and inhibit immunoglobin E production, induce high levels of interleukin-12, and alleviate atopic dermatitis symptoms in mice. In humans, heat-killed *Lactobacillus* species were reported to potentiate immunity and prevent infection. No adverse effects were observed in studies in which humans were orally given heat-killed *Lactobacillus*.

EpiDerm™ assays were performed using Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate (either as a pure ingredient or as part of a trade name mixture). All test substances were tested undiluted and were predicted to be non-irritating. No irritation was reported in a use assay in which 21 females applied cream containing *Lacticaseibacillus rhamnosus* lysate filtrate to the face, 2x/d, for 4 wk.

The majority of these test substances listed above were evaluated in direct peptide reactivity assays (DPRAs; at 100 mM) and antioxidant/electrophile response element (ARE)-nuclear factor erythroid 2-related factor (Nrf2) luciferase assays (at up to 2000 μM). All test substances were predicted to be non-sensitizing. HRIPTs were performed using Lactobacillus Ferment in 2 assays (tested at 4 - 10%; 50 - 51 subjects) and using a product containing 5% Lactobacillus Ferment Lysate Filtrate (tested at 100%; 50 subjects). All test substances were considered to be non-irritating and non-sensitizing.

EpiOcular™ assays were performed using Lactobacillus Ferment, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate (either as pure ingredients or as part of a trade name mixture). All test substances were tested at 100% and considered to be non-irritating.

Skin redness, skin redness profile values, and BoSS questionnaire scores were statistically significantly decreased in patients (n = 52) with sensitive skin following the use of a lotion containing a trade mixture consisting of fermented cultures of both *Lacticaseibacillus rhamnosus* and *Lactobacillus paracasei* (this mixture comprised 3% of the total lotion composition) 2x/d for 30 d. Alleviation of itching, prickling, tightness, dandruff, and burning was reported in female volunteers (n = 30) with sensitive scalp syndrome treated with a topical application of postbiotic *Saccharomyces* and *Lactobacillus* ferment complex.

DISCUSSION

This assessment reviews the safety of Lactobacillus Ferment, Lactobacillus Ferment Filtrate, Lactobacillus Ferment Lysate, and Lactobacillus Ferment Lysate Filtrate as used in cosmetic formulations, in accordance with the product categories and concentrations of use identified in the Use section and Use table. The Panel considered the available data and concluded that these ingredients are safe in cosmetics in the present practices of use and concentration.

Data included in this report indicate that these ingredients may have a skin lightening effect. The Panel noted that skin lightening is considered a drug effect, and should not occur during the use of cosmetic products. Because of that caveat, the Panel's knowledge of the mechanism of action (i.e., inhibition of tyrosinase activity resulting in reduced melanin synthesis), and clinical experience, concern for this effect in cosmetics was mitigated. Nevertheless, cosmetic formulators should only use this ingredient in products in a manner that does not cause skin depigmentation.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Lactobacillus Ferment is used in hair sprays (concentration not stated)). Inhalation toxicity data were not available. However, the Panel noted that the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern. Although frequency and/or concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Lactobacillus Ferment, Lactobacillus Ferment Lysate, Lactobacillus Ferment Lysate Filtrate, and Lactobacillus Ferment Filtrate are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Definitions, reported functions, and idealized structures of the ingredients in this safety assessment.[1]

Ingredient & CAS No.	Definition	Functions
Lactobacillus Ferment	Lactobacillus Ferment is the product obtained through fermentation by the microorganism, *Lactobacillus*	antiacne agents antidandruff agents antioxidants emulsion stabilizers film formers humectants skin-conditioning agents - miscellaneous viscosity increasing agents - aqueous
Lactobacillus Ferment Lysate	Lactobacillus Ferment Lysate is a lysate of the product obtained by the fermentation of *Lactobacillus*.	skin-conditioning agents - miscellaneous
Lactobacillus Ferment Lysate Filtrate CAS No. 1686112-36-6)	Lactobacillus Ferment Lysate Filtrate is a filtrate of the lysate of the product obtained by the fermentation of *Lactobacillus*.	skin-conditioning agents - miscellaneous
Lactobacillus Ferment Filtrate	Lactobacillus Ferment Filtrate is a filtrate of the product obtained by the fermentation of the growth media by the microorganism, *Lactobacillus*.	antioxidants skin-conditioning agents - miscellaneous

Table 2. Chemical properties

Property	Value	Reference
Lactobacillus Ferment		
Physical Form	liquid	4
Color	colorless - yellow	4
Odor	characteristic	4
pH	3.0 – 7.0	4
Specific Gravity (@ 25°C)	0.990 – 1.11	4
Boiling Point (°C)	100	9
Freezing Point (°C)	0	9
Lactobacillus Ferment Lysate Filtrate		
Physical Form	semi-viscous liquid	91
Color	clear to slightly hazy; yellow	91
Odor	characteristic	91
pH (@ 25°C)	3.5 – 5.5	91
Specific Gravity (@ 25°C)	1.02 – 1.07	91

Table 3. Species* used in the manufacturing of *Lactobacillus* ferment ingredients[5]

	Lactobacillus Ferment	Lactobacillus Ferment Filtrate	Lactobacillus Ferment Lysate	Lactobacillus Ferment Lysate Filtrate
Lactobacillus acidophilus	X		X	
Lactobacillus bifidus				X
Lactobacillus brevis	X		X	
Lactobacillus bulgaricus		X	X	X
Lactobacillus casei			X	
Lactobacillus crispatus	X	X		
Lactobacillus curvatus	X			
Lactobacillus delbrueckii			X	
Lactobacillus gasseri			X	
Lactobacillus helveticus			X	
Lactobacillus kunkeei	X			
Lactobacillus paracasei	X	X		
Lactobacillus pentosus	X			
Lactobacillus plantarum	X	X	X	
Lactobacillus reuteri	X			
Lactobacillus rhamnosus	X	X	X	
Lactobacillus reuteri		X		
Lactobacillus sakei			X	

*Some species listed herein are outdated species names. Table 4 lists the outdated names along with currently accepted names. Several of these species are reported to be used in more than one ingredient.

Table 4. Taxonomy of species reported to be used in *Lactobacillus* ferment-derived cosmetic ingredients[92]

Class	Order	Family	Genus	Species
Bacilli	Lactobacillales	Lactobacillaceae	*Apilactobacillus*	*Apilactobacillus kunkeei* (previously known as *Lactobacillus kunkeei)*
Actinomycetes	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	*Bifidobacterium bifidum* (previously known as *Lactobacillus bifidus)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus casei* (previously known as *Lactobacillus casei)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* (previously known as *Lactobacillus paracasei)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus rhamnosus* (previously known as *Lactobacillus rhamnosus)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactiplantibacillus*	*Lactiplantibacillus pentosus* (previously known as *Lactobacillus pentosus)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactiplantibacillus*	*Lactiplantibacillus plantarum* (previously known as *Lactobacillus plantarum)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus acidophilus*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus bulgaricus* (synonymous to *Lactobacillus delbrueckii)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus crispatus*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* (synonymous to *Lactobacillus bulgaricus)*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus gasseri*
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus helveticus*
Bacilli	Lactobacillales	Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus curvatus* (previously known as *Lactobacillus curvatus*)
Bacilli	Lactobacillales	Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus sakei* (previously known as *Lactobacillus sakei)*
Bacilli	Lactobacillales	Lactobacillaceae	*Levilactobacillus*	*Levilactobacillus brevis* (previously known as *Lactobacillus brevis)*
Bacilli	Lactobacillales	Lactobacillaceae	*Limosilactobacillus*	*Limosilactobacillus reuteri*

Table 5. Method of manufacture of *Lactobacillus* ferment-derived ingredients[93-98]

Ingredient	Method	Manufacturing process
Lactobacillus Ferment	A	media preparation → sterilization and filtration → fermentation (addition of *Lactobacillus* sp.)→ sterilization and filtration → decolorization and deodorization → addition of other ingredients (as indicated in table 5 (e.g., 1,2-hexanediol)) → stabilization → quality control → packing
Lactobacillus Ferment	B	media preparation → media preparation → sterilization and filtration → fermentation (addition of *Lactobacillus plantarum*) → sterilization and filtration → addition of ethyl acetate and fraction → dehydration and concentration → addition of water and butylene glycol and dissolving → filtration → stabilization → quality control → packing
Lactobacillus Ferment	C	media preparation → sterilization and filtration → fermentation at 35°C for 48 h (addition of *Lactobacillus kunkeii*) → lysis and filtration → decolorization and deodorization → addition of 1,2-hexanediol → stabilization → quality control → packing
Lactobacillus Ferment	D	media preparation → sterilization and filtration → fermentation (addition of *Lactobacillus brevis*) → sterilization and filtration → decolorization and deodorization → addition of 1,2-hexanediol → stabilization → quality control → packing
Lactobacillus Ferment Filtrate	E	media preparation → sterilization and filtration → fermentation (addition of *Lactobacillus* sp.) → sterilization and filtration → decolorization and deodorization → addition of 1,2-hexanediol → filtration → stabilization → quality control → packing
Lactobacillus Ferment Lysate	F	media preparation (glucose, yeast extract, soy peptone) → sterilization → fermentation at 35°C for 72 h (addition of *Lactobacillus plantarum*) → quality control → tyndallization at 80°C for 1 h/d for 3 d → addition of 1,2-hexanediol → quality control → packing

*Some species listed herein are outdated species names. Table 4 lists the outdated names along with currently accepted names.

Table 6. Summary composition of *Lactobacillus* ferment-derived ingredients[18]

Ingredient	Species used in manufacturing*	Composition	Manufacturing method**
Lactobacillus Ferment	*Lactobacillus brevis*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus brevis*	97.8% Lactobacillus Ferment 2.2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus brevis*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus curvatus*	98% Lactobacillus Ferment 2% 1,2-hexanediol	E
Lactobacillus Ferment	*Lactobacillus curvatus*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus kunkeei*	98% Lactobacillus Ferment 2% 1,2-hexanediol	E
Lactobacillus Ferment	*Lactobacillus curvatus*	98% Lactobacillus Ferment 2% 1,2-hexanediol	C
Lactobacillus Ferment	*Lactobacillus paracasei*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus paracasei*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus pentosus*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus plantarum*	98% Lactobacillus Ferment 2% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus plantarum*	98% Lactobacillus Ferment 2% 1,2-hexanediol	E
Lactobacillus Ferment	*Lactobacillus plantarum*	87.9% Lactobacillus Ferment 10% butylene glycol 2% 1,2-hexanediol 0.1% ethylhexylglycerin	A
Lactobacillus Ferment	*Lactobacillus plantarum*	97% Lactobacillus Ferment 3% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus plantarum*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus plantarum*	0.1% Lactobacillus Ferment 69.9% water 30% butylene glycol	B
Lactobacillus Ferment	*Lactobacillus plantarum*	99% Lactobacillus Ferment 0.8% propanediol 0.15% caprylyl glycol 0.05% ethylhexylglycerin	E
Lactobacillus Ferment	*Lactobacillus reuteri*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus rhamnosus*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	A
Lactobacillus Ferment	*Lactobacillus rhamnosus*	98% Lactobacillus ferment 2% 1,2-hexanediol	A

Table 6. **Summary composition of *Lactobacillus* ferment-derived ingredients**[18]

Ingredient	Species used in manufacturing*	Composition	Manufacturing method**
Lactobacillus Ferment Filtrate	*Lactobacillus plantarum*	98% Lactobacillus Ferment 2% 1,2-hexanediol	E
Lactobacillus Ferment Filtrate	*Lactobacillus reuteri*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	E
Lactobacillus Ferment Filtrate	*Lactobacillus rhamnosus*	97.8% Lactobacillus Ferment 2.2% 1,2-hexanediol	E
Lactobacillus Ferment Lysate	*Lactobacillus brevis*	97.5% Lactobacillus Ferment 2.5% 1,2-hexanediol	D
Lactobacillus Ferment Lysate	*Lactobacillus brevis*	98% Lactobacillus Ferment 2% 1,2-hexanediol	E
Lactobacillus Ferment Lysate	*Lactobacillus plantarum*	98% Lactobacillus Ferment 2% 1,2-hexanediol	F

*Some species listed herein are outdated species names. Table 4 lists the outdated names along with currently accepted names.
**Method of manufacture number correlates to the corresponding method in Table 5

Table 7. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use		# of Uses		Max Conc of Use	
	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]
	Lactobacillus Ferment				Lactobacillus Ferment Filtrate			
Totals*	**2106**	**266**	**0.001 – 1.5**	**0.000025 – 5.6**	**32**	**8**	**0.0005 – 0.09**	**NR**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	***	*216*	*0.001 – 1.5*	*0.000025 – 5.6*	***	*4*	*0.09*	*NR*
Rinse-Off	***	*49*	*0.04*	*0.0002 – 5.6*	***	*4*	*0.0005*	*NR*
Diluted for (Bath) Use	***	*1*	*NR*	*NR*	***	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	***	8	0.1	0.000025 – 4.6	***	NR	NR	NR
Incidental Ingestion	***	17	NR	NR	***	NR	NR	NR
Incidental Inhalation-Spray	***	1; 98[a]; 54[b]	0.2[a]	4.6; 1.5[b]	***	2[a]; 2[b]	NR	NR
Incidental Inhalation-Powder	***	2; 54[b]; 1[c]	0.18[c]	0.0013; 1.5[b]; 0.015 – 5.6[c]	***	2[b]	NR	NR
Dermal Contact	***	233	0.01 – 1.5	0.000025 – 5.6	***	8	0.0005 – 0.09	NR
Deodorant (underarm)	***	NR	NR	0.5	***	NR	NR	NR
Hair - Non-Coloring	***	16	0.001	0.0002	***	NR	NR	NR
Hair-Coloring	***	NR	NR	NR	***	NR	NR	NR
Nail	***	NR	NR	NR	***	NR	NR	NR
Mucous Membrane	***	32	NR	0.0003 – 5.6	***	3	NR	NR
Baby Products	***	1	NR	NR	***	NR	NR	NR
as reported by product category								
Baby Products	***6***							
Baby Shampoos	3	NR	NR	NR				
Baby Lotions, Oils. Powders, Creams	2	1	NR	NR				
Baby Wipes	1	NA	NR	NR				
Other Baby Products								
Bath Preparations	***10***							
Other Bath Preparations	10	1	NR					
Eye Makeup Preparations (not children's)	***71***							
Eyeliner	2	1	NR	NR				
Eye Shadow	1	NR	NR	NR				
Eye Lotion	24	1	0.1	0.1 – 4.6				
Eye Makeup Remover	1	NR	NR	NR				
Mascara	35	NR	NR	NR				
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)	8	NA	NR	NR				
Other Eye Makeup Preparations				0.000025				
Fragrance Preparations	***25***							
Cologne and Toilet Water	19	NR	NR	NR				
Perfumes	NR	1	NR	NR				
Other Fragrance Preparation	6	NR	NR	NR				
Hair Preparations (non-coloring)	***310***				***2***			
Hair Conditioners	20 (l.o.); 70 (r.o.)	**7**	NR	0.0002 (l.o.); 0.0002 (r.o.)				
Hair Sprays (aerosol fixatives)	10	NR	NR	NR				
Permanent Waves	1	NR	NR	NR				
Rinses (non-coloring)	13	1	NR	NR				

Table 7. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use		# of Uses		Max Conc of Use	
	RLD (2024)[21]	**VCRP (2023)[20]**	**% (2022)[99]**	**% (2025)[22]**	**RLD (2024)[21]**	**VCRP (2023)[20]**	**% (2022)[99]**	**% (2025)[22]**
Shampoos (non-coloring)	4 (l.o.); 76 (r.o.)	4	NR	NR				
Tonics, Dressings, Other Hair Grooming Aids	32	3	NR	NR	1	NR	NR	NR
Wave Sets	2	NR	NR	NR				
Other Hair Preparations	68 (l.o.); 30 (r.o.)	1	0.001	NR	1 (r.o.)	NR	NR	NR
Hair Coloring Preparations	***81***							
Hair Dyes and Colors (all types requiring caution statements and patch tests)	80	NR	NR	NR				
Hair Shampoos (coloring)	1 (r.o.)	NR	NR	NR				
Other Hair Coloring Preparation								
Makeup Preparations (not eye or children's)	***186***							
Blushers and Rouges (all types)	12	NR	NR	NR				
Face Powders	7	2	NR	0.0013				
Foundations	111 (traditional application); 37 (airbrush application)	1	NR	0.0056 (traditional application)				
Lipstick and Lip Glosses	24	15	NR	NR				
Makeup Bases	7 (traditional application); 2 (airbrush application)	2	1.5	NR				
Makeup Fixatives	9	NR	NR	0.019				
Other Makeup Preparations	15 (l.o.); 6 (r.o.)	3	0.19	NR				
Manicuring Preparations	***5***							
Basecoats and Undercoats	2	NR	NR	NR				
Nail Polish and Enamel	3	NR	NR	NR				
Oral Products	***11***							
Dentifrices	7	NR	NR	NR				
Mouthwashes and Breath Fresheners	3	NR	NR	NR				
Other Oral Products	1	2	NR	NR				
Personal Cleanliness	***96***							
Bath Soaps and Body Washes	30	6	NR	NR				
Deodorants (underarm)	41	NR	NR	0.5 (not spray)				
Douches				NR				
Feminine Deodorants	NR	1	NR	NR	NR	2	NR	NR
Disposable Wipes	8	NA	NR	NR				
Other Personal Cleanliness Products	5 (l.o.); 12 (r.o.)	7	NR	0.003 – 5.6 (r.o.)	NR	1	NR	NR
Shaving Preparations	***3***							
Beard Softeners	1	NR	NR	NR				
Other Shaving Preparations	2	NR	NR	NR				
Skin Care Preparations	***1310***				***30***			
Cleansing	127	15	0.04	0.014 – 1	3	3	0.0005	NR
Face and Neck (excluding shaving preps)	737 (l.o.); 74 (r.o.)	49	0.18	0.015 – 5.6 (l.o.)	24 (l.o.); 3 (r.o.)	NR	0.09	NR

Table 7. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use		# of Uses		Max Conc of Use	
	RLD (2024)[21]	**VCRP (2023)[20]**	**% (2022)[99]**	**% (2025)[22]**	**RLD (2024)[21]**	**VCRP (2023)[20]**	**% (2022)[99]**	**% (2025)[22]**
Body and Hand (excluding shaving preps)	66 (l.o.); 6 (r.o.)	4	NR	0.02 (l.o.); 4.6 (spray)	1 (l.o.)	NR	NR	NR
Foot Powders and Sprays	NR	NR	NR	1.5				
Moisturizing	326	76	0.01 (not spray)	0.1 – 4.6 (not spray)	3	2	NR	NR
Night	40	14	NR	NR				
Paste Masks (mud packs)	41	7	NR	0.0063				
Skin Fresheners	52	3	0.2	NR	2	NR	NR	NR
Other Skin Care Preparations	163 (l.o.); 74 (r.o.)	32	NR	5.6 (not spray; l.o.); 5.6 (not spray; r.o.)	4 (l.o.)	NR	NR	NR
Suntan Preparations	***9***							
Suntan Gels, Creams, and Liquids	7	NR	NR	NR				
Indoor Tanning Preparations	2 (spray application); 2 (professional spray application); 1 (airbrush application); 1 (professional airbrush application)	NR	NR	NR				
Tattoo Preparations	***1***							
Other Tattoo Preparations	1	NA	NR	NR				
Other Preparations (i.e., those preparations that do not fit another category)	***10***							
	Lactobacillus Ferment Lysate				**Lactobacillus Ferment Lysate Filtrate**			
Totals*	**876**	**47**	**0.17 – 0.5**	**0.05 – 0.17**	**39**	**6**	**NR**	**0.003 – 0.1**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	***	*34*	*0.5*	*0.05 – 0.17*	***	*6*	*NR*	*0.015 – 0.1*
Rinse-Off	***	*13*	*0.17*	*NR*	***	*NR*	*NR*	*0.003*
Diluted for (Bath) Use	***	*NR*	*NR*	*NR*	***	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	***	3	0.5	NR	***	NR	NR	NR
Incidental Ingestion	***	1	NR	NR	***	NR	NR	NR
Incidental Inhalation-Spray	***	21[a]; 9[b]	NR	NR	***	3[a]; 3[b]	NR	NR
Incidental Inhalation-Powder	***	9[b]	NR	0.05 – 0.12[c]	***	3[b]	NR	0.1[c]
Dermal Contact	***	44	0.17 – 0.5	0.05 – 0.17	***	6	NR	0.003 – 0.1
Deodorant (underarm)	***	NR	NR	NR	***	NR	NR	0.015
Hair - Non-Coloring	***	2	NR	NR	***	NR	NR	NR
Hair-Coloring	***	NR	NR	NR	***	NR	NR	NR
Nail	***	NR	NR	NR	***	NR	NR	NR
Mucous Membrane	***	4	NR	NR	***	NR	NR	0.003
Baby Products	***	NR	NR	NR	***	NR	NR	NR

Table 7. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use		# of Uses		Max Conc of Use	
	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]
as reported by product category								
Baby Products	***9***							
Baby Shampoos	1	NR	NR	NR				
Baby Lotions, Oils. Powders, Creams	5	NR	NR	NR				
Baby Wipes								
Other Baby Products	3 (r.o.)	NR	NR	NR				
Bath Preparations (diluted for use)								
Other Bath Preparations								
Eye Makeup Preparations	***13***							
Eyeliner								
Eye Shadow	1	NR	NR	NR				
Eye Lotion	10	1	0.5	NR				
Eye Makeup Remover								
Mascara								
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)	2	NA	NR	NR				
Other Eye Makeup Preparations	NR	2	NR	NR				
Fragrance Preparations								
Cologne and Toilet Water								
Perfumes								
Other Fragrance Preparation								
Hair Preparations (non-coloring)	***139***							
Hair Conditioners	6 (l.o.); 37 (r.o.)	NR	NR	NR				
Hair Sprays (aerosol fixatives)	1	NR	NR	NR				
Permanent Waves								
Rinses (non-coloring)	7	1	NR	NR				
Shampoos (non-coloring)	64 (r.o.)	1	NR	NR				
Tonics, Dressings, Other Hair Grooming Aids	15	NR	NR	NR				
Wave Sets								
Other Hair Preparations	18 (l.o.); 8 (r.o.)	NR	NR	NR				
Hair Coloring Preparations	***5***							
Hair Dyes and Colors (all types requiring caution statements and patch tests)	2	NR	NR	NR				
Hair Shampoos (coloring)	1 (r.o.)	NR	NR	NR				
Other Hair Coloring Preparation	2 (l.o.)	NR	NR	NR				
Makeup Preparations (not eye or children's)	***39***							
Blushers and Rouges (all types)	2	NR	NR	NR				
Face Powders								
Foundations	23 (traditional application)	NR	NR	NR				
Lipstick and Lip Glosses	3	NR	NR	NR				
Makeup Bases	4 (traditional application)	NR	NR	NR				
Makeup Fixatives	3	NR	NR	NR				
Other Makeup Preparations	4 (l.o.)	NR	NR	NR				
Manicuring Preparations (Nail)								

Table 7. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use		# of Uses		Max Conc of Use	
	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]	RLD (2024)[21]	VCRP (2023)[20]	% (2022)[99]	% (2025)[22]
Basecoats and Undercoats								
Nail Polish and Enamel								
Oral Hygiene Products	***4***							
Dentifrices	3	NR	NR	NR				
Mouthwashes and Breath Fresheners	1	1	NR	NR				
Other Oral Products								
Personal Cleanliness Products	***99***							
Bath Soaps and Body Washes	11	NR	NR	NR				
Deodorants (underarm)	1	NR	NR	NR	NR	NR	NR	0.015 (not spray)
Douches	4	NR	NR	NR				
Feminine Deodorants	3 (r.o.)	NR	NR	NR				
Disposable Wipes	76	NA	NR	NR				
Other Personal Cleanliness Products	1 (l.o.); 3 (r.o.)	3	NR	NR	NR	NR	NR	0.003 (r.o.)
Shaving Preparations								
Beard Softeners								
Other Shaving Preparations								
Skin Care Preparations	***575***				***38***			
Cleansing	59	6	NR	NR	**2**	NR	NR	NR
Face and Neck (excluding shaving preps)	353 (l.o.); 27 (r.o.)	9	NR	0.05 – 0.12 (not spray; l.o.); 0.05 (not spray; r.o.)	16 (l.o.); 2 (r.o.)	NR	NR	NR
Body and Hand (excluding shaving preps)	29 (l.o.); 5 (r.o.)	NR	NR	NR	2 (l.o.)	3	NR	0.1 (not spray; l.o.)
Foot Powders and Sprays								
Moisturizing	209	19	NR	0.17 (not spray)	15	1	NR	NR
Night	7	NR	NR	NR	1	2	NR	NR
Paste Masks (mud packs)	9	1	0.17	NR				
Skin Fresheners	19	NR	NR	NR	6	NR	NR	NR
Other Skin Care Preparations	37 (l.o.); 6 (r.o.)	2	NR	NR	3 (l.o.)	NR	NR	NR
Suntan Preparations	***2***							
Suntan Gels, Creams, and Liquids	2	NR	NR	NR				
Indoor Tanning Preparations								
Tattoo Preparations								
Other Tattoo Preparations								
Other Preparations (i.e., those preparations that do not fit another category)	***6***				***1***			

NR – not reported; NA – not applicable (this category was not part of the VCRP)
l.o. – leave-on; r.o. – rinse-off
*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.
**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
*** In the RLD each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 8. In vitro genotoxicity studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lacto-bacillus acidophilus*, 48% water, 30% glycerin, and 20% pereskia aculeata callus extract	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; sterile water used as negative control; a known mutagen used as positive control	non-genotoxic; controls performed as expected	45
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 20% momordica cochinchinesis seed extract, and 78% water	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	47
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lacto-bacillus acidophilus*	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	43
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lacto-bacillus acidophilus*	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	. *typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	49
Lactobacillus Ferment	Lactobacillus Ferment	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	44
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate (derived from *Lactobacillus acidophilus*) and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	42
Lactobacillus Ferment Lysate Filtrate	Lactobacillus Ferment Lysate Filtrate derived from *Lactobacillus bifidus* (98% purity)	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	48

Table 8. In vitro genotoxicity studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% camelia sinensis leaf extract, 10% punica granatum extract, and 1% caffeine *Lactobacillus* species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	distilled water	1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP*2uvrA*	OECD TG 471; Ames assay performed with and without metabolic activation; as described above	non-genotoxic; controls performed as expected	46

OECD = Organisation for Economic Cooperation and Development; TG = test guidelines

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION							
IN VITRO							
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 20% *Momordica cochinchinesis* seed extract, and 78% water	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay; cell viability evaluated using MTT assay; phosphate-buffered saline used as negative control; sodium dodecyl sulfate used as positive control	predicted to be non-irritating; controls gave expected results	62
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	66
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	67
Lactobacillus Ferment	Lactobacillus Ferment	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	65
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate (derived from *Lactobacillus acidophilus*) and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	64
Lactobacillus Ferment Lysate Filtrate	Lactobacillus Ferment Lysate Filtrate derived from *Lactobacillus bifidus* (98% purity)	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	61

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% *Camelia sinensis* leaf extract, 10% *Punica granatum* extract, and 1% caffeine Lactobacillus species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	none	tested neat; 30 µl	reconstructed human epidermis (n = 3)	EpiDerm™ assay. as described above	predicted to be non-irritating; controls gave expected results	63
				HUMAN			
Lactobacillus Ferment Lysate	cream containing *Lacticaseibacillus rhamnosus* ferment lysate (composition of cream not stated)	none	tested neat	21 female subjects	use study; subjects instructed to apply cream to face 2x/d for 4 wk	no erythema, edema, stinging, burning, tingling, or tightness reported	54
				SENSITIZATION			
				IN CHEMICO/IN VITRO			
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 48% water, 30% glycerin, and 20% *Pereskia aculeata* callus extract	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; negative control: dimethyl sulfoxide; positive control: cinnamic aldehyde	predicted to be non-sensitizing; reference control gave expected results	72
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment, 20% *Momordica cochinchinesis* seed extract, and 78% water	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	74
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	69
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	68
Lactobacillus Ferment	Lactobacillus Ferment	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	70
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 48% water, 30% glycerin, and 20% *Pereskia aculeata* callus extract	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; solvent control; cinnamic aldehyde as positive control	predicted to be non-sensitizing; controls gave expected results	79
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 20% *Momordica cochinchinesis* seed extract, and 78% water	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	81
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	76
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	75

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
Lactobacillus Ferment	Lactobacillus Ferment	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	77
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate (derived from *Lactobacillus acidophilus*) and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	71
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate (derived from *Lactobacillus acidophilus*) and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	78
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% camelia sinensis leaf extract, 10% punica granatum extract, and 1% caffeine Lactobacillus species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	acetonitrile	100 mM	cysteine and lysine peptides	OECD TG 442C; DPRA; as described above	predicted to be non-sensitizing; reference control gave expected results	73
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% camelia sinensis leaf extract, 10% punica granatum extract, and 1% caffeine Lactobacillus species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	dimethyl sulfoxide	0.98 – 2000 µM	KeratinoSens™ tissues; 50 µl	OECD TG 442D; ARE-Nrf2 luciferase assay; as described above	predicted to be non-sensitizing; controls gave expected results	80

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
				HUMAN			
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	distilled water	4%; 0.2 ml	50 subjects	HRIPT; test material applied under occlusive patches placed directly on the skin of the infrascapular region of the back; patch removed after 24 h; procedure repeated 3x/wk for 3 wk until a series of 9 consecutive 24 h applications were made ; following a 10 – 14 d non-treatment period; a challenge patch was applied to previously unexposed site; reactions scored 24 and 48 h after application	non-irritating and non-sensitizing	84
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	distilled water	10%; 0.2 ml	51 subjects	HRIPT performed under occlusive conditions according to the same procedures as stated above	non-irritating and non-sensitizing 9 d after study initiation, one subject had an allergic reaction on the face that was treated with methylprednisolone; investigator discontinued this subject's participation, and judged that it was unlikely that the reaction was related to the test material	82
Lactobacillus Ferment Lysate Filtrate	product containing 5% Lactobacillus Ferment Lysate Filtrate derived from *Lactobacillus bulgaricus* (purity 99.1%)	NR	tested neat; 0.2 ml	50 subjects	HRIPT performed under occlusive conditions according to the same procedures as stated above	non-irritating and non-sensitizing	83
				PHOTOIRRITATION			
				IN VITRO			
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 48% water, 30% glycerin, and 20% *Pereskia aculeata* callus extract	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis tissues (n = 2/concentration)	phototoxicity assay; tissue inserts incubated with test substance or negative (sterile deionized water) or positive (chloropromazine) controlst; appropriate tissues were irradiated with UVA (6 J/cm2) for 60 min (some tissues left un-irradiated for comparison); after incubation and irradiation, cell viability was evaluated in MTT assay	non-phototoxic; controls gave expected results	85
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 20% *Momordica cochinchinesis* seed extract, and 78% water	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis tissues (n = 2/concentration)	phototoxicity assay performed according to the same procedures as above	non-phototoxic; controls gave expected results	88

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	NR	0.4, 1.3, 3.8, and 12%	reconstructed human epidermis tissues (n = 2/concentration)	phototoxicity assay performed according to the same procedures as above	test substance considered to be non-phototoxic at concentrations of 0.4, 1.3, and 3.8%; a decrease in viability at the 12% test concentration was observed with and without irradiation (tissue viability was approximately 70 and 50% in non-irradiated and irradiated tissues, respectively, compared to 100% viability in the negative control group); negative and positive controls performed as expected	100
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis tissues (n = 2/ concentration)	phototoxicity assay performed according to the same procedures as above	test substance considered to be non-phototoxic at concentrations of 0.5, 1.5, and 5%; a decrease in viability at the 10% concentration was observed with and without irradiation (tissue viability was approximately 85 and 80% in non-irradiated and irradiated tissues, respectively, compared to 100% viability in non-irradiated and irradiated tissues in the negative control group); negative and positive controls performed as expected	101
Lactobacillus Ferment	Lactobacillus Ferment	NR	0.4, 1.2, 3.7, and 11%	reconstructed human epidermis tissues (n = 2/ concentration)	phototoxicity assay performed according to the same procedures as above	test substance was considered to be non-phototoxic at concentrations of 0.4, 1.2, and 3.7%; a decrease in cell viability at the 11% concentration was observed with and without irradiation (tissue viability was approximately 70 and 60% in non-irradiated and irradiated tissues, respectively, compared to 100% viability in both non-irradiated and irradiated tissues in the negative control group); negative and positive controls performed as expected	9
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate (derived from *Lactobacillus acidophilus*) and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis tissues (n = 2/ concentration)	phototoxicity assay performed according to the same procedures as above	non-phototoxic; controls gave expected results	86

Table 9. Dermal irritation, sensitization, and photoirritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% *Camelia sinensis* leaf extract, 10% *Punica granatum* extract, and 1% caffeine *Lactobacillus* species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis tissues (n = 2/ concentration)	phototoxicity assay performed according to the same procedures as above	non-phototoxic; controls gave expected results	87

ARE = antioxidant/electrophile response element; DPRA = direct peptide reactivity assay; HRIPT = human repeat insult patch test; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; NR = not reported; Nrf2 = nuclear factor erythroid 2-related factor 2; OECD = Organisation for Economic Cooperation and Development; TG = test guideline; UVA = ultraviolet A

Table 10. In vitro ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
			IN VITRO				
Lactobacillus Ferment	trade name mixture consisting of 2% Lactobacillus Ferment derived from *Lactobacillus acidophilus*, 20% *Momordica cochinchinesis* seed extract, and 78% water	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; 90 min incubation; cell viability evaluated via MTT assay; sterilized deionized water as negative control; methyl acetate as positive control	non-irritating; controls gave expected results	62
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	66
Lactobacillus Ferment	Lactobacillus Ferment derived from *Lactobacillus acidophilus*	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	67
Lactobacillus Ferment	Lactobacillus Ferment	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	65
Lactobacillus Ferment Lysate	trade name mixture consisting of 2% Lactobacillus Ferment Lysate derived from *Lactobacillus acidophilus* and 98% *Medicago sativa* (alfalfa) callus culture conditioned media extract	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	64
Lactobacillus Ferment Lysate Filtrate	Lactobacillus Ferment Lysate Filtrate derived from *Lactobacillus bifidus* (98% purity)	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	61

Table 10. In vitro ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
Lactobacillus Ferment and Lactobacillus Ferment Lysate	trade name mixture consisting of 10% Lactobacillus Ferment Lysate, 2% Lactobacillus Ferment, 67% water, 10% *Camelia sinensis* leaf extract, 10% *Punica granatum* extract, and 1% caffeine Lactobacillus species used in manufacturing of Lactobacillus Ferment ingredients in this trade name mixture include *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* (unknown which species corresponds to the ferment or ferment lysate)	none	tested neat; 50 µl	cornea epithelial model (n = 2)	EpiOcular™ assay; as described above	non-irritating; controls gave expected results	63

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; OECD = Organisation for Economic Cooperation and Development; TG = test guidelines

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