Safety Assessment of *Nelumbo nucifera*-Derived Ingredients as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e., **June 9, 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to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific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

ALT	alanine aminotransferase
AST	aspartate transferase
cAMP	cyclic adenosine monophosphate
AST	aspartate transferase
BCOP	bovine cornea opacity and permeability test
C3GE	cyanidin 3-*O*-glucoside equivalent
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPK	creatine phosphokinase
CPSC	Consumer Product Safety Commission
CREB	cAMP-response element-binding protein
DAPI	4',6-diamidino-2-phenylindole, dihydrochloride
DAPK1	death-associated protein kinase 1
Dictionary	web-based (wINCI) *International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
DNCB	dinitrochlorobenzene
DNPH	2,4-dinitrophenylhydrazine
DOPA	4-dihydroxyphenylalanine
DPRA	direct peptide reactivity assay
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
G-6-PSD	glucose-6-phosphate dehydrogenase
GAE	gallic acid equivalents
HET-CAM	hen's egg test on the chorioallantoic membrane
HPLC	high-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography with diode array detector
HRIPT	human repeated-insult patch test
3β-HSD	3β-hydroxysteroid dehydrogenase
IC_{50}	half-maximal inhibitory concentration
IgE	immunoglobulin E
INCI	International Nomenclature Cosmetic Ingredient
LD_{50}	median lethal dose
l.o.	leave-on
α-MSH	α-melanocyte stimulating hormone
MITF	microphthalmia-associated transcription factor
MED	minimal erythema dose
MoCRA	Modernization of Cosmetics Regulation Act
N/A	not applicable
Na-CMC	carboxymethyl cellulose
ND	not detected
NOAEL	no-observed-adverse-effect level
NR	not reported
NRU	neutral red uptake
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PEG	polyethylene glycol
PI	propidium iodide
pKA	protein kinase A
PVP	polyvinylpyrrolidone
QE	quercetin equivalents
RIFM	Research Institute for Fragrance Materials
r.o.	rinse-off
RLD	Registration and Listing Data
TAE	tannic acid equivalents
TG	test guideline
TNF-α	tumor necrosis factor α
TRP-1	tyrosinase-related protein-1

US	United States
UVB	ultraviolet B
USP	*United States Pharmacopeia*
VCRP	Voluntary Cosmetic Registration Program
WHO	World Health Organization

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 14 *Nelumbo nucifera*-derived ingredients, most of which are reported to function in cosmetics as skin-conditioning agents and/or antioxidants. The Panel reviewed the available data to determine the safety of these ingredients. Industry should minimize impurities that could be present in cosmetic formulations, such as heavy metals and pesticide residues, according to limits set by the US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA). The Panel concluded that the available data are insufficient to make a determination of safety for all 14 *Nelumbo nucifera*-derived ingredients under the intended conditions of use in cosmetic formulations.

INTRODUCTION

This assessment reviews the safety of 14 *Nelumbo nucifera*-derived ingredients as used in cosmetic formulations:

Nelumbo Nucifera Callus Culture Extract	Nelumbo Nucifera Leaf Extract
Nelumbo Nucifera Extract	Nelumbo Nucifera Phytoplacenta Culture Extract
Nelumbo Nucifera Flower Extract	Nelumbo Nucifera Root Extract
Nelumbo Nucifera Flower/Leaf/Stem Juice	Nelumbo Nucifera Root Water
Nelumbo Nucifera Flower Oil	Nelumbo Nucifera Seed Extract
Nelumbo Nucifera Flower Water	Nelumbo Nucifera Seed Powder
Nelumbo Nucifera Germ Extract	Nelumbo Nucifera Stamen Extract

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), most of these ingredients are reported to function in cosmetics as skin-conditioning agents and/or antioxidants (Table 1).[1] Nelumbo Nucifera Flower Oil is not included in the *Dictionary*; however, it had reported uses in 2023 in the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP) database and in RLD for 2024, and thus included in this review. A few of these ingredients have other reported functions; e.g., Nelumbo Nucifera Flower Water and Nelumbo Nucifera Seed Extract are reported to function as cosmetic astringents, and Nelumbo Nucifera Seed Powder is reported to function as an abrasive. Additionally, Nelumbo Nucifera Root Water is only reported to function as a fragrance ingredient. The Expert Panel for Cosmetic Ingredient Safety (Panel) does not typically review ingredients that function only as fragrance ingredients, because, as fragrances, the evaluation of the safety of these ingredients is the purview of the Research Institute for Fragrance Materials (RIFM). A RIFM safety monograph is not available at this time; therefore, this ingredient is included in this safety assessment.

These ingredients are all derived from the same species and have therefore been grouped together in this assessment. Botanicals, such as *Nelumbo nucifera*-derived ingredients, may contain hundreds of constituents. In this assessment, the Panel is reviewing the potential toxicity of each of these *Nelumbo nucifera*-derived ingredients as a whole, complex substance; toxicity from single components may not predict the potential toxicity of botanical ingredients.

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 in January 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 plant from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Nelumbo nucifera*). Often in the published literature, the general name "lotus" is used. 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 that is being cited (e.g., lotus petal extract). However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., Nelumbo Nucifera Flower Extract) will be used.

CHEMISTRY

Definition and Plant Identification

According to the *Dictionary*, most of the *Nelumbo nucifera*-derived ingredients named in this assessment have the generic CAS No. 85085-51-4.[1] The definitions of these *Nelumbo nucifera*-derived ingredients are presented in Table 1.[1] *Nelumbo nucifera* belongs to the family Nelumbonaceae and is commonly known as Indian lotus, Chinese water lily, and sacred lotus.[2] The *Nelumbo nucifera* plant is native to China, Japan, and India and is a large, perennial rhizomatous aquatic herb which grows in ponds, jheels, ditches and pools.[3-5]

Generic definitions of the parts of plants which pertain to the ingredients reviewed in this report are presented in Table 2.[1] The roots of *Nelumbo nucifera* are planted in the soil of a muddy pond or river bottom.[6] The *Nelumbo nucifera* plant can grow up to 1.5 m in height and can have a horizontal spread of up to 3 m. Flowers grow solitary on stems (3 – 6 ft in length) arising from the leaves, are white to pink in color, fragrant, and have a diameter of 4 – 10 in. The leaves float on the water

surface, are shiny, round, and can have a diameter of 1 to 3 ft. Additionally, lotus leaves have unique water adhesion properties which make them hydrophobic.[7] *Nelumbo nucifera* seeds are 1 cm in diameter and are contained in a woody seed receptacle which looks like a showerhead.[8] Stamens are yellow and are comprised of many ripe carpels (10 mm long) which surround the seed receptacle.[6]

Chemical Properties

An aqueous *Nelumbo nucifera* flower extract was described as a dark, yellowish liquid with a specific gravity of 0.98 – 1.04.[9] Chemical properties for a *Nelumbo nucifera* flower extract,[9] Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate),[10] Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin),[11] and a *Nelumbo nucifera* lotus seed flour [12] can be found in Table 3.[9,12]

Method of Manufacture

Most of the methods described below are general to the processing of *Nelumbo nucifera*-derived ingredients, and it is unknown if they apply to cosmetic ingredient manufacturing. In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.

Nelumbo nucifera plant part extracts

Descriptions of the method of manufacture of a whole plant extract of *Nelumbo nucifera* were not found; however, descriptions of the manufacture of extracts with some plant parts were available. Accordingly, because Nelumbo Nucifera Extract is the extract of the whole plant, this information is provided.

Nelumbo Nucifera Callus Culture Extract

For the preparation of a *Nelumbo nucifera* callus culture extract, sterilized *Nelumbo nucifera* seeds were grown under water to promote the germination of leaves.[13] Upon being transferred to an agar plate and with appropriate growth medium, these leaf segments began to induce callus formation. A callus suspension culture was initiated by adding 7 g callus inoculum to a 70 ml Murashige and Skoog liquid medium containing 30 g/l sucrose. The culture was incubated for approximately 2 - 3 wk. Dried or lyophilized callus (2 g/l) was added to distilled water in an Erlenmeyer flask, which was heated in a 40°C water bath for 4 h. The extract was then filtered twice, using a strainer and a 0.22 µm filter.

In a study seeking to establish a reliable method for lotus callus induction, tissue from lotus leaves, immature cotyledons, immature embryos and rhizome tips were cultured separately in Murashige and Skoog medium that was supplemented with 3 mg/l 2,4-dichlorophenoxyacetic acid and 1 mg/l zeatin.[14] Immature cotyledons (leaf origins in the seed) taken 9 d after pollination showed the earliest signs of callus formation 5 d after culture, followed by sections of immature seed embryos which formed calluses 18 d after pollination and 7 d post-culture.

Nelumbo Nucifera Flower Extract

An aqueous *Nelumbo nucifera* flower extract was prepared by extracting freeze-dried and ground *Nelumbo nucifera* flowers.[9] For the first extraction, 50 g of ground flowers were heated with 2 l of distilled water at 100°C until the solution volume was reduced by half. Another portion of 2 l fresh water was added and heated again until the total solution volume became 50% (second extraction). The third extraction was performed under the same conditions and the final solution was cooled to room temperature and preservatives were added. The preservatives used included 20% propylene glycol and 0.5% of a trade name mixture containing phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben. The resultant solution was filtered with a 200-mesh (0.75 µm) filter, refrigerated for 24 h, and filtered again with mixed cellulose ester filter.

Nelumbo Nucifera Flower/Leaf/Stem Juice

According to the *Dictionary*, Nelumbo Nucifera Flower/Leaf/Stem Juice is the juice expressed from the flowers, leaves, and stems of *Nelumbo nucifera*.[1] No further information regarding method of manufacture was found or submitted.

Nelumbo Nucifera Flower Water

According to the *Dictionary*, Nelumbo Nucifera Flower Water is the aqueous extract of the steam distillate obtained from the flowers of *Nelumbo nucifera*.[1] No further information regarding method of manufacture was found or submitted.

Nelumbo Nucifera Germ Extract

According to an industry submission describing a method of manufacture for a trade name mixture containing 0.5-1.5 w/v% Nelumbo Nucifera Germ Extract as used in cosmetics, raw dried material was extracted with an ethanolic solution, filtered and concentrated.[15] This was dissolved in (50% volume)1,3-butylene glycol solution and allowed sedimentation. The resultant product was packaged after filtration and adjustment.

Nelumbo nucifera germs (200 g) were extracted with 50% ethanol under reflux for 2 h.[16] The resulting mixture was filtered through diatomite, and this filtrate was concentrated under reduced pressure at 60°C. The residue was freeze-dried, and 23.1 g of a *Nelumbo nucifera* germ extract was obtained.

Nelumbo Nucifera Leaf Extract

According to an industry submission, a trade name mixture containing a maximum of 1.2% Nelumbo Nucifera Leaf Extract is prepared by solubilization of *Nelumbo nucifera* leaf powder in a mix of water/butylene glycol (50/50).[17] The soluble and insoluble phases were separated, the soluble phase was filtered and then using sterilized membrane filtration.

An aqueous *Nelumbo nucifera* leaf extract was prepared by freeze-drying and grinding leaves (50 g) and performing 3 extractions with 2 l of water heated to 100°C until the solution volume reduced to half.[9] The final extract was cooled and preservatives were added. The preservatives used included 20% propylene glycol and 0.5% of a trade name mixture containing phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben. The resultant solution was filtered with a 200-mesh (0.75 µm) filter, refrigerated for 24 h, and filtered again with a mixed cellulose ester filter.

Another aqueous *Nelumbo nucifera* leaf extract was reported to be prepared from *Nelumbo nucifera* leaves that were washed with distilled water, air-dried at 50°C, and ground into powder.[18] Distilled water (5 l) was used to resuspend 200 g of the leaf powder for 24 h at 4°C. The precipitate was removed via filtration and the supernatant was condensed using a vacuum concentrator. The condensed solution was then lyophilized as a *Nelumbo nucifera* leaf extract.

Nelumbo Nucifera Root Extract

An aqueous *Nelumbo nucifera* root extract was prepared by freeze-drying and grinding the root (50 g) and performing an extraction 3 times with 2 l of water heated to 100°C until the solution volume reduced to half.[9] The final extract was cooled and preservatives were added. The preservatives used included 20% propylene glycol and 0.5% of a trade name mixture containing phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben. The resultant solution was filtered with a 200-mesh (0.75 µm) filter, refrigerated for 24 h, and filtered again with mixed cellulose ester filter.

In another study, fresh lotus roots were ground into powder using a mortar and pestle.[19] Ground samples of *Nelumbo nucifera* lotus root were weighed to 20 g and added to either distilled water, anhydrous ethanol, methanol, 20, 40, 60 or 80% ethanol, or 20, 40, 60, or 80% methanol, at a material-to-liquid ratio of 1:10 (g:ml). The resulting *Nelumbo nucifera* lotus root extracts were obtained via ultrasonic extraction at an extraction temperature of 50°C for 1 h, concentrated with a rotary evaporator, dried into a lyophilized powder using a vacuum freeze dryer, and stored at – 20°C.

Nelumbo Nucifera Seed Extract

A crude *Nelumbo nucifera* seed extract was prepared by drying, grinding, and extracting *Nelumbo nucifera* seeds in a Soxhlet extractor with petroleum ether.[20] The resultant extract was dried by the removal of solvent under vacuum.

An aqueous *Nelumbo nucifera* seed extract was prepared by freeze-drying and grinding the seeds (50 g) and performing an extraction 3 times with 2 l of water heated to 100°C until the solution volume reduced to half.[9] The final extract was cooled and the preservatives were added. The preservatives used included 20% propylene glycol and 0.5% of a trade name mixture containing phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben. The resultant solution was filtered with a 200-mesh (0.75 µm) filter, refrigerated for 24 h, and filtered again with a mixed cellulose ester filter.

For a study, *Nelumbo nucifera* seeds were dried, powdered, and then extracted with 50% ethanol in a Soxhlet apparatus.[21] The resulting ethanolic extract was filtered and then evaporated under reduced pressure. A *Nelumbo nucifera* lotus seed tea was prepared by removing the seed coat, roasting the seeds until brown, and then extracting the roasted seeds with hot water.[22]

Nelumbo Nucifera Seed Powder

In a preparation of *Nelumbo nucifera* seed powder, fresh lotus seeds were washed and the seed coat was separated from the seed.[12] The seeds were then dried in a tray dryer at 60°C, ground into flour, and sieved through 72 µm mesh. Another *Nelumbo nucifera* seed powder was obtained using dry lotus seeds.[23] Seed kernels were obtained by breaking with a hammer and were immediately ground using a mortar and pestle. The resulting *Nelumbo nucifera* seed powder was sieved through a fine cloth to obtain uniform particle size.

Nelumbo Nucifera Stamen Extract

Dried powder of *Nelumbo nucifera* stamens (5 kg) was extracted with 95% ethanol by percolation at room temperature over 2 wk.[24] The extract was then filtered and the combined filtrate was evaporated to remove ethanol under reduced pressure and lyophilized to yield an ethanolic *Nelumbo nucifera* stamen extract.

Dried *Nelumbo nucifera* stamens (100 g) were extracted in an ultrasonic bath using 90% ethanol.[25] The resulting solution was centrifuged and filtered through 0.45µm nylon syringe membranes to obtain an ethanolic *Nelumbo nucifera* stamen extract.

Composition and Impurities

The main chemical classes of compounds present in the *Nelumbo nucifera* plant are proteins, amino acids, and phytosterols (present mostly in the seeds), carbohydrates (present mostly in the leaves and seeds), alkaloids and flavonoids

(present mostly in the flowers, leaves, and seeds), and terpenoids (present mostly in the leaves).[26] Alkaloids are the prominent bioactive chemical class of constituents present in *Nelumbo nucifera*. Among them, the high representation of biosynthetic sub classes of aporphine alkaloids, benzylisoquinoline alkaloids, and bisbenzylisoquinoline alkaloids is significant.[27-30] Nuciferine is the main aporphine alkaloid, and neferine and liensinine are the main bioactive bisbenzylisolquinoline alkaloids present in the *Nelumbo nucifera* plant.[29] A list of major constituents, organized by chemical class, and presence in *Nelumbo nucifera* plant parts (embryo, flower, leaf, seed, and stamen) is provided in Table 4.

Nelumbo Nucifera Extract

The mineral and heavy metal content of *Nelumbo nucifera* has been considered.[31] A *Nelumbo nucifera* plant was described as containing iron (171.38 ppm), zinc (45 ppm), copper (8.43 ppm), nickel (4.16 ppm), lead (0.728 ppm), chromium (0.27 ppm), arsenic (0.178 ppm), mercury (0.065 ppm), and cadmium (0.022 ppm).

Nelumbo Nucifera Flower Extract

According to an industry submission, Nelumbo Nucifera Flower Extract (1 - 5%) extracted in isostearyl isostearate (95 – 99%) complies with aflatoxin limits set in the *United States Pharmacopeia* (USP) and pesticide and residual solvent limits set in the *European Pharmacopoeia*.[10] Heavy metal content was not analyzed; however, according to the raw materials used and the manufacturing process, the eventual presence of total heavy metals in this product would be technically unavoidable and lower than 10 ppm.

According to an industry submission, a trade name mixture of Nelumbo Nucifera Flower Extract (0.5 – 1%) extracted in propanediol (70 -90%) and glycerin (10 – 30%) with 0.5 – 1% Nymphaea Caerulea Flower Extract also comprised 70 – 90% propanediol and 10 – 30% glycerin.[11] According to gas chromatography-mass spectrometry analysis, benzyl alcohol was present at 3.4 – 13 ppm (average value of 6.7 ppm); all other European fragrance allergens were below the limit of detection (< 2 ppm). Heavy metal content was not analyzed, however, according to the raw materials used and the manufacturing process, the eventual presence of total heavy metals in this product would be technically unavoidable and lower than 10 ppm.

The total flavonoid content in whole *Nelumbo nucifera* flowers and *Nelumbo nucifera* petals, using ultrasound extraction with ethanol and flavonoid enrichment, was determined to be 40.08 ± 1.94 and 38.67 ± 0.70 mg/g dry weight (DW), respectively.[25] Separate aqueous and ethanolic extracts of white and red *Nelumbo nucifera* petals were evaluated for total phenolic, tannin, flavonoid, and monomeric anthocyanin content.[32] For both aqueous extracts, the average total phenolic content was 22.41 gallic acid equivalents (GAE)/g DW, the average total tannin content was 18.84 tannic acid equivalents (TAE)/g DW, the average total flavonoid content was 9.22 quercetin equivalents (QE)/g DW, and the total monomeric anthocyanin content for the aqueous red petal extract was 49.75 µg cyanidin 3-*O*-glucoside equivalents (C3GE)/g DW. For both ethanolic extracts, the average total phenolic content was 0.52 GAE/g DW, the average total tannin content was 1.24 TAE/g DW, and the average total flavonoid content was 1.24 QE/g DW. In another ethanolic *Nelumbo nucifera* flower extract, the average total flavonoid content was reported to be 15.98 mg/100 g of dry extract, while the total phenolic content was reported to be 10.68 mg/100 g of dry extract.[33]

A hydroalcoholic *Nelumbo nucifera* flower extract was determined to contain alkaloids, proteins and amino acids, flavonoids, tannins, and phytosterols (amounts not specified).[34] Phenolic substances (total, 10.20 µg/100 g), protein (34 µg/100 g), vitamin C (0.36 µg/100 g), vitamin E (0.42 µg/100g), tannins (4.30 µg/100g), and carbohydrates (672 µg/100 g) were also identified.

An ethyl alcohol *Nelumbo nucifera* lotus petal extract was shown to have a higher total phenolic content (351 mg GAE/g dry extract) compared to an ethyl acetate lotus petal extract (208 mg GAE/g dry extract) when analyzed via the Folin-Ciocalteau method.[35] A quantitative comparison of reference standard compounds in both lotus petal extracts identified in a high-performance liquid chromatography with diode array detector (HPLC-DAD) analysis is presented in Table 5.

A 70% ethanolic *Nelumbo nucifera* petal extract was analyzed.[36] Total phenolic content (18.56 GAE)/g), total flavonoid content (6.77QE/g), total alkaloid content (4.55 piperidine equivalents), and total tannins (23.14 GAE/g) were measured.

In another phytochemical study, the alkaloids present in a methanolic *Nelumbo nucifera* flower bud extract were identified.[37] A crude alkaloid fraction of 0.9 kg methanolic *Nelumbo nucifera* flower bud extract contained nuciferine (183 mg), nornuciferine (121 mg), *N*-methylasimilobine (36 mg), (-)-lirinidine (3 mg), lysicamine (38.2 mg), pronuciferine (23 mg), and β-sitosterol (1.8 mg).

One aqueous extract of *Nelumbo nucifera* flower was reported to contain 10 ppm heavy metals, 2 ppm arsenic, and 100 cfu/ml microbes.[9] Quantification of phenolic, flavonoid, and anthocyanin content in the flower and leaf stalk, leaf, petal, seed embryo, and stamen of the *Nelumbo nucifera* plant is presented in Table 6.[38] Total phenolic content (GAE/g DW) was highest in the leaf (39.09 ± 0.79 GAE/g DW) and total flavonoid content was highest in the petal (approximately 5054.72 mg/100 g DW). Minimal anthocyanins (C3GE/g DW) were detected in the stamen (0.23 ± 0.02) and petal (0.05 ± 0.00).

Nelumbo Nucifera Germ Extract

According to an industry submission, Nelumbo Nucifera Germ Extract is composed of tannins and flavonoids or tannins and saccharides.[15] The presence of heavy metals were not more than 20 ppm and arsenic was not present at more than 2 ppm.[15]

Several flavonoids and alkaloids such as neferine, and polyphenols, such as orientin, isoorientin, vitexin, isovitexin, vicenin-3, vicenin-1, and schaftoside were identified (amounts not specified) in a *Nelumbo nucifera* germ extract prepared with 50% ethanol.[39] Quantification of phenolic, flavonoid, and anthocyanin content in a *Nelumbo nucifera* seed embryo is presented in Table 6.[38]

Nelumbo Nucifera Leaf Extract

According to an industry submission, the composition of a trade name mixture containing 0.5 – 1.2% Nelumbo Nucifera Leaf in a 50/50 mix of water/butylene glycol was as follows: sugars (51.1%), mineral ashes 28.0%, proteins 28%, and polyphenols 7.6%.[17] The presence of heavy metals antimony, arsenic, cadmium, chromium, cobalt, mercury, nickel, lead and vanadium was below the threshold ($\leq$ 0.5 ppm).

The total phenolic content for an aqueous and a methanolic extract of *Nelumbo nucifera* leaves was 85.01 ± 2.32 mg GAE/g DW and 147.63 ± 2.23 mg GAE/g DW, respectively; the total flavonoid content was determined to be 35.38 ± 1.32 mg QE/g DW in the aqueous extract and 41.86 ± 1.07 mg QE/g DW in the methanolic extract.[40] In another phytochemical study, the following compounds were identified in the ethyl acetate fraction of a methanolic *Nelumbo nucifera* leaf extract (36.9 g): *N*-methylasimilobine *N*-oxide (3.3 mg), nuciferine (67.3 mg), nuciferine *N*-oxide (40.7 mg), *N*-nornuciferine (2.3 mg), dehydronuciferine (3.9 mg), ± (41.8 mg), quercetin 3-*O*-β-d-galactopyranoside (7.5 mg), and (+)-catechine (40.5 mg).[37] Quantification of phenolic, flavonoid, and anthocyanin content in a *Nelumbo nucifera* old leaf and leaf stalk are presented in Table 6.[38]

Nelumbo Nucifera Root Extract

A phytochemical screening of an ethanolic extract of *Nelumbo nucifera* roots was performed.[41] The *Nelumbo nucifera* root extract was found to contain carbohydrates, alkaloids, glycosides, flavonoids, and proteins and amino acids (amounts not specified).

Nelumbo Nucifera Seed Extract

Nelumbo nucifera seeds, extracted with a hydroalcoholic solvent, were analyzed for phenolic content.[42] The total phenolic content of the hydroalcoholic *Nelumbo nucifera* seed extract was determined to be 7.61 ± 0.04% (w/w). In another phytochemical study, *Nelumbo nucifera* lotus seed proteins were fractionated according to their solubility in various solvents.[43] The major phytochemicals present in the seeds of *Nelumbo nucifera* are the alkaloids dauricine, nuciferine, pronuciferine, liensinine, isoliensinine, rosmerine and neferine.

The essential and non-essential amino acid composition of a lotus seed protein and its fractions (water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamine, and alkali-soluble glutelin) is presented in Table 7. Total essential amino acid content in the seed protein was 322.82 g/kg (crude protein, DW), while the total non-essential amino acid content was 553.06 g/kg. The essential and non-essential amino acid contents were highest in the globulin fraction. Palmitic acid (33.27%) and linoleic acid (19.9%) were the 2 most prevalent constituents in a fatty acid composition of a whole *Nelumbo nucifera* seed oil (obtained via extraction of seed powder with 2:1 v/v chloroform: methanol solution). The fatty acid profile from this analysis is presented in Table 8.[44]

Nelumbo Nucifera Seed Powder

A nutritive analysis of *Nelumbo nucifera* seeds demonstrated that it contains 1.93% crude fat, 2.7% crude fiber, 4.5% ash, 10.6% protein, 10.5% moisture content, and 72.17% carbohydrate.[45] The composition of the mineral content in *Nelumbo nucifera* seeds was reported as potassium (28.5%), calcium (22.1%), magnesium 9.2%, sodium 1%, and negligible percentages of chromium, copper, manganese, iron, and zinc.

The nutritional composition of a *Nelumbo nucifera* lotus seed flour (per 100 g) was analyzed.[12] A nutritive analysis of *Nelumbo nucifera* seeds suggested a by-weight content of 72.17% carbohydrates, 10.16% proteins, 2.7% crude fiber, and 1.93% crude fat. Pyrolysis resulted in 4.5% residual ash and release of 10.5% moisture.

Nelumbo Nucifera Stamen Extract

The total phenolic content in a *Nelumbo nucifera* stamen was determined to be 36.37 ± 0.73 mg GAE/100 g DW.[38] Flavonoids such as myricetin (7.63 ± 0.35 mg/100 g DW), luteolin (amount not determined), quercetin (43.94 ± 2.08 mg/100 g DW), naringenin (2185.84 ± 24.21 mg/100 g DW), kaempferol (160.71 ± 13.66 mg/100 g DW), isorhamnetin (192.09 ± 15.70 mg/100 g DW) , cyanidin (115.79 ± 10.21 mg/100 g DW), and delphinidin (211.63 ± 17.21 mg/100 g DW) were also identified. In another phytochemical study, total flavonoid content was higher in an ethanolic *Nelumbo nucifera* stamen extract (68.11 ± 3.53 mg/g DW), compared to ethanolic *Nelumbo nucifera* whole flower and petal extracts (40.08 ± 1.94 and 38.67 ± 0.70 mg/g DW, respectively).[25]

Phytochemical investigations on *Nelumbo nucifera* stamens have been able to identify the benzylisoquinoline alkaloids annaine, dehydroanonaine, armepavine, asimilobine, demthycoclaurine, lirinidine, dehydronuciferine, liriodenine, dehydroemerine, nornuciferine, N-methylasimilobine, N-methylcoclaurine, N-methylisococlaurine, N-norarmepavine and romarin.[46] In addition, the bis-benzylisoquinolic alkaloids iosliensinine and lisensinine have also been reported from the stamens of *Nelumbo nucifera*.

Seven flavonoids were identified in the ethanolic *Nelumbo nucifera* stamen extract via reversed-phase high-performance liquid chromatography (HPLC), recorded at 320 nm: isorhamnetin-3-*O*-glucose, kaempferol-3-*O*-glucose, kaempferol 3-*O*-

glucuronic acid, kaempferol-3-*O*-robinobioside, myricetin-3-*O*-glucose, quercetin-3-*O*-glucuronic acid, and rutin (amounts not determined). Quantification of phenolic, flavonoid, and anthocyanin content in a *Nelumbo nucifera* stamen is presented in Table 6.[38]

UV Absorption

Nelumbo Nucifera Germ Extract

The ultraviolet (UV) absorption of Nelumbo Nucifera Germ Extract in water and butylene glycol was determined.[47] According to an industry submission, three trade name mixtures that consisted of Nelumbo Nucifera Germ Extract in water and butylene glycol (concentrations not stated) have absorption maxima of 272.1, 273.0 and 273.0 nm.

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 *Nelumbo nucifera*-derived ingredients in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (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 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-yr period is less than $1000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. 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.[48] 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.

Nelumbo Nucifera Flower Extract had the highest number of reported uses, with 544 uses (211 of which are for face and neck cleansing products) are reported in the RLD in 2024[49] and in 2023, 200 uses reported in the VCRP in 2023[50] (Table 9). The results of the concentration of use survey conducted by the Council in 2022 and 2024 indicate that Nelumbo Nucifera Root Extract has the highest maximum reported concentration of use; it is reported to be used at up to 0.2% in foundations.[51,52]

Cosmetic products containing *Nelumbo nucifera*-derived ingredients may incidentally come in contact with the eyes (e.g., Nelumbo Nucifera Flower Extract at 0.0015% in eye lotions), and could be incidentally ingested or come in contact with mucous membranes (e.g., Nelumbo Nucifera Flower Extract at 0.1% in lipstick). Use in baby products is also reported (e.g., Nelumbo Nucifera Flower Extract is used at up to 0.00055% in baby shampoos).

Additionally, *Nelumbo nucifera*-derived ingredients are used in cosmetics that can possibly be inhaled; for example, Nelumbo Nucifera Flower Oil is reported to be used in perfumes (concentration of use not reported) and Nelumbo Nucifera Flower Extract is reported to be used at 0.1% in face powders. 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 *Nelumbo nucifera*-derived ingredients may be 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 for some, but not all, product categories 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 these ingredients as listed in the RLD do require designation if airbrush application is used, and this type of application was reported for Nelumbo Nucifera Flower Extract in leg and body paints. 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 *Nelumbo nucifera*-derived ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[53]

Non-Cosmetic

Nelumbo nucifera flowers, leaves, rhizomes, stems and seeds are used as food and widely used in traditional medicine.[54,55] *Nelumbo nucifera* flowers are ornamental and the species is of religious significance in South East Asia.[56] *Nelumbo nucifera* seeds are used in East Asian cuisine and are sometimes sold as a snack food.[57] *Nelumbo nucifera* seed powder is used in baked goods, and *Nelumbo nucifera* seeds are used to produce milk and other food products.[58,59] *Nelumbo nucifera* seeds have also been used in the production of biofuels.[57]

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Nelumbo nucifera*-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetic data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Nelumbo Nucifera Germ Extract

In a study to determine acute toxicity, groups of 4 male and 4 female mice were given a trade name mixture containing 0.5 -1.5 w/v% Nelumbo Nucifera Germ Extract (composed of tannins and flavonoids) [15] The LD_{50} was > 2 g/kg. (Additional details were not provided.)

Oral

Details on the acute oral toxicity studies summarized below can be found in Table 10.

No signs of toxicity or mortality were observed in mice that received a single oral dose (up to 99.9 g/kg bw) of an herbal mixture capsule containing 33% *Nelumbo nucifera* Gaertn.[60] The acute oral toxicity of several ethanolic extracts of *Nelumbo nucifera* plant parts were evaluated using rats.[41] The acute oral LD_{50} values of a *Nelumbo nucifera* leaf, flower, and root extract, a *Nelumbo nucifera* leaf and root extract, and a *Nelumbo nucifera* flower extract were > 2 g/kg, which was the maximum dose tested for each test article. No mortality was observed in rats administered a single oral dose of a hydroalcoholic *Nelumbo nucifera* flower extract at 2 g/kg.[34] A hydroalcoholic *Nelumbo nucifera* seed extract, in 0.3% sodium carboxymethyl cellulose, had an acute oral LD_{50} > 1 g/kg in mice.[42] The acute oral LD_{50} values for an ethanolic *Nelumbo nucifera* lotus root extract and a *Nelumbo nucifera* stamen extract-polyvinylpyrrolidone (PVP)-10 complex were both > 5 g/kg in mice and rats, respectively.[24,61]

Short-Term, Subchronic, and Chronic Toxicity Studies

Details on the repeated dose oral toxicity studies summarized below can be found in Table 11.

An herbal mixture capsule containing 33% *Nelumbo nucifera* Gaertn. was dissolved in water and orally administered at doses of 0, 1.44, or 4.32 g/kg/d to Wistar rats (10/group; sex not specified) for 4 wk.[60] Statistically significant increases in body weight were observed in 1.44 g/kg/d rats after 2 wk of treatment, compared to controls. No gross lesions or size changes were observed in the heart, liver, lungs, or kidneys and no significant histopathological differences were observed in rats treated for 4 wk, compared to controls. In a 6-mo study, a *Nelumbo nucifera* lotus seed tea was administered as the drinking fluid to male SKH-1 hairless mice (10/group).[22] No significant differences in food or liquid consumption or body weight were observed between treated mice and controls. In another oral toxicity study, Sprague-Dawley rats (5/sex/group) were orally dosed with 0, 500, 1000, or 2000 mg/kg/d Nelumbinis semen (*Nelumbo nucifera* seeds) for 13 wk.[62] No mortality, body weight, or ophthalmic changes were observed in treated animals, compared to controls. Statistically significant lower food consumption was observed in males at weeks 7 and 12 for the 500 and 2000 mg/kg/d groups and at weeks 7, 9, 10, and 12 for 1000 mg/kg/d males, compared to controls. Lower right adrenal gland weight in 500 and 1000 mg/kg males was neither dose-dependent or sex-matched and was, thus, not considered treatment-related. The no-observed-adverse-effect-level (NOAEL) was determined to be 2000 mg/kg/d for both sexes (combined). Beagle dogs (1/sex/group) were orally dosed with 0, 500, 1000, 2000, or 4000 mg/kg/d Nelumbinis semen for 28 d.[62] No mortality was observed. Vomiting in the 2000 mg/kg male, low specific gravity of the urine in all treated females, and white blood cell reactions in all the treated males and the 2000 mg/kg female were not considered systemically or toxicologically significant. The NOAEL was determined to be 4000 mg/kg/d. In a 90-d oral toxicity study, Sprague-Dawley rats (6/sex/group) were orally administered 0, 50, 100, or 200 mg/kg/d of a *Nelumbo nucifera* stamen extract-PVP complex in distilled water.[24] Statistically significant decreases in the body weights of 200 mg/kg females and reduced relative heart, liver, and kidney weights were not considered treatment-related because the values were within normal laboratory range. No gross or histopathological abnormalities were noted. The NOAEL for both male and female rats was determined to be > 200 mg/kg/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details on the in vitro and oral reproductive toxicity studies summarized below can be found in Table 12.

In an in vitro reproductive toxicity study, rat sperm was tested with an aqueous *Nelumbo nucifera* petal extract at 0, 0.22, 0.44, 0.88, 1.76, or 3.52 mg/ml.[32] A statistically significant increase in sperm viability was observed from exposure to the 0.22 - 1.76 mg/ml concentrations; differences in sperm viability from the 3.52 mg/ml group and controls were not significant. In an animal study, male Wistar albino rats (10/group) were orally administered 7.5 mg/kg bw of a petroleum ether *Nelumbo nucifera* seed extract every other day for 15 d.[20] Statistically significant decreases in testis, epididymis, and adrenal gland weights, body growth rate, sperm count and motility, and 3β-hydroxysteroid dehydrogenase (3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PSD) levels in treated animals, compared to controls, were considered possibly due to inhibition of testicular steroidogenesis. In a similar study, female Wistar rats (12/group) were orally dosed with up to 7.5 mg/kg of a petroleum ether *Nelumbo nucifera* seed extract every other day for 15 d.[20] Statistically significant inhibition of the vaginal opening and first estrus and decreases in body weights, ovary weights, and uterus weights were observed in treated animals, compared to controls. The researchers considered the suppressed activity of 3β-HSD and G-6-PSD to possibly indicate an inhibition of ovarian steroidogenesis. The potential effects of an ethanolic *Nelumbo nucifera* seed extract were evaluated in female Wistar albino rats.[21] Groups of female Wistar albino rats (10/group) were orally dosed with 0 or 800 mg/kg bw of an ethanolic *Nelumbo nucifera* seed extract for 40 d. Statistically significant decreases in ovary, uterus, and vagina weights were observed in treated animals, compared to controls. Estrous cycles were prolonged in treated animals, which was accompanied by a statistically significant increase in the diestrus phase of the estrous cycle in treated animals, compared to controls. Groups of male Wistar rats (10/group) were dosed with an ethanolic *Nelumbo nucifera* seed extract at 0, 50, 100, or 200 mg/kg bw/d, via gavage, for 60 d.[63] Decreases in the testes, epididymis, seminal vesicle, and ventral prostate weights of treated animals were observed in a dose-dependent manner. A statistically significant decrease in sperm motility was observed in all treated groups. Dose-dependent and statistically significant decreases in testicular and caudal epididymal sperm and serum testosterone levels were observed, compared to controls.

GENOTOXICITY STUDIES

In Vitro

Nelumbo Nucifera Flower Extract

An Ames test was performed in accord with the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 471 to evaluate the mutagenic potential of a trade name mixture of Nelumbo Nucifera Flower Extract (0.5 – 1%, extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract).[11] *Salmonella typhimurium* strains TA1535, TA1537, TA8, TA-100, and TA102 were tested in the presence and absence of metabolic activation. The test substance was not mutagenic.

Methanolic extracts of *Nelumbo nucifera* plumule and blossom were not mutagenic when tested at 0.5, 1, or 2.5 mg/plate, with or without metabolic activation, using *Salmonella typhimurium* TA98 and TA100 strains in an Ames test.[64] In another Ames test, dichloromethane, methanol, and aqueous *Nelumbo nucifera* flower extracts were not mutagenic towards *S. typhimurium* strains TA98 and TA100 without metabolic activation.[65] No further details were provided.

Nelumbo Nucifera Germ Extract

The mutagenicity of trade name mixtures containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (composed of tannins and flavonoids and of tannins and saccharides) was determined by reverse mutation testing using *S. typhimurium* strains TA100, TA1535, TA98, and TA 1537 and *Escherichia coli* WP2*uvrA*.[15] The concentration of each test solution was 5000 µg/plate. Negative results were observed for both trade name mixtures.

CARCINOGENICITY STUDIES

No relevant carcinogenicity studies on the *Nelumbo nucifera*-derived ingredients evaluated in this report were found in the published literature, and unpublished data were not submitted.

ANTI-CARCINOGENICITY STUDIES

Several *Nelumbo nucifera*-derived ingredients exhibit anti-carcinogenic properties. Aqueous and methanolic *Nelumbo nucifera* leaf extracts have been shown to inhibit angiogenesis in normal and breast cancer cells.[66-68] (Human breast cancer MDA-MB-231 cells were treated with a *Nelumbo nucifera* leaf extract at 0.5, 1, 2, 3, 4, and 5 mg/ml concentrations.) A methanolic *Nelumbo nucifera* floral receptacle extract and an ethanolic *Nelumbo nucifera* petal extract were shown to have cytotoxic effects against breast and cervical cancer cell lines (PC_{50} of 10.5 µg/ml), respectively.[68,69] An aqueous *Nelumbo nucifera* rhizome extract exhibited antiproliferative effects in both epidermoid and breast cancer cells[70] and an ethanolic *Nelumbo nucifera* stamen extract exhibited 86.3% inhibition at 400 µg/ ml and induced apoptosis in HCT 116 human colon cancer cells.[71]

OTHER RELEVANT STUDIES

Effects on Pigmentation

The skin lightening effects of aqueous *Nelumbo nucifera* leaf, root, flower, stem, and seed extracts were evaluated, separately, at concentration of 10, 50, 100, or 200 µg/ml, in both a tyrosinase inhibition assay and a 4-dihydroxyphenylalanine (DOPA)-oxidase inhibition assay.[9] Arbutin was used as the positive control (at the same

concentration as the test substances). Statistically significant tyrosinase inhibition was exhibited by the *Nelumbo nucifera*-derived extracts, compared to that of arbutin. In the DOPA-oxidase assay, the inhibitory effect at the 100 µg/ml concentration was 59% for a *Nelumbo nucifera* leaf extract, 57% for a *Nelumbo nucifera* seed extract, and 50% for a *Nelumbo nucifera* flower extract, compared to the 44% inhibitory effect of arbutin. Based on skin-lightening effects seen in the study, the researchers concluded that inhibition of one of these pathways was sufficient to affect melanin synthesis.

A phosphodiesterase inhibitor, theophylline, was utilized to stimulate melanogenesis in murine B16 melanoma 4A5 cells, which were subsequently treated with methanolic *Nelumbo nucifera* flower bud, stamen, seed, and leaf extracts (at up to 100 µg/ml).[37] The methanolic *Nelumbo nucifera* flower bud extract significantly inhibited melanogenesis with a half-maximal inhibitory concentration (IC_{50}) value of 20 µg/ml. The *Nelumbo nucifera* leaf extract exhibited a moderate effect, while the inhibitory activity of the stamen and seed extracts were weak. *Nelumbo nucifera* flower bud, stamen, and seeds showed no cytotoxic effects and the leaf extract showed weak cytotoxicity at a high concentration of 100 µg/ml.

Nelumbo Nucifera Callus Culture Extract

The whitening effect of a *Nelumbo nucifera*-derived callus extract was evaluated in cultured B16F1 melanoma cells using a melanin synthesis inhibition test.[13] Cells were treated with 0.025, 0.050, or 0.1% of a *Nelumbo nucifera*-derived callus extract. α-melanin stimulating hormone (10 nM) was used as the negative control and kojic acid was used as the positive control; negative and positive controls produced expected results. A dose-dependent, inhibitory effect on melanin synthesis of cells treated with the *Nelumbo nucifera*-derived callus extract was observed at approximately 26.65% at the low dose, 36.02% at the medium dose, and 78.89% at the high dose, on average. Kojic acid used as a positive control showed a suppression rate of 54.52% at 200 ppm.

Nelumbo Nucifera Leaf Extract

The potential for an aqueous *Nelumbo nucifera* leaf extract to inhibit melanogenesis was evaluated in B16F1 melanoma cells obtained from mice.[18] Cells were treated with 10 µM α-melanocyte stimulating hormone (α-MSH) and either aqueous *Nelumbo nucifera* leaf extract (0.1, 0.2, 0.3, 0.4, or 0.5 mg/ml) or gallic acid, a constituent of the leaf extract, (60, 70, 80, 90, 100 µM) for 24, 48, or 72 h. Melanin content was measured by normalizing total melanin values with protein content (µg of melanin/mg of protein) and levels of proteins associated with melanogenesis were measured using an immunoblotting assay. Overall, the *Nelumbo nucifera* leaf extract exhibited better efficacy in inhibiting melanogenesis stimulated by α-MSH compared to gallic acid, which the authors surmised was due to the synergistic effect of the extract. Furthermore, the *Nelumbo nucifera* leaf extract significantly inhibited the expression of tyrosinase, microphthalmia-associated transcription factor (MITF) and tyrosinase-related protein-1 (TRP-1) in a dose-dependent manner, indicating that the *Nelumbo nucifera* leaf extract reduced melanin content via downregulation of MITF and tyrosinase family proteins. Congruently, treatment with *Nelumbo nucifera* leaf extract also exhibited inhibition of cyclic adenosine monophosphate (cAMP) response element-binding (CREB) protein, and protein kinase A (pKA) phosphorylation under both basal and stimulated conditions.

The effects of an aqueous *Nelumbo nucifera* leaf extract upon melanogenesis and epidermal hyperplasia induced by ultraviolet B (UVB) radiation were evaluated in guinea pigs.[18] Four female Dunkin-Hartley guinea pigs had a 1.5 cm^2 area of the back exposed to 280 – 305 nm UVB radiation 3 times/wk for 2 wk, for a total UVB dose of 500 mJ/cm^2 per exposure. The animals received a topical gel application of 1 or 2 % *Nelumbo nucifera* leaf extract mixed with polyethylene glycol (PEG-40) to irradiated skin the following day. Skin biopsies were collected, stained, and measured for melanin content. Results revealed that treatment with the *Nelumbo nucifera* leaf extract reversed UVB-induced epidermal hyperplasia and melanin content in the epidermis of irradiated guinea pigs. Western blot analysis demonstrated that the *Nelumbo nucifera* leaf extract downregulated the expression of proteins involved in melanogenesis under UVB-stimulated conditions (tyrosinase, TRP-1, β-actin, extracellular signal-regulated kinase (ERK), phospho-ERK) and modulated cAMP mediated PKA signaling and ERK activity, confirming mechanistic involvement in the depigmentation of guinea pig skin under study conditions.

Photoprotective Effects

Nelumbo Nucifera Leaf Extract

The protective effects of an ethanolic *Nelumbo nucifera* leaf extract against UVB radiation were evaluated using mitochondria isolated from the livers of female Sprague-Dawley rats.[72] The reaction models comprised 0.5 ml mitochondrial protein, with either 10, 100, or 1000 µg/ml *Nelumbo nucifera* leaf extract in 70% v/v ethanol added as the test material. Butylated hydroxytoluene and gallic acid served as positive controls, while 70% v/v ethanol solution without test extracts served as a model group; the blank control group was identical to the model group, without irradiation. Each mixture was irradiated for 4 h with a 20 W UVB lamp; the irradiation dose was measured to be 0.88 J/cm^2. In a thiobarbituric acid assay, the overall absorbance at 532 nm was lower in groups treated with the leaf extract and positive controls, compared to the model group. However, only the 100 µg/ml and 1000 µg/ml *Nelumbo nucifera* leaf extract groups showed a statistically significant inhibition capacity against UVB-induced oxidation.

The protective effects of the same *Nelumbo* nucifera leaf extract against UVB-induced phototoxicity were evaluated in vivo using male BALB/C mice.[72] Groups of 6 mice were divided into non-irradiated controls, a radiation-only model group, 3 groups receiving 0.1% sodium carboxymethyl cellulose solvent with 50, 250, or 5000 mg/kg bw ethanolic *Nelumbo nucifera* leaf extract, or positive control group receiving 250 mg/kg bw gallic acid. The animals were irradiated for 1 h daily

for the first 5 d (irradiation dose = 0.22 J/cm^2) and then irradiated for 2 h up till the tenth day (irradiation dose = 0.44 J/cm^2). All mice were treated with a topical dose of corresponding solvent on the dorsal surface 30 min prior to irradiation. Effects resulting from UVB irradiation were significantly reversed with treatment with the *Nelumbo nucifera* leaf extracts and gallic acid. The group treated with 50 mg/kg leaf extract showed significantly reduced malondialdehyde levels and superoxide dismutase activity compared to the UVB-model group. Additionally, glutathione peroxidase, catalase, and hydroxyproline levels were significantly higher in the groups treated with the 250 and 500 mg/kg bw *Nelumbo nucifera* leaf extracts than that of the UVB model group.

Nelumbo Nucifera Seed Extract

The potential for the oral administration of an aqueous *Nelumbo nucifera* lotus seed tea to protect against the effects of UVB-irradiation was examined in hairless male SKH-1 mice.[22] The lotus seed tea was made by roasting *Nelumbo nucifera* seeds until browned and extracting with hot water. Animals were randomly divided into 2 groups (n = 10) which either received the lotus seed tea or water (controls) as drinking fluid for 6 mo. After 6 mo of treatment, each group was further divided into 2 groups each (n = 5), 2 of which received UVB-irradiation and 2 of which were not irradiated (water group, water-UVB group, lotus seed tea group, and lotus seed tea-UVB group). The backs of the mice were irradiated with UVB at a dose of 1.8 mW/s and 50 mJ/cm^2 3 times per wk; the dose of irradiation was increased by 20% every wk for 15 wk. The moisture content of skin was measured using a Corneometer. A 1 cm^2 cross-section was obtained from the center of the dorsal side, stained with hematoxylin-eosin dye, and observed for histopathological changes in the skin; 5 random locations on a skin tissue were selected and average values were used. The skin homogenate samples were treated with either hydrochloric acid (control) or 2,4-dinitrophenylhydrazine (DNPH) and the respective absorbance of each sample was measured at 370 nm. The difference in the spectrum of the DNPH-treated sample and the hydrochloride control was determined and the protein carbonyl content of tissue samples was calculated using the molar absorption coefficient. There were no significant differences in the final weight, food intake, water intake, body weight gain, or food efficiency of mice in either group treated for 6 mo, or across the treatment groups after the 3 mo-irradiation period. There were no significant differences in the moisture content of animal skin prior to radiation exposure. Moisture content measured in the skin 2 mo after UVB irradiation was 32.60 ± 6.95% in mice treated with the *Nelumbo nucifera* lotus seed tea, compared to 22.67 ± 1.25% for the water controls ($p < 0.05$). Tissues of mice that were irradiated had an abnormally enlarged epidermis and horny layers, but the tissue samples from mice treated with *Nelumbo nucifera* lotus seed tea had a relatively thinner horny layer, suggesting a protective effect. Protein carbonyl values of skin tissues in the water-UVB group were higher than those of the *Nelumbo nucifera* lotus seed tea, with no significance.

Inhibitory Effect on Induction of Delayed-Type Hypersensitivity

Nelumbo Nucifera Leaf Extract

The effect of an orally administered aqueous *Nelumbo nucifera* leaf extract upon the severity of 2,4-dinitrochloro-benzene (DNCB)-induced atopic dermatitis and inflammation was evaluated in NC/Nga mice.[73] A 200 µl-application of 1% DNCB (w/w) in olive oil/acetone was made to shaved dorsal skin of the mice (7/group) to evoke sensitization. Four days later, mice received 3 challenge applications of 200 µl 0.4% DNCB (w/v) per week over 4 wk. The aqueous *Nelumbo nucifera* leaf extract (5, 25, or 50 mg/mouse/d) was given to the mice, via gavage, from the day of sensitization until 4 wk. Controls received distilled water and were also sensitized with DNCB. Dermatitis symptoms on the face, ears, and dorsal part of the body (erythema/hemorrhage, pruritis and dry skin, edema, excoriation/erosion, and lichenification) were scored blindly on a scale of 1-3 every week for 4 wk; the sum of these individual scores was considered the skin severity score (maximum score: 15). Skin severity scores across groups were similar up to 14 d from the day of sensitization; however, from day 14 to day 28 after sensitization, there were significantly lower dermatitis scores in treated animals, compared to controls. The epidermal thickness of dorsal skin of mice treated with the 50 mg/mouse/d *Nelumbo nucifera* leaf extract was 61.3 ± 21 µm compared to 88.7 ± 15 µm in controls. Thus, the effects seen in controls, including hyperkeratosis, parakeratosis, acanthosis with varying degrees of spongiosis, exocytosis of mononuclear cells in the epidermis, and infiltration of inflammatory cells into the upper dermis, were suppressed in treated animals. The suppression of DNCB-induced elevated immunoglobulin E (IgE) levels was statistically significant in animals treated with 25 and 50 mg/mouse/d *Nelumbo nucifera* leaf extract compared to controls.

Immunomodulatory Effects

Nelumbo Nucifera Seed Extract

The potential immunomodulatory effects of an ethanolic *Nelumbo nucifera* seed extract and an ethanolic *Nelumbo nucifera* rhizome extract were evaluated in Swiss albino mice.[74] Groups of mice (6/sex/group) were orally dosed with either saline (negative control), 100 or 300 mg/kg of the seed or rhizome extract, or dexamethasone (positive control). Blood was collected 14 d after dosing and analyzed for immunologic markers. A statistically significant, dose-dependent increase in leukocyte count was seen in the serum of mice treated with both extracts, which was more significant for the *Nelumbo nucifera* seed extract groups. Neutrophil and basophil counts were significantly decreased for cells treated with both extracts, but monocyte counts were not significantly changed compared to controls. A statistically significant increase in the percentage of neutrophil adhesion was observed in cells from mice treated with *Nelumbo nucifera* rhizome extract; no significant changes in neutrophil adhesion were observed in cells from mice treated with *Nelumbo nucifera* seed extract, compared to controls.

Anti-Inflammatory Effects

Nelumbo Nucifera Flower Extract

The anti-inflammatory effects of *Nelumbo nucifera* lotus petals extracted (separately) with ethyl acetate and ethyl alcohol were examined in human monocyte-derived macrophages stimulated with lipopolysaccharide.[35] Cells were treated with 500 µl of 5% (low) and 10% (high) concentrations of *Nelumbo nucifera* lotus petal extracts for 6 h, either prior to or after stimulation of an inflammatory response with 10 ng/ml lipopolysaccharide for 6 h. Aspirin and dexamethasone were utilized as positive controls. Results from an enzyme-linked immunosorbent assay (ELISA) showed that pre-treating and post-treating human macrophages with both *Nelumbo nucifera* lotus petal extracts significantly decreased tumor necrosis factor-alpha (TNF-α) secretion; by comparison, ethyl acetate and ethyl alcohol *Nelumbo nucifera* lotus petal extracts were more effective than the positive controls in suppressing TNF-α secretion when applied after exposure to lipopolysaccharide.

Cytotoxicity

Nelumbo Nucifera Flower Extract

An in vitro 3T3neutral red uptake (NRU) cytotoxicity assay was performed in accord with OECD TG 129 to estimate the basal cytotoxicity of 10 – 100 mg/ml Nelumbo Nucifera Flower Extract (0.5 – 1%, extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract) in Balb/c 3T3 fibroblasts.[11] Dose-dependent cytotoxicity was observed; the IC_{50} was 14.71 mg/ml and the test substance was classified as a non-toxic substance.

Anti-Aging in Fibroblasts

Nelumbo Nucifera Germ Extract

The effect of *Nelumbo nucifera* lotus germ extract (50 µg/ml) upon mitochondrial function was evaluated in human diploid fibroblast cell lines, NB1RGB and IMR90.[16] Exposure to the *Nelumbo nucifera* lotus germ extract increased mitochondrial transmembrane potential in aging IMR90 cells. Additionally, treatment with the *Nelumbo nucifera* lotus germ extract upregulated death-associated protein kinase 1 (DAPK1), by stimulating the acetylation of histones and inducing autophagy through activation of the DAPK1-Beclin1 signaling pathway, compared to dimethyl sulfoxide (DMSO) controls. Furthermore, treatment of young and aging NB1RGB cells with *Nelumbo nucifera* lotus germ extract for 72 h stimulated collagen production and cell proliferation in a 3-dimensional gel culture. The researchers posited that *Nelumbo nucifera* lotus germ extract rejuvenates aging fibroblasts via the DAPK1-Beclin1 pathway, clearing abnormal proteins and agglutinates that are characteristic of aging via autophagy.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details on the dermal irritation and sensitization studies summarized below can be found in Table 13.

Neither a trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)[11] nor a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides) indicated potential for dermal irritation in in vitro studies, and a trade name mixture containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate) was not irritating to rabbit skin.[15] In clinical patch tests, no irritation was observed with trade name mixtures containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate; tested at 25% in mineral oil);[10] 0.5 – 1%, Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract; tested at 15%);[11] 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids; tested at 50%);[15] or 0.5 - 1.2% Nelumbo Nucifera Leaf Extract (tested at 25%).[17] Use tests (28-d) with foundations containing 0.2% Nelumbo Nucifera Flower Water[75] or 0.2% Nelumbo Nucifera Root Water[76] reported very good tolerance and no comedogenicity. Irritation was not observed in 24-h patch tests that examined the irritation potential of extracts of several plant parts (1% leaf, root, flower, or stem extracts or 4% combined extract; 20 subjects).[9]

A direct peptide reactivity assay (DPRA) and a KeratinoSens assay of a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides) were both negative, and a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids) was not a sensitizer in guinea pigs (induction with 5 and 100%; challenge at 10 and 100%).[15] No irritation or sensitization was reported in HRIPTs with trade name mixtures containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract; tested at 15%),[11] 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tested at up to 30%),[15] or 0.5 - 1.2% Nelumbo Nucifera Leaf Extract (tested at 25%);[17] with an emulsion containing 0.0001% Nelumbo Nucifera Germ Extract (as supplied);[77] or with foundations (as supplied) containing 0.00001% Nelumbo Nucifera Flower Extract,[78] 0.2% Nelumbo Nucifera Flower Water,[75] or 0.2% Nelumbo Nucifera Root Water.[76]

Photosensitization/Phototoxicity studies

Details on the photosensitization/phototoxicity studies summarized below can also be found in Table 13.

A trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract did not indicate phototoxic potential in vitro, and was not phototoxic or a photosensitizer in guinea pigs (tested at up to 30%.[15] Foundations (as supplied) containing 0.2% Nelumbo Nucifera Flower Water[75] or Nelumbo Nucifera Root Water[76] were not phototoxic or photosensitizing in clinical studies with 28 or 26 subjects, respectively.

OCULAR IRRITATION STUDIES

Details on the ocular irritation and sensitization studies summarized below can be found in Table 14.

In vitro ocular irritation studies were performed with trade name mixtures containing Nelumbo Nucifera Flower Extract at 1 – 5% extracted in isostearyl isostearate[10] or at 0.5 – 1% extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract[11] a trade name mixture containing a maximum of 0.5 - 1.2% Nelumbo Nucifera Leaf Extract,[17] and with a foundation containing ~0.2% Nelumbo Nucifera Flower Water.[75] Results were primarily negative in all studies.

SUMMARY

This assessment reviews the safety of 14 *Nelumbo* nucifera-derived ingredients; 1 ingredient, Nelumbo Nucifera Flower Oil, is not included in the *Dictionary* but is listed in use in the VCRP and RLD, and accordingly, it is included in this report, According to the *Dictionary*, the 13 *Nelumbo nucifera*-derived ingredients named in the *Dictionary* and reviewed in this safety assessment are mostly reported to function as skin-conditioning agents or antioxidants.

The main chemical classes of compounds present in the *Nelumbo nucifera* plant are proteins, amino acids, and steroids (present mostly in the seeds), carbohydrates (present mostly in the leaves and seeds), alkaloids and flavonoids (present mostly in the flowers, leaves, and seeds), and terpenoids (present mostly in the leaves). Alkaloids are the prominent bioactive chemical class of constituents.

Nelumbo Nucifera Flower Extract had the highest number of reported uses, with 544 uses reported in the RLD in 2024 and 200 uses reported in the VCRP in 2023. According to the Council survey, Nelumbo Nucifera Root Extract had the maximum reported concentration of use, at up to 0.2% in foundations.

A trade name mixture containing 0.5 -1.5 w/v% Nelumbo Nucifera Germ Extract (composed of tannins and flavonoids) had a dermal LD_{50} > 2 g/kg in mice. No signs of toxicity or mortality were observed in mice that received a single oral dose of an herbal mixture capsule (up to 99.9 g/kg bw) containing 33% *Nelumbo nucifera* Gaertn. The acute oral LD_{50} values were > 2 g/kg, in rats, for several ethanolic *Nelumbo nucifera* leaf, flower, and root, *Nelumbo nucifera* leaf and root, and *Nelumbo nucifera* flower extracts. No mortality or toxicity was observed in rats administered a single oral dose of an hydroalcoholic *Nelumbo nucifera* flower extract at 2 g/kg. A hydroalcoholic *Nelumbo nucifera* seed extract, in 0.3% sodium carboxymethylcellulose, had an acute oral LD_{50} of > 1 g/kg in mice. The acute oral LD_{50} values for an ethanolic *Nelumbo nucifera* lotus root extract and a *Nelumbo nucifera* stamen extract-PVP-10 complex were both > 5 g/kg in mice and rats, respectively.

Groups of 10 Wistar rats were orally administered up to 4.32 g/kg/d of an herbal mixture capsule containing 33% *Nelumbo nucifera* Gaertn., dissolved in water, for 4 wk. A statistically significant increase in body weights was observed in 1.44 g/kg/d rats after 2 wk of treatment, compared to controls; no other significant gross or histopathological differences were observed, compared to controls. No significant differences in food or liquid consumption were observed between male SKH-1 hairless mice that received a *Nelumbo nucifera* lotus seed tea as drinking water for 6 mo compared to controls. Groups of 5 Sprague-Dawley rats were orally dosed with up to 2000 mg/kg/d Nelumbinis semen for 13 wk; the NOAEL for both sexes was determined to be 2000 mg/kg/d. Nelumbinis semen was orally administered to Beagle dogs at up to 4000 mg/kg/d a for 28 d; the NOAEL was determined to be 4000 mg/kg/d. The NOAEL for a *Nelumbo nucifera* stamen extract-PVP-10 complex was determined to be > 200 mg/kg/d for both male and female rats in a 90-d oral toxicity study.

Rat sperm was tested with an aqueous *Nelumbo nucifera* petal extract at 0, 0.22, 0.44, 0.88, 1.76, or 3.52 mg/ml in an in vitro reproductive toxicity study. A statistically significant increase in sperm viability was observed in the 0.22 – 1.76 mg/ml groups; no significant differences were observed between the 3.52 mg/ml group and controls. Male Wistar albino rats (10/group) were orally administered 7.5 mg/kg bw of a petroleum ether *Nelumbo nucifera* seed extract every other day for 15 d. Statistically significant decreases in the weight of the testis, epididymis, adrenal glands, body growth rate, sperm count and motility, 3β-HSD and G-6-PSD levels in treated animals, compared to controls have been observed. In a related study, female Wistar rats were orally administered up to 7.5 mg/kg bw of a petroleum ether *Nelumbo nucifera* seed extract every other day for 15 d. Statistically significant decreases in body, ovary, and uterus weights, 3β-HSD and G-6-PSD levels, as well as inhibition of the vaginal opening and first estrus were observed in treated animals compared to controls. In another study, female Wistar albino rats that were orally dosed with 800 mg/kg bw of an ethanolic *Nelumbo nucifera* seed extract for 40 d; statistically significant decreases in ovary, uterus, and vagina weights were observed, compared to controls. Estrous cycles were also prolonged in treated animals, which was accompanied by a statistically significant increase in the diestrous phase of the estrous cycle in treated animals, compared to controls. Dose-dependent and statistically significant decreases in testicular and caudal epididymal sperm and serum testosterone levels were observed in male Wistar rats dosed with up to 200 mg/kg bw/d of an ethanolic *Nelumbo* nucifera seed extract for via gavage for 60 d, compared to controls.

Nelumbo Nucifera Flower Extract (0.5 – 1%, extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract) was not mutagenic in an Ames test. Methanolic extracts of *Nelumbo nucifera* plumule and blossom were not mutagenic at up to 2.5 mg/plate, with or without metabolic activation in an Ames test using *S. typhimurium* TA98 and TA100 strains. In another Ames test, several *Nelumbo nucifera* flower extracts were not mutagenic towards *S. typhimurium* TA98 and TA100, without metabolic activation. Trade name mixtures containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (composed of tannins and flavonoids and of tannins and saccharides) were not mutagenic in an Ames test. Additionally,

aqueous and methanolic *Nelumbo nucifera* leaf extracts, a *Nelumbo nucifera* flower receptacle extract, a *Nelumbo nucifera* petal extract, a *Nelumbo nucifera* rhizome extract, and a *Nelumbo nucifera* stamen extract have been shown to exhibit anti-carcinogenic effects in various cancer cell lines.

The skin lightening effects of aqueous *Nelumbo nucifera* leaf, root, flower, stem, and seed extracts were evaluated at up to 200 µg/ml in a tyrosinase inhibition and DOPA-oxidase assay. DOPA-oxidase was inhibited by 59% after treatment with a *Nelumbo nucifera* leaf extract, 57% was inhibited after treatment with a *Nelumbo nucifera* seed extract, and 50% was inhibited after treatment with a *Nelumbo nucifera* flower extract, in comparison to the 44% inhibitory effect of the positive control (arbutin). In a melanogenesis inhibition assay, a methanolic *Nelumbo nucifera* flower bud extract significantly inhibited melanogenesis in murine B16 melanoma 4A5 cells, with an IC_{50} value of 20 µg/ml; methanolic *Nelumbo* nucifera leaf extract and stamen extract exhibited a moderate and a weak effect, respectively. Dose-dependent increases in inhibition were seen in cultured B16F1 melanoma cells treated with up to 0.1% *Nelumbo nucifera* callus culture extract in a melanin synthesis inhibition test. In another melanogenesis inhibition test, B16F1 melanoma cells were treated with up to 0.5 mg/ml of a *Nelumbo nucifera* leaf extract; overall, the *Nelumbo nucifera* leaf extract significantly inhibited the expression of tyrosinase, MITF, and TRP-1 in a dose-dependent manner and also inhibited cAMP protein and pKA phosphorylation under both basal and stimulated conditions. In a study evaluating the effect of an aqueous *Nelumbo nucifera* leaf extract upon melanogenesis and epidermal hyperplasia induced by UVB exposure, topical treatment with 1 or 2% *Nelumbo nucifera* leaf extract reversed UVB-induced epidermal hyperplasia and melanin content in the epidermis of irradiated guinea pigs.

The effect of an orally administered aqueous *Nelumbo nucifera* leaf extract (up to 50 mg/mouse/d) upon the severity of DNCB-induced atopic dermatitis and inflammation was evaluated in NC/Nga mice over 4 wk. The epidermal thickness of dorsal skin of mice treated with the 50 mg/mouse/d *Nelumbo nucifera* leaf extract was 61 ± 21 µm compared to 89 ± 15 µm in controls. The suppression of DNCB-induced elevated IgE levels was statistically significant in animals treated with 25 and 50 mg/d *Nelumbo nucifera* leaf extract compared to controls.

The potential immunomodulatory effects of an orally administered ethanolic *Nelumbo nucifera* seed extract and an *Nelumbo nucifera* rhizome extract (100 or 300 mg/kg) were evaluated in Swiss albino mice. A statistically significant, dose-dependent increase in leukocyte count was seen in the serum of rats treated with both extracts, which was more significant for the *Nelumbo* nucifera seed extract groups. Neutrophil and basophil counts were significantly decreased for cells treated with both extracts, but monocyte counts were not significantly changed compared to controls; neutrophil adhesion was only significant in the cells of mice treated with the *Nelumbo nucifera* rhizome extract.

The protective effects of an ethanolic *Nelumbo nucifera* leaf extract (10, 100, or 1000 µg/ml) against UVB radiation were evaluated using reaction models comprised of mitochondrial protein isolated from the livers of female Sprague-Dawley rats. Significant inhibition against UVB-induced oxidation was observed in the reaction models treated with 100 µg/ml and 1000 µg/ml *Nelumbo nucifera* leaf extract. In an in vivo phototoxicity study, the protective effects of an ethanolic *Nelumbo* nucifera leaf extract (50, 250, or 5000 mg/kg bw) against UVB-induced phototoxicity were evaluated using male BALB/C mice. The group treated with 50 mg/kg leaf extract showed significant protective activity in the contents of malondialdehyde and superoxide dismutase by a reduction of the level of their activity, compared to the UVB-model group. Additionally, glutathione peroxidase, catalase, and hydroxyproline levels were significantly higher in the groups treated with the 250 and 500 mg/kg bw *Nelumbo nucifera* leaf extracts than that of the UVB-model group. In another study, the potential for an aqueous *Nelumbo nucifera* lotus seed tea (administered in drinking fluid for 6 mo before irradiation) to protect from the effects of UVB-irradiation was examined in hairless male SKH-1 mice. Moisture content measured in the skin 2 mo after UVB irradiation was 32.60 ± 6.95% in mice treated with the *Nelumbo nucifera* lotus seed tea, compared to 22.67 ± 1.25% for the water controls ($p < 0.05$). Tissues of mice that were irradiated had an abnormally enlarged epidermis and horny layers, but the tissue samples from mice treated with *Nelumbo nucifera* lotus seed tea had a relatively thinner horny layer, suggesting a protective effect.

The anti-inflammatory effects of 6-h exposure to ethyl acetate or ethyl alcohol *Nelumbo nucifera* petal extracts were examined in human monocyte-derived macrophages treated either prior to or after stimulation with lipopolysaccharide. ELISA results showed that pre-treating and post-treating human macrophages with both *Nelumbo nucifera* petal extracts significantly decreased TNF-α secretion, especially when applied after exposure to lipopolysaccharide, when compared to positive controls.

The effect of *Nelumbo nucifera* germ extract upon mitochondrial function was evaluated in human diploid fibroblast cell lines, NB1RGB and IMR90. Treatment with 50 µg/ml of a *Nelumbo nucifera* germ extract increased mitochondrial transmembrane potential, stimulated collagen production and cell proliferation, and upregulated the DAPK1-Beclin1 signaling pathway. The researchers posited that the *Nelumbo nucifera* germ extract rejuvenates aging fibroblasts via activation of the DAPK1-Beclin1 pathway, in which autophagy clears age-related abnormal proteins and agglutinates.

Neither a trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract) nor a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides) indicated potential for dermal irritation in in vitro studies, and a trade name mixture containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate) was not an irritating to rabbit skin. In clinical patch tests, no irritation was observed with trade name mixtures containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate; tested at 25% in mineral oil); 0.5 – 1%, Nelumbo Nucifera Flower Extract

(extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract; tested at 15%); 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids; tested at 50%); or 0.5 - 1.2% Nelumbo Nucifera Leaf Extract (tested at 25%). Use tests (28-d) with foundations containing 0.2% Nelumbo Nucifera Flower Water or 0.2% Nelumbo Nucifera Root Water reported very good tolerance and no comedogenicity. Irritation was not observed in 24-h patch tests (20 subjects) that examined the irritation potential of extracts of several plant parts (1% leaf, root, flower, or stem extracts or 4% combined extract).

A DPRA and a KeratinoSens assay of a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides) were both negative, and a trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids) was not a sensitizer in guinea pigs (induction with 5 and 100%; challenge at 10 and 100%). No irritation or sensitization was reported in HRIPTs with trade name mixtures containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract; tested at 15%), 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tested at up to 30%), or 0.5 - 1.2% Nelumbo Nucifera Leaf Extract (tested at 25%); with an emulsion containing 0.0001% Nelumbo Nucifera Germ Extract (as supplied); or with foundations (as supplied) containing 0.00001% Nelumbo Nucifera Flower Extract, 0.2% Nelumbo Nucifera Flower Water, or 0.2% Nelumbo Nucifera Root Water.

A trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract did not indicate phototoxic potential in vitro, and was not phototoxic or a photosensitizer in guinea pigs (tested at up to 30%.) Foundations (as supplied) containing 0.2% Nelumbo Nucifera Flower Water or 0.2% Nelumbo Nucifera Root Water were not phototoxic or photosensitizing in clinical studies with 28 or 26 subjects, respectively.

In vitro ocular irritation studies were performed with trade name mixtures containing Nelumbo Nucifera Flower Extract either at 1 – 5% extracted in isostearyl isostearate or at 0.5 – 1% extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract, a trade name mixture containing a maximum of 0.5 - 1.2% Nelumbo Nucifera Leaf Extract, and with a foundation containing ~0.2% Nelumbo Nucifera Flower Water. Results were negative in all studies.

DISCUSSION

This assessment reviews the safety of 14 *Nelumbo nucifera*-derived ingredients 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 reviewed the data in this report and concluded that the available data are insufficient for determining the safety of these ingredient under the intended conditions of use in cosmetics. The Panel noted a lack of relevant safety data and determined that the data needs from the Insufficient Data Announcement issued following the December 2024 Panel meeting have not been fulfilled. In order to come to a conclusion of safety for these 14 ingredients, the following additional data are needed:

- For all ingredients
    - Composition and impurities
    - Methods of manufacturing
    - 28-d dermal toxicity data
        - if positive; additional data (e.g. development and reproductive toxicity data) may be needed.
    - UV absorption data (as well as more detailed information about the previously submitted UV spectra)
        - if absorbed, phototoxicity/photosensitization data are needed data (additional protocol details are needed for the previously-submitted studies)
- For the callus-, phytoplacenta-, stamen-, and seed-derived ingredients
    - Dermal irritation and sensitization data at maximum concentration of use.
- For all except the flower- and germ-derived ingredients
    - In vitro genotoxicity data
- For flower- and whole plant-derived ingredients
    - Development and reproductive toxicity data
- For all except flower- and leaf-derived ingredients
    - In vitro ocular irritation data

The Panel expressed concern about heavy metals, pesticide residues, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA.

Data included in this report indicate that *Nelumbo nucifera*-derived 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 depigmentation.

The Panel also discussed the issue of incidental inhalation that could result from exposure to these ingredients; for example, Nelumbo Nucifera Flower Oil is reported to be used in perfumes (concentration of use not reported) and Nelumbo Nucifera Flower Extract is reported to be used at 0.1% in face powders. 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 this ingredient is used (or is expected to be used) in potentially inhaled products, the available information indicates that the 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.

As stated in the Use section, products containing these ingredients may be marketed for use with airbrush delivery systems. While it may be known in some (but not all) instances whether or not there is use in airbrush applications, information regarding the consumer habits and practices data, product particle size data, and/or other relevant particle data (e.g., diameter) related to this use technology are absent, and thus the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the available data are insufficient to make a determination of safety for the following 14 *Nelumbo nucifera*-derived ingredients under the intended conditions of use in cosmetic formulations:

Nelumbo Nucifera Callus Culture Extract
Nelumbo Nucifera Extract
Nelumbo Nucifera Flower Extract
Nelumbo Nucifera Flower/Leaf/Stem Juice
Nelumbo Nucifera Flower Oil
Nelumbo Nucifera Flower Water
Nelumbo Nucifera Germ Extract
Nelumbo Nucifera Leaf Extract
Nelumbo Nucifera Phytoplacenta Culture Extract
Nelumbo Nucifera Root Extract
Nelumbo Nucifera Root Water
Nelumbo Nucifera Seed Extract
Nelumbo Nucifera Seed Powder
Nelumbo Nucifera Stamen Extract

TABLES

Table 1. Definitions and functions of *Nelumbo nucifera*-derived ingredients[1*]

Ingredient/CAS No.	Definition	Function
Nelumbo Nucifera Callus Culture Extract 85085-51-4 (generic)	Nelumbo Nucifera Callus Culture Extract is the extract of a culture of the callus of *Nelumbo nucifera*.	antifungal agent; antimicrobial agent antioxidant; skin-conditioning agent - humectant
Nelumbo Nucifera Extract 85085-51-4 (generic)	Nelumbo Nucifera Extract is the extract of the whole plant, *Nelumbo nucifera*.	antioxidant; skin-conditioning agent - miscellaneous
Nelumbo Nucifera Flower Extract 85085-51-4 (generic)	Nelumbo Nucifera Flower Extract is the extract of the flower of *Nelumbo nucifera*.	skin-conditioning agent - miscellaneous
Nelumbo Nucifera Flower/Leaf/Stem Juice 85085-51-4 (generic)	Nelumbo Nucifera Flower/Leaf/Stem Juice is the juice expressed from the flowers, leaves, and stems of *Nelumbo nucifera*.	antioxidant
Nelumbo Nucifera Flower Water 85085-51-4 (generic)	Nelumbo Nucifera Flower Water is the aqueous extract of the steam distillate obtained from the flowers of *Nelumbo nucifera*.	antioxidant; cosmetic astringent; fragrance ingredient; skin-conditioning agent-miscellaneous
Nelumbo Nucifera Germ Extract 85085-51-4 (generic)	Nelumbo Nucifera Germ Extract is the extract of the germ of *Nelumbo nucifera*.	antioxidant; skin-conditioning agent - humectants
Nelumbo Nucifera Leaf Extract 85085-51-4 (generic)	Nelumbo Nucifera Leaf Extract is the extract of the leaves of *Nelumbo nucifera*.	skin-conditioning agent - miscellaneous
Nelumbo Nucifera Phytoplacenta Culture Extract 85085-51-4 (generic)	Nelumbo Nucifera Phytoplacenta Culture is the extract of a culture of the phytoplacenta of *Nelumbo nucifera*.	antioxidant; antimicrobial agent; hair-conditioning agent; skin-conditioning agent - humectant
Nelumbo Nucifera Root Extract 85085-51-4 (generic)	Nelumbo Nucifera Root Extract is the extract of the roots of *Nelumbo nucifera*.	skin-conditioning agent – miscellaneous
Nelumbo Nucifera Root Water 85085-51-4 (generic)	Nelumbo Nucifera Root Water is the aqueous solution of the steam distillate obtained from the roots of *Nelumbo nucifera*.	fragrance ingredient
Nelumbo Nucifera Seed Extract 85085-51-4 (generic)	Nelumbo Nucifera Seed Extract is the extract of the seeds of *Nelumbo nucifera*.	antifungal agent; antimicrobial agent; antioxidant; cosmetic astringent; hair conditioning agent; skin protectant; skin-conditioning agent – emollient; skin-conditioning agent - miscellaneous
Nelumbo Nucifera Seed Powder 85085-51-4 (generic)	Nelumbo Nucifera Seed Powder is the powder obtained from the dried, ground seeds of *Nelumbo nucifera*.	abrasives; antioxidants
Nelumbo Nucifera Stamen Extract 85085-51-4 (generic)	Nelumbo Nucifera Stamen Extract is the extract of the stamens of *Nelumbo nucifera*.	antioxidants; skin protectants

*Nelumbo Nucifera Flower Oil is not included in this table because it is not an INCI ingredient.

Table 2. Generic definitions of plant parts as they apply to *Nelumbo nucifera*-derived ingredients[1]

Plant Part	Definition
Callus	An undifferentiated mass of cells; a thickened area of an organ of a plant or scar tissue that covers a wound in a plant
Callus culture	An undifferentiated mass of cells produced through tissue culture
Flower	The reproductive shoot in flowering plants, usually with sepals, petals, stamens and pistil(s)
Germ	The embryo in a seed; the part of a seed that can develop into a new plant
Juice	The liquid contained in the vegetative parts or fruits of a plant
Leaf	Flattened photosynthetic organs that are attached to stems
Phytoplacenta	Novel word for placentas from plants, used in INCI Committee to indicate a plant-sourced placenta as opposed to animal-sourced
Root	Organ of a plant that absorbs and transports water and nutrients, lacks leaves and nodes, and is usually underground
Seed	A propagating sexual structure resulting from the fertilization of an ovule, formed by embryo, endosperm, or seed coat
Stamen	The male reproductive organ in flowers, usually formed by a filament and anther (part of stamen that produces and contains pollen, and typically originates at the stalk/stem)
Stem	A slender or elongated structure that supports a plant, fungus, a plant part, or a plant organ

INCI – International Nomenclature Cosmetic Ingredient

Table 3. Chemical properties

Property	Value	Reference
Nelumbo Nucifera Flower Extract (extracted in water)		
Physical Form	liquid	[9]
Color	dark, yellowish	[9]
pH	4 - 7	[9]
Specific Gravity	0.98 – 1.04	[9]
Nelumbo Nucifera Flower Extract (1 - 5%; extracted in isostearyl isostearate (95 – 99%))		
Physical Form	transparent liquid	[10]
Color	pale yellow-yellow	[10]
Density (g/ml; 20°C)	0.84 - 0.88	[10]
Solubility	soluble in oils	[10]
Nelumbo Nucifera Flower Extract (0.5 – 1%; extracted in propanediol (70 -90%) and glycerin (10 – 30%), with Nymphaea Caerulea Flower Extract)		
Physical Form	transparent, slightly turbid liquid	[11]
Color	brown – dark brown	[11]
Nelumbo Nucifera Seed Powder		
Physical Form	fine ground flour	[12]
pH	7.43	[12]

Table 4. Main constituents in *Nelumbo nucifera*, organized by chemical class and presence in plant parts[26-30,40,42,46,69,79-83]

Constituent	Embryo	Flower	Leaf	Seed	Stamen
ALKALOIDS – *Aporphine alkaloids*					
anonaine			♦♦	♦♦	
anonaine-*N*-acetyl		♦			
asimilobine		♦♦	♦♦	♦♦	
caaverine			♦♦	♦♦	
cepharadione			♦		
dehydroanonaine			♦		
dehydroaporphine			♦		
dehydronuciferine			♦		
dehydroroemerine			♦		
2-hydroxy-1-methoxyaporphine			♦		
7-hydroxydehydronuciferine			♦		
glaziovine				♦	
lirindine			♦♦	♦♦	
liriodenine			♦		
lysicamine			♦		
methyl asimilobine			♦♦	♦♦	
nelumnucine			♦♦	♦♦	
N-methylasimilobine		♦♦	♦♦		
N-methylasimilobine-*N*-oxide			♦♦	♦♦	
nornuciferine		♦			
N-nornuciferine			♦♦	♦♦	
O-nornuciferine		♦♦	♦♦	♦♦	
nuciferine	♦♦	♦♦	♦♦	♦♦	
nuciferine-*N*-acetyl		♦			
nuciferine-*N*-methanol		♦			
nuciferine-*N*-oxide			♦♦	♦♦	
pronuciferine	♦♦	♦♦	♦♦	♦♦	
(6*R*, 6*ar*) roemerine-N_{β}-oxide			♦		
roemerine		♦♦	♦♦	♦♦	
roemerine-*N*-oxide			♦♦	♦♦	
ALKALOIDS – *Benzylisoquinoline alkaloids*					
Constituent	**Embryo**	**Flower**	**Leaf**	**Seed**	**Stamen**
Anonaine					♦
Dehydroanonaine					♦
argemexerine			♦♦	♦♦	
armepavine		♦♦	♦♦	♦♦	♦
bromo methyl armepavine			♦♦	♦♦	
(+)-1(*R*)- coclaurine			♦		
coclaurine		♦♦	♦♦	♦♦	

Table 4. Main constituents in *Nelumbo nucifera*, organized by chemical class and presence in plant parts[26-30,40,42,46,69,79-83]

Constituent	Embryo	Flower	Leaf	Seed	Stamen
demethylcoclaurine	♦				
6-demethyl-4-methyl-*N*-methylcoclaurine				♦	
isococlaurine		♦♦	♦♦		
(+)-juziphine		♦			
lotusine	♦♦	♦♦			
methoxymethyl lisoquinoline			♦♦	♦♦	
4'-methyl coclaurine			♦♦	♦♦	
methylhigenamine				♦	
methyl lotusine			♦		
4'-*N*-methylcoclaurine			♦♦	♦♦	
N-methylcoclaurine		♦♦	♦♦	♦♦	♦
N-methylisococlaurine		♦♦	♦♦		♦
Nornuciferine			♦		♦
norarmepavine		♦			
N-norarmepavine			♦		♦
nor-*O*-methylarmepavine				♦	
4'-*O*-methylarmepavine			♦		
norcoclaurine			♦♦	♦♦	
norcoclaurine-6-*O*-glucoside				♦	
(-)-1(*S*)-norcoclaurine			♦		
norjuziphine		♦			
Rosmerine			♦	♦	♦
ALKALOIDS – *Bisbenzylisoquinoline alkaloids*					
Constituent	**Embryo**	**Flower**	**Leaf**	**Seed**	**Stamen**
dauricine		♦♦		♦♦	
6-hydroxynorisoliensinine	♦♦	♦♦			
isoliensinine	♦♦	♦♦	♦♦	♦♦	♦
liensinine	♦♦	♦♦	♦♦	♦♦	♦
methyl neferine	♦				
neferine	♦♦	♦♦	♦♦	♦	
nelumboferine	♦♦		♦♦		
nelumborine		♦			
N-norisoliensinine	♦♦	♦♦			
FLAVONOIDS – *and Flavonoid glycosides*					
Constituent	**Embryo**	**Flower**	**Leaf**	**Seed**	**Stamen**
(-)-catechin			♦		
dihydrophaseic acid				♦	
dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside				♦	
hyperin	♦♦		♦♦		
isoquecetrin			♦		♦
isoschaftoside			♦		
kaempferol		♦♦		♦♦	♦
kaempferol 3-*O*-*β*-D-galactopyranoside					♦
kaempferol 3-*O*-*β*-D -glucopyranoside					♦
kaempferol 3 *O*-*β*-D-glucuronopyranoside					♦
kaempferol 3-*O*-*β*-D-glucuronopyranosyl methylester					♦
kaempferol 3-*O*-*α*-L -rhamnopyranosyl-(1→6)-β-D-glucopyranoside					♦
Luteolin		♦			
Luteolin glucoside		♦			♦
myricetin 3',5'-dimethylether 3-*O*-*β*-D -glucopyranoside					♦
quercetin			♦		♦
quercetin 3-*O*-*β*-D-glucuronide			♦		
quercetin 3-*O*-*β*-D-xylopyranosyl-(1→2)-β-d-			♦		
rutin	♦♦		♦♦		

Table 4. Main constituents in *Nelumbo nucifera*, organized by chemical class and presence in plant parts[26-30,40,42,46,69,79-83]

Constituent	Embryo	Flower	Leaf	Seed	Stamen
Megastigmanes, terpenoids & glucosides and other compounds					
Constituent	**Embryo**	**Flower**	**Leaf**	**Seed**	**Stamen**
annuionone D			♦		
boscialin			♦♦	♦♦	
betulinic acid				♦	
byzantionoside A			♦		
chrysoeriol 7-*O*-glucopyranoside			♦		
(+)-dehydrovomifoliol			♦		
dihydrophaseic acid				♦	
(*E*)-3-hydroxymegastigm-7-en-9-one			♦		
elephantorrhizol			♦		
epiloliolide			♦♦	♦♦	
epitaxifolin			♦		
5,6-epoxy-3-hydroxy-7-megastigmen-9-one			♦		
galactopyranoside			♦		
grasshopper ketone			♦		
icariside B_2			♦		
isohydnocarpin			♦		
lanosterol				♦	
luteolin				♦	
nelumnnucifoside A			♦♦	♦♦	
nelumnnucifoside B			♦♦	♦♦	
3-*O*-*β*-dxylopyranosyl-(1-2)-*β*-D-galactopyranoside			♦		
3-*O*-*β*-D-glucuronide			♦		
3-oxo-retro-α-ionol I taxifolin			♦		
5,7,3'5'-tetrahydroxyflavanone			♦		
vomifoliol			♦♦	♦♦	

♦ - present in single plant part; ♦♦ - present in multiple plant parts

Table 5. Comparison of standard phenolic acid and lactone compounds found in a HPLC-DAD analysis of two *Nelumbo nucifera* lotus petal extracts[35]

Compound	Ethyl acetate lotus petal extract (µg/ml)	Ethyl alcohol lotus petal extract (µg/ml)
chlorogenic acid	1.45 ± 0.120	3.10 ± 1.070
coumarin	1.72 ± 0.330	4.61 ± 0.590
ferulic acid	20.62 ± 1.560	51.27 ± 1.190
kaempferol	92.17 ± 0.850	31.84 ± 1.810
quercetin	43.34 ± 0.280	25.95 ± 0.730
rutin	2.42 ± 0.020	5.61 ± 3.150

HPLC-DAD - high-performance liquid chromatography with diode array detector

Table 6. Phenolic, flavonoid, and anthocyanin contents in parts of a *Nelumbo nucifera* plant (mg/100 g DW)[38]

	Plant parts					
	flower stalk	**leaf stalk**	**old leaf**	**petal**	**seed embryo**	**stamen**
Phenolic acids						
ferulic acid	ND	ND	ND	ND	24.71 ± 2.03	ND
gallic acid	ND	163.09 ± 8.58	49.38 ± 4.83	277.84 ± 6.36	ND	ND
p-coumaric acid	ND	ND	ND	ND	105.34 ± 2.93	10.78 ± 0.38
Flavonoids						
cyanidin	12.02 ± 0.09	7.15 ± 0.74	184.82 ± 11.38	349.98 ± 24.28	1901.52 ± 14.15	115.79 ± 10.21
delphinidin	20.70 ± 0.24	6.15 ± 1.05	39.46 ± 2.42	1837.27 ± 52.67	691.58 ± 9.84	211.63 ± 17.21
isorhamnetin	3.51 ± 0.28	6.80 ± 0.35	2.67 ± 0.09	237.85 ± 13.86	11.56 ± 0.85	192.09 ± 15.70
kaempferol	6.40 ± 0.64	ND	3.87 ± 0.31	197.83 ± 19.81	4.92 ± 0.41	160.71 ± 13.66
luteolin	4.89 ± 0.35	12.43 ± 0.77	ND	ND	37.50 ± 1.87	ND
myricetin	8.89 ± 0.83	ND	ND	8.55 ± 0.29	ND	7.63 ± 0.35
naringenin	2213.41 ± 11.35	1918.10 ± 37.81	1064.17 ± 75.38	2226.9 ± 13.66	2241.51 ± 18.41	2185.84 ± 24.21
quercetin	59.91 ± 5.64	35.95 ± 1.94	458.56 ± 33.45	196.34 ± 19.03	81.79 ± 3.57	43.94 ± 2.08
Total phenolic contents (mg GAE/g DW)	4.33 ± 0.11	2.72 ± 0.10	39.09 ± 0.79	12.25 ± 0.36	12.84 ± 0.22	36.37 ± 0.73
Total anthocyanidin contents (mg C3GE/g DW)	ND	ND	ND	0.05 ± 0.00	ND	0.23 ± 0.02

C3GE – cyanidin 3-*O*-glucoside equivalent; DW – dry weight; GAE – gallic acid equivalent; ND – not detected

Table 7. Amino acid profile of a *Nelumbo nucifera* lotus seed protein and its protein fractions (g/kg crude protein on a DW basis)[43]

	Protein fraction					
	Seed protein	**Albumin**	**Globulin**	**Prolamine**	**Glutelin**	**Soybean***
Essential amino acids (EAA)						
isoleucine	32.73	31.7	32.4	4.98	26.33	46.2
leucine	64.04	58.02	59.24	8.35	49.73	77.2
lysine	56.94	44.15	41.88	11	36.56	60.8
methionine	24.5	23.52	23.13	8.3	23.12	12.2
phenylalanine	44.81	42.34	45.13	10.53	38.51	48.4
threonine	35.31	28.91	29.20	7.17	25.56	37.6
tryptophan	21.66	24.14	29.71	3.04	9.37	33.9
valine	42.83	38.75	40.84	10.77	34.48	45.9
Total essential amino acids	**322.82**	**291.53**	**301.53**	**64.14**	**243.66**	**362.2**
Non-essential amino acids (NEAA)						
alanine	43.34	36.19	36	4.75	31.86	42.3
arginine	72	78.97	80.17	8.52	53.83	71.3
aspartic acid	98.68	91.74	93.32	16.91	69.78	113
cystine	8.12	6.82	7.81	3.9	6.21	17
glutamic acid	171.32	157.98	154.46	30.32	111.93	169
glycine	44.28	36.44	36.73	6.25	30.97	40.1
histidine	23.66	22.57	22.47	3.57	18.34	25
proline	18.09	17	18.55	3.7	16.99	48.6
serine	58.44	55.08	56.02	10.03	41.91	56.7
tyrosine	15.13	18.47	14.04	6.04	14.41	12.4
Total non-essential amino acids	**553.06**	**521.26**	**519.57**	**93.99**	**396.23**	**595.4**
Hydrophobic amino acids	314.62	283.96	304.89	81.68	251.99	360.9
Hydrophilic amino acids	270	109.3	107.07	27.14	88.09	123.7
Basic amino acids	152.6	145.69	144.52	23.09	108.73	157.1
Acidic amino acids	53.67	249.72	247.78	47.23	181.71	282
Total amino acids (EAA + NEAA)	**875.88**	**812.79**	**821.10**	**158.13**	**639.89**	**957.6**

*soybean protein was used as the reference

Table 8. Fatty acid composition of a whole *Nelumbo nucifera* seed oil[44]

Acid	**Amount (%)**
arachidic acid	5.5
capric acid	2.09
lauric acid	2.04
linoleic acid	19.9
linolenic acid	3.4
mygaric acid	0.2
myristic acid	3.21
oleic acid	11.7
palmitic acid	33.27
palmitoleic acid	5.7
stearic acid	3
unknown	10

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
	Nelumbo Nucifera Callus Culture Extract			Nelumbo Nucifera Extract			Nelumbo Nucifera Flower Extract		
Totals*	**40**	**8**	**NR**	**64**	**7**	**NR**	**544**	**200**	**0.00025 – 0.13**
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*8*	*NR*	***	*5*	*NR*	***	*151*	*0.00025 – 0.13*
Rinse-Off	***	*NR*	*NR*	***	*2*	*NR*	***	*49*	*0.00025 – 0.00055*
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*	***	*NR*	*NR*
Exposure Type									
Eye Area	***	NR	NR	***	1	NR	***	4	0.00025 – 0.0015
Incidental Ingestion	***	NR	NR	***	NR	NR	***	1	0.1
Incidental Inhalation-Spray	***	5[a]; 3[b]	NR	***	2[a]	NR	***	1; 79[a]; 35[b]	NR
Incidental Inhalation-Powder	***	3[b]	NR	***	NR	NR	***	1; 35[b]; 5[c]	0.1; 0.001 – 0.05[c]
Dermal Contact	***	8	NR	***	5	NR	***	182	0.00025 – 0.1
Deodorant (underarm)	***	NR	NR	***	NR	NR	***	NR	NR
Hair - Non-Coloring	***	NR	NR	***	2	NR	***	17	0.00055
Hair-Coloring	***	NR	NR	***	NR	NR	***	NR	NR
Nail	***	NR	NR	***	NR	NR	***	NR	0.13
Mucous Membrane	***	NR	NR	***	NR	NR	***	17	0.00025 – 0.1
Baby Products	***	NR	NR	***	NR	NR	***	11	0.00055
as reported by product category									
Baby Products							***1***		
Baby Shampoos							1	2	0.00055
Baby Lotions/Oils/Powders/Creams							NR	5	NR
Baby Wipes									
Other Baby Products							NR	4	NR
Bath Preparations							***1***		
Other Bath Preparations							1	NR	NR
Eye Makeup Preparations (not children's)							***4***		
Eye Shadow							NR	1	0.00025
Eye Lotion				NR	1	NR	2	1	0.0015
Eye Makeup Remover							2	NR	NR
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)									
Other Eye Makeup Preparations							NR	2	NR
Fragrance Preparations							***2***		
Perfumes							NR	1	NR
Other Fragrance Preparation							2	NR	NR
Hair Preparations (non-coloring)	***6***						***80***		
Hair Conditioners							4 (l.o.); 23 (r.o.)	3	NR
Hair Sprays (aerosol fixatives)									
Rinses (non-coloring)							1	1	NR
Shampoos (non-coloring)	1 (r.o.)	NR	NR				1 (l.o.); 34 (r.o.)	5	NR
Tonics, Dressings, and Other Hair Grooming Aids							4	4	NR
Other Hair Preparations				NR	2	NR	9 (l.o.); 5 (r.o.)	2	NR
Hair Coloring Preparations									
Hair Dyes and Colors (all types requiring caution statements and patch tests)									
Hair Rinses (coloring)									

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
Other Hair Coloring Preparation									
Makeup Preparations (not eye; not children's)				***3***			***120***		
Blushers and Rouges (all types)				3	NR	NR	24	NR	NR
Face Powders							5	1	0.1
Foundations				3 (tradition application)	NR	NR	31 (traditional application)	1	NR
Leg and Body Paints							1 (airbrush application)	NR	NR
Lipsticks and Lip Glosses							57	1	0.1
Makeup Bases				3 (traditional application)	NR	NR	6 (traditional application)	1	NR
Makeup Fixatives									
Other Makeup Preparations							2 (l.o.)	4	NR
Manicuring Preparations (Nail)							***2***		
Other Manicuring Preparations							2	NR	0.13
Personal Cleanliness				***6***			***17***		
Bath Soaps and Body Washes				5	NR	NR	13	10	0.00025
Deodorants (underarm)									
Feminine Deodorants							NR	1	NR
Other Personal Cleanliness Products							4 (r.o.)	5	NR
Shaving Preparations				***1***					
Other Shaving Preparations				1	NR	NR			
Skin Care Preparations	***39***			***53***			***316***		
Cleansing	4	NR	NR	3	2	NR	43	19	NR
Face and Neck (excluding shaving preps)	21 (l.o.); 1 (r.o.)	3	NR	26 (l.o.); 3 (r.o.)	NR	0.01% (not spray 0.01% (l.o); 0.02% (r.o)	211 (l.o.); 29 (r.o.)	28	0.001 (not spray)
Body and Hand (excluding shaving preps)	2 (l.o.)	NR	NR	7 (l.o.)	NR	0.001%	7 (l.o.); 3 (r.o.)	6	0.01 – 0.05 (not spray)
Moisturizing	10	3	NR	13	2	NR	87	70	0.0015 (not spray)
Night	NR	2	NR	2	NR	NR	1	2	NR
Paste Masks (mud packs)				1	NR	NR	5	3	NR
Skin Fresheners				1	NR	NR	9	3	NR
Other Skin Care Preparations	2 (l.o.)	NR	NR	8 (l.o.); 2 (r.o.)	NR	NR	14 (l.o.); 6 (r.o.)	13	NR
Suntan Preparations									
Indoor Tanning Preparations									
Other Suntan Preparations									
Other Preparations (i.e., those that do not fit another category)				***1***	NA	NA	***8***	NA	NA

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
	Nelumbo Nucifera Flower/Leaf/Steam Juice			Nelumbo Nucifera Flower Oil			Nelumbo Nucifera Flower Water		
Totals*	**NR**	**1**	**0.000023 – 0.0034**	**17**	**8**	**NR**	**74**	**13**	**0.001**
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*1*	*0.0034*	***	*5*	*NR*	***	*11*	*0.001*
Rinse-Off	***	*NR*	*0.000023*	***	*3*	*NR*	***	*2*	*NR*
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*	***	*NR*	*NR*
Exposure Type									
Eye Area	***	NR	NR	***	NR	NR	***	1	NR
Incidental Ingestion	***	NR	NR	***	NR	NR	***	NR	NR
Incidental Inhalation-Spray	***	1[b]	NR	***	5	NR	***	5[a]; 1[b]	NR
Incidental Inhalation-Powder	***	1[b]	0.0034[c]	***	NR	NR	***	1[b]	0.001[c]
Dermal Contact	***	1	0.0034	***	8	NR	***	13	0.001
Deodorant (underarm)	***	NR	NR	***	NR	NR	***	NR	NR
Hair - Non-Coloring	***	NR	0.000023	***	NR	NR	***	NR	NR
Hair-Coloring	***	NR	NR	***	NR	NR	***	NR	NR
Nail	***	NR	NR	***	NR	NR	***	NR	NR
Mucous Membrane	***	NR	NR	***	2	NR	***	NR	NR
Baby Products	***	NR	NR	***	NR	NR	***	NR	NR
as reported by product category									
Baby Products							***3***		
Baby Shampoos							1	NR	NR
Baby Lotions/Oils/Powders/Creams							1	NR	NR
Baby Wipes									
Other Baby Products							1 (l.o.); 2 (r.o.)	NR	NR
Bath Preparations (diluted for use)									
Other Bath Preparations									
Eye Makeup Preparations									
Eye Shadow									
Eye Lotion									
Eye Makeup Remover									
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)									
Other Eye Makeup Preparations							NR	1	NR
Fragrance Preparations				***1***			*4*		
Perfumes				NR	5	NR			
Other Fragrance Preparation				1	NR	NR	4	NR	NR
Hair Preparations (non-coloring)				***1***			***7***		
Hair Conditioner	NR	NR	0.000023				1 (l.o.)	NR	NR
Hair Spray (aerosol fixatives)							1	NR	NR
Rinses (non-coloring)							2	NR	NR
Shampoos (non-coloring)							1 (r.o.)	NR	NR
Tonics, Dressings, and Other Hair Grooming Aids							2	NR	NR
Other Hair Preparations				1 (l.o.)	NR	NR			
Hair Coloring Preparations							***7***		
Hair Dyes and Colors (all types requiring caution statements and patch tests)									
Hair Rinses (coloring)							7 (r.o.)	NR	NR
Other Hair Coloring Preparation									

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
Makeup Preparations (not eye; not children's)				***3***			***4***		
Blushers and Rouges (all types)									
Face Powders									
Foundations				3 (traditional application)	NR	NR			
Leg and Body Paints									
Lipsticks and Lip Glosses									
Makeup Bases							2 (traditional application)	NR	NR
Makeup Fixatives							1	NR	NR
Other Makeup Preparations							1 (l.o.)	NR	NR
Manicuring Preparations (Nail)									
Other Manicuring Preparations									
Personal Cleanliness Products				***2***			*1*		
Bath Soaps and Body Washes				1	2	NR			
Deodorants (underarm)									
Feminine Deodorants									
Other Personal Cleanliness Products				1 (l.o.)	NR	NR	1 (r.o.)	NR	NR
Shaving Preparations									
Other Shaving Preparations									
Skin Care Preparations				***9***			***47***		
Cleansing				1	1	NR	5	NR	NR
Face and Neck (excluding shaving preps)	NR	1	0.0034 (not spray)	7 (l.o.); 1 (r.o.)	NR	NR	26 (l.o.)	1	0.001 (not spray)
Body and Hand (excluding shaving preps)							1 (l.o.)	NR	NR
Moisturizing				2	NR	NR	11	4	NR
Night									
Paste Masks (mud packs)							4	2	NR
Skin Fresheners							2	1	NR
Other Skin Care Preparations							NR	4	NR
Suntan Preparations							***3***		
Indoor Tanning Preparations							3 (traditional application)	NR	NR
Other Suntan Preparations							1	NR	NR
Other Preparations (i.e., those that do not fit another category)				***1***	NA	NA			

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
	Nelumbo Nucifera Germ Extract			Nelumbo Nucifera Leaf Extract			Nelumbo Nucifera Phytoplacenta Culture Extract		
Totals*	**34**	**8**	**NR**	**63**	**20**	**0.00025**	**NR**	**1**	**NR (2024)[52]**
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*8*	*NR*	***	*17*	*NR*	***	*NR*	**NR**
Rinse-Off	***	*NR*	*NR*	***	*3*	*0.00025*	***	*NR*	**NR**
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*	***	*NR*	**NR**
Exposure Type									
Eye Area	***	2	NR	***	NR	NR	***	NR	**NR**
Incidental Ingestion	***	NR	NR	***	NR	NR	***	NR	**NR**
Incidental Inhalation-Spray	***	5[a]	NR	***	7[a]; 8[b]	NR	***	NR	**NR**
Incidental Inhalation-Powder	***	NR	NR	***	1; 8[b]	NR	***	NR	**NR**
Dermal Contact	***	8	NR	***	20	0.00025	***	1	**NR**
Deodorant (underarm)	***	NR	NR	***	NR	NR	***	NR	**NR**
Hair - Non-Coloring	***	NR	NR	***	NR	NR	***	NR	**NR**
Hair-Coloring	***	NR	NR	***	NR	NR	***	NR	**NR**
Nail	***	NR	NR	***	NR	NR	***	NR	**NR**
Mucous Membrane	***	NR	NR	***	1	0.00025	***	NR	**NR**
Baby Products	***	NR	NR	***	NR	NR	***	NR	**NR**
as reported by product category									
Baby Products				***1***					
Baby Shampoos									
Baby Lotions/Oils/Powders/Creams				1	NR	NR			
Baby Wipes									
Other Baby Products									
Bath Preparations									
Other Bath Preparations									
Eye Makeup Preparations (not children's)	***2***			***1***					
Eye Shadow									
Eye Lotion	2	1	NR						
Eye Makeup Remover	NR	1	NR						
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)									
Other Eye Makeup Preparations				1	NR	NR			
Fragrance Preparations									
Perfumes									
Other Fragrance Preparation									
Hair Preparations (non-coloring)				***1***					
Hair Conditioners									
Hair Sprays (aerosol fixatives)									
Rinses (non-coloring)									
Shampoos (non-coloring)									
Tonics, Dressings, and Other Hair Grooming Aids									
Other Hair Preparations				1 (r.o.)	NR	NR			
Hair Coloring Preparations									
Hair Dyes and Colors (all types requiring caution statements and patch tests)									
Hair Rinses (coloring)									

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
Other Hair Coloring Preparation									
Makeup Preparations (not eye; not children's)				***1***					
Blushers and Rouges (all types)									
Face Powders				NR	1	NR			
Foundations									
Leg and Body Paints									
Lipsticks and Lip Glosses									
Makeup Bases									
Makeup Fixatives				1	NR	NR			
Other Makeup Preparations									
Manicuring Preparations (Nail)									
Other Manicuring Preparations									
Personal Cleanliness	***1***			***5***					
Bath Soaps and Body Washes	1	NR	NR	3	1	0.00025			
Deodorants (underarm)				1 (spray)	NR	NR			
Feminine Deodorants				1 (l.o.)	NR	NR			
Other Personal Cleanliness Products				1 (r.o.)	NR	NR			
Shaving Preparations									
Other Shaving Preparations									
Skin Care Preparations	***31***			***55***					
Cleansing	2	NR	NR	7	2	NR			
Face and Neck (excluding shaving preps)	18 (l.o.); 1 (r.o.)	NR	NR	31 (l.o.); 5 (r.o.)	3	NR			
Body and Hand (excluding shaving preps)				7 (l.o.)	5	NR			
Moisturizing	28	5	NR	11	6	NR			
Night									
Paste Masks (mud packs)									
Skin Fresheners	8	NR	NR	5	NR	NR			
Other Skin Care Preparations	NR	1	NR	3 (l.o.); 1 (r.o.)	1	NR	NR	1	NR
Suntan Preparations									
Indoor Tanning Preparations									
Other Suntan Preparations									
Other Preparations (i.e., those that do not fit another category)				***1***	NA	NA			

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
	Nelumbo Nucifera Root Extract			Nelumbo Nucifera Root Water			Nelumbo Nucifera Seed Extract		
Totals*	**190**	**15**	**0.2**	**21**	**1**	**NR**	**72**	**25**	**NR**
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*8*	*0.2*	***	*NR*	*NR*	***	*20*	*NR*
Rinse-Off	***	*7*	*NR*	***	*1*	*NR*	***	*5*	*NR*
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*	***	*NR*	*NR*
Exposure Type									
Eye Area	***	NR	NR	***	NR	NR	***	NR	NR
Incidental Ingestion	***	NR	NR	***	NR	NR	***	NR	NR
Incidental Inhalation-Spray	***	4[a]; 3[b]	NR	***	NR	NR	***	8[a]; 5[b]	NR
Incidental Inhalation-Powder	***	3[b]	NR	***	NR	NR	***	5[b]	NR
Dermal Contact	***	8	0.2	***	1	NR	***	21	NR
Deodorant (underarm)	***	NR	NR	***	NR	NR	***	NR	NR
Hair - Non-Coloring	***	5	NR	***	NR	NR	***	4	NR
Hair-Coloring	***	2	NR	***	NR	NR	***	NR	NR
Nail	***	NR	NR	***	NR	NR	***	NR	NR
Mucous Membrane	***	1	NR	***	NR	NR	***	1	NR
Baby Products	***	NR	NR	***	NR	NR	***	NR	NR
as reported by product category									
Baby Products	***1***						***3***		
Baby Shampoos							2	NR	NR
Baby Lotions/Oils/Powders/Creams							1	NR	NR
Baby Wipes	1	NA	NA						
Other Baby Products									
Bath Preparations (diluted for use)									
Other Bath Preparations									
Eye Makeup Preparations							***2***		
Eye Shadow									
Eye Lotion							1	NR	NR
Eye Makeup Remover									
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)							1	NA	NA
Other Eye Makeup Preparations									
Fragrance Preparations									
Perfumes									
Other Fragrance Preparation									
Hair Preparations (non-coloring)	***23***						***12***		
Hair Conditioner	2 (l.o.); 12 (r.o.)	1	NR				5 (r.o.)	1	NR
Hair Spray (aerosol fixatives)									
Rinses (non-coloring)									
Shampoos (non-coloring)	9 (r.o.)	2	NR				6 (r.o.)	1	NR
Tonics, Dressings, and Other Hair Grooming Aids	NR	2	NR				2	NR	NR
Other Hair Preparations									
Hair Coloring Preparations									
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	2	NR						
Hair Rinses (coloring)									
Other Hair Coloring Preparation									

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
Other Hair Coloring Preparation							NR	2	NR
Makeup Preparations (not eye; not children's)	***13***			***15***					
Blushers and Rouges (all types)									
Face Powders	NR	NR	0.2	15	NR	NR			
Foundations	**NR**	NR	0.2				NR	2	NR
Leg and Body Paints									
Lipsticks and Lip Glosses	10	NR	NR						
Makeup Bases	3 (traditional application)	NR	NR						
Makeup Fixatives									
Other Makeup Preparations									
Manicuring Preparations (Nail)									
Other Manicuring Preparations									
Personal Cleanliness Products	***4***			***2***			***1***		
Bath Soaps and Body Washes	4	NR	NR	1	NR	NR	1	1	NR
Deodorants (underarm)									
Feminine Deodorants									
Other Personal Cleanliness Products	NR	1	NR						
Shaving Preparations									
Other Shaving Preparations									
Skin Care Preparations	***148***			***4***			*54*		
Cleansing	14	1	NR	2	1	NR	2	1	NR
Face and Neck (excluding shaving preps)	114 (l.o.); 15 (r.o.)	3	NR	2 (l.o.)	NR	NR	NR	5	NR
Body and Hand (excluding shaving preps)	9 (l.o.)								
Moisturizing	83	2	NR				27	7	NR
Night									
Paste Masks (mud packs)	3	NR	NR				1	1	NR
Skin Fresheners	9	NR	NR				5	1	NR
Other Skin Care Preparations	1 (l.o.)	1	NR				2 (l.o.)	1	NR
Suntan Preparations									
Indoor Tanning Preparations									
Other Suntan Preparations									
Other Preparations (i.e., those that do not fit another category)	***1***	NA	NA						

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
	Nelumbo Nucifera Seed Powder			Nelumbo Nucifera Stamen Extract					
Totals*	**13**	**1**	**NR**	**6**	**1**	**NR**			
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*1*	*NR*	***	*1*	*NR*			
Rinse-Off	***	*NR*	*NR*	***	*NR*	*NR*			
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*			
Exposure Type									
Eye Area	***	NR	NR	***	NR	NR			
Incidental Ingestion	***	NR	NR	***	NR	NR			
Incidental Inhalation-Spray	***	NR	NR	***	1[b]	NR			
Incidental Inhalation-Powder	***	NR	NR	***	1[b]	NR			
Dermal Contact	***	1	NR	***	1	NR			
Deodorant (underarm)	***	NR	NR	***	NR	NR			
Hair - Non-Coloring	***	NR	NR	***	NR	NR			
Hair-Coloring	***	NR	NR	***	NR	NR			
Nail	***	NR	NR	***	NR	NR			
Mucous Membrane	***	NR	NR	***	NR	NR			
Baby Products	***	NR	NR	***	NR	NR			
as reported by product category									
Baby Products									
Baby Shampoos									
Baby Lotions/Oils/Powders/Creams									
Baby Wipes									
Other Baby Products									
Bath Preparations									
Other Bath Preparations									
Eye Makeup Preparations (not children's)									
Eye Shadow									
Eye Lotion									
Eye Makeup Remover									
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)									
Other Eye Makeup Preparations									
Fragrance Preparations									
Perfumes									
Other Fragrance Preparation									
Hair Preparations (non-coloring)									
Hair Conditioners									
Hair Sprays (aerosol fixatives)									
Rinses (non-coloring)									
Shampoos (non-coloring)									
Tonics, Dressings, and Other Hair Grooming Aids									
Other Hair Preparations									
Hair Coloring Preparations									
Hair Dyes and Colors (all types requiring caution statements and patch tests)									
Hair Rinses (coloring)									
Other Hair Coloring Preparation									
Makeup Preparations (not eye; not children's)									

Table 9. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category[49,50]

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]	RLD (2024)[49]	VCRP (2023)[50]	% (2022)[51]
Blushers and Rouges (all types)									
Face Powders									
Foundations									
Leg and Body Paints									
Lipsticks and Lip Glosses									
Makeup Bases									
Makeup Fixatives									
Other Makeup Preparations									
Manicuring Preparations (Nail)									
Other Manicuring Preparations									
Personal Cleanliness	***1***								
Bath Soaps and Body Washes									
Deodorants (underarm)									
Feminine Deodorants									
Other Personal Cleanliness Products	1 (r.o.)	NR	NR						
Shaving Preparations									
Other Shaving Preparations									
Skin Care Preparations	***12***			***6***					
Cleansing									
Face and Neck (excluding shaving preps)	10 (l.o.). 2 (r.o.)	NR	NR	5 (l.o.)	1	NR			
Body and Hand (excluding shaving preps)									
Moisturizing	9	NR	NR	1	NR	NR			
Night									
Paste Masks (mud packs)									
Skin Fresheners	1	NR	NR						
Other Skin Care Preparations	NR	1	NR						
Suntan Preparations									
Indoor Tanning Preparations									
Other Suntan Preparations									
Other Preparations (i.e., those that do not fit another category)									

NR – not reported; NA – not applicable (this category was not part of the VCRP)
PCPC concentration of use survey is underway, but results have not yet been received.
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 10 . Acute oral toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/Results	Reference
Nelumbo nucifera Gaertn., 0.5 g in a capsule (33% of contents)*	N/A	Swiss mice (10/group; sex not specified)	59.9, 79.9, or 99.9 g capsule materials/kg bw	Contents of the capsule was dissolved in distilled water and administered orally ; 7-d observation	No signs of toxicity and no mortality were observed.	60
Nelumbo nucifera leaf, flower, and root extract (ethanol)	N/A	Wistar albino rats (n = 3; of sex not specified)	2 g/kg	OECD TG 425; via gavage; 24-h observation	LD_{50} > 2 g/kg No deaths occurred.	41
Nelumbo nucifera leaf and root extract (ethanol)	N/A	Wistar albino rats (n = 3;sex not specified)	2 g/kg	OECD TG 425; via gavage; 24-h observation	LD_{50} >2 g/kg No deaths occurred.	41
Nelumbo nucifera flower extract (ethanol)	N/A	Wistar albino rats (n = 3; of sex not specified)	2 g/kg	OECD TG 425; via gavage; 24-h observation	LD_{50} > 2 g/kg No deaths occurred.	41
Nelumbo nucifera flower extract (water, in ethanol)	N/A	Male Wistar albino rats (3/sex/group)	2 g/kg	OECD TG 420; via gavage; 14-d observation	The test substance was considered non-toxic at up to 2 g/kg. No mortality occurred.	34
Nelumbo nucifera lotus root extract (ethanol)	N/A	ICR mice (12/sex/group)	0, 2, or 5 g/kg	Animals were dosed orally; 14-d observation	LD_{50} > 5 g/kg	61
Nelumbo nucifera seed extract (water, in ethanol)	0.3% w/v Na-CMC, in distilled water	Male Swiss albino mice (6/group)	0, 0.2, 0.4, 0.6, 0.8, or 1 g/kg bw	Animals were dosed orally; 24-h observation	LD_{50} > 1 g/kg No signs of toxicity were observed.	42
Nelumbo nucifera stamen extract-PVP complex**	distilled water	Sprague-Dawley rats (5/sex/group)	0 or 5 g/kg	OECD TG 420; via gavage; 14-d observation	LD_{5}> 5 g/kg	24

N/A – not applicable; Na-CMC – sodium carboxymethyl cellulose; OECD – Organisation for Economic Co-operation and Development;PVP-10 - polyvinylpyrrolidone-10; TG – test guideline; WHO – World Health Organization

*each capsule contained 0.5 g *Nelumbo nucifera* Gaertn., 0.5 g *Codonopsis pilosula (Franch)* Nannf, 0.15 g *Lactuca indica* L., 0.1 g *Curcuma longa* L., 0.1 g *Zingiber officinale* Rosc., 0.075 g *Saussurea lappa* Clarke, and 0.075 g *Atractylodes macrocephela* Koidz.

**Ethanol was used in the initial extraction of the *Nelumbo nucifera* stamen extract-PVP complex and was subsequently removed during the preparation process.

Table 11. Repeated dose oral toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Nelumbo nucifera Gaertn., in a capsule,	distilled water	Wistar rats (10/group; sex not specified) Swiss mice	4 wk	0, 1440, or 4320 mg/kg/d, in a capsule, Nelumbo nucifera 0.5g is only 33% of contents of the capsule)*	orally dosed; body weight changes, hematology, and serum biochemistry values (AST, ALT, total bilirubin, albumin, total cholesterol, and creatine levels) were evaluated before treatment, and after 2 and 4 wk of treatment. For acute toxicity study, a group of mice (10 per group) were fasted for 12-16 h and administered the test mixture orally in ascending doses that the mice could tolerate. The general symptoms of toxicity and the mortality in each group was observed for 72 h. The animals that survived were observed for another 7 d for delayed toxicity.	Statistically significant increases in body weight were observed in rats in the 1.44 g/kg/d group after 2 wk of treatment, compared to controls. No significant differences in red blood cell counts, hematocrit, hemoglobin level, platelet count, total white blood cell count and white blood cells, AST, total bilirubin, albumin concentration, and total cholesterol concentration were observed between treated animals and controls. After 4 wk of treatment, a statistically significant increase in ALT levels was observed in the 4.32 g/kg/d group compared to controls; however, these values were at the normal range for rats and no significant differences were observed compared to baseline values. No gross lesions or changes in size were observed in heart, liver, lungs, or kidney and abdominal cavities in treated rats, as compared to controls, upon necropsy. No significant differences were observed upon histopathological examination of the liver and kidneys of rats treated for 4 wk compared to controls; serum creatinine levels in both treated groups were also not significantly different from controls.	60

Table 11. Repeated dose oral toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Nelumbo nucifera lotus seed tea (produced by roasting uncoated seeds and extracting with hot water)	N/A	Male SKH-1 hairless mice (10/group)	6 mo	not specified	administered as the drinking fluid; mice received either *Nelumbo nucifera* lotus seed tea (test animals) or tap water (controls). Both groups received a chow diet. The animals were subsequently used for testing in a phototoxicity study.	No significant differences in food or liquid consumption or body weight were observed between test animals and controls.	[22]
Nelumbinis semen (*Nelumbo nucifera* seeds)	N/A	Sprague-Dawley rats (5/sex/group)	13 wk	0, 500, 1000, or 2000 mg/kg/d	Administered by gavage, mortality, clinical signs, body weight changes, food and water consumption, urinalysis, hematology and serum biochemistry, and necropsy findings and relative organ weights were recorded.	No mortality, body weight, or ophthalmic changes were observed in treated animals, compared to controls. Food consumption was lower, compared to controls, at weeks 7 and 12 for males in the 500 and 2000 mg/kg/d groups, and at weeks 7, 9, 10, and 12 for males dosed with 1000 mg/kg/d. A significant increase in hemoglobin concentration distribution (all test groups) and red blood cell distribution (500 and 2000 mg/kg/d groups) in males were not considered test article-related. Higher AST and ALT levels in all treated females and lower CPK levels in both treated sexes were not statistically significant. Lower right adrenal gland weight (with respect to body mass) in male rats from the 500 and 1000 mg/kg/d groups, in comparison to controls was neither dose-dependent or sex-matched, and, thus, was not considered treatment-related. No gross pathological abnormalities were observed. The NOAEL was determined to be 2000 mg/kg/d for both sexes combined.	[62]
Nelumbinis semen (*Nelumbo nucifera* seeds)	N/A	Beagle dogs (1/sex/group)	28 d	0, 500, 1000, 2000, or 4000 mg/kg/d	orally administered; body weights and average food consumption were recorded weekly. Serum biochemical values were obtained both before and after dosing. Animals were observed daily for changes in behavior, food intake, and urine output.	No mortality was observed. Vomiting was observed in the male dog that received the 2000 mg/kg dose, which could have been induced by gastro-intestinal stimuli. In the urinalysis, proteinuria was observed in controls and 500 and 1000 mg/kg males and in 50, 100, and 4000 mg/kg females. Low specific gravity of the urine was observed in all treated females. Urine occult blood was seen for the 2000 and 4000 mg/kg male and female, respectively. However, these effects were observed before treatment and none of these effects were dose-dependent or accompanied with other corresponding changes. No systemic and toxicologically significant changes related to treatment with Nelumbinis semen were observed. The NOAEL was determined to be 4000 mg/kg/d.	[62]

Table 11. Repeated dose oral toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Nelumbo nucifera stamen extract-PVP complex**	distilled water	Sprague-Dawley rats (6/sex/group)	90 d	0, 50, 100, or 200 mg/kg/d	OECD TG 408; orally dosed; body weights were recorded on day 0, 90, and at necropsy. Controls received 80% PVP-10 (w/w) in distilled water. A 200 mg/kg treatment satellite group and a control satellite group were observed for 28 d post-dosing for reversibility, persistence, or delayed toxicity occurrence. Any rat that died during the study underwent pathological examination.	No deaths or treatment-related signs were observed in treated animals during the study or recovery period. There was a slight but statistically significant decrease in the body weight of 200 mg/kg/d females compared to controls on day 90. However, weight changes of both groups showed no significant difference and the % weight changes of both groups were similar. Additionally, no statistically significant differences were observed in male and female satellite rats compared to controls at any dose. A few statistically significant differences were observed in the hematologic and biochemical parameters of 200 mg/kg rats treated for 90 d compared to controls. However, these minimal differences were not considered pathologically significant or treatment-related. Absolute kidney weights were slightly lower in 200 mg/kg/d rats for both sexes at day 90 and for treated females after 118 d, compared to controls. Relative liver weights were lower than controls for both sexes on day 90 and in treated males on day 118; relative heart, liver, and kidney weights were also lower than controls in treated females at day 90. However, these results were not considered treatment-related because values were within normal laboratory range and no abnormality was noted with respect to gross or histopathological examination of all organs. The NOAEL for both male and female rats was determined to be > 200 mg/kg/d.	[24]

ALT – alanine aminotransferase; AST – aspartate transferase; CPK – creatine phosphokinase; N/A – not applicable; NOAEL – no-observed-adverse-effect level; OECD – Organisation for Economic Cooperation and Development; PVP- polyvinylpyrrolidone; TG – test guideline; WHO – World Health Organization

*each capsule contained 0.5 g *Nelumbo nucifera* Gaertn., 0.5 g *Codonopsis pilosula (Franch)* Nannf, 0.15 g *Lactuca indica* L., 0.1 g *Curcuma longa* L., 0.1 g *Zingiber officinale* Rosc., 0.075 g *Saussurea lappa* Clarke, and 0.075 g *Atractylodes macrocephela* Koidz.

**Ethanol was used in the initial extraction of the *Nelumbo nucifera* stamen extract-PVP complex and was subsequently removed during the preparation process.

Table 12. Reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
IN VITRO						
Nelumbo nucifera petal extract (aqueous)	N/A	Rat sperm	0, 0.22, 0.44, 0.88, 1.76, or 3.52 mg/ml	Sperm dosed with extracts of *Nelumbo nucifera* petals (from red and white flowers) were first stained with DAPI, followed by staining with PI. Sperm which stained red with PI was considered dead, but sperm that remained unstained with PI was considered viable.	Increases in sperm viability were statistically significant at the 0.22 - 1.76 mg/ml exposure concentrations, when compared to controls. No statistically significant differences were seen between the viability of sperm from the highest dose group (3.52 mg/ml) and controls.	[32]
ORAL						
Nelumbo nucifera seed extract (petroleum ether)	peanut oil	Male Wistar albino rats (10/group)	7.5 mg/kg bw	orally administered; rats were dosed every other day for a 15-d period. An untreated group received saline (5 mg/kg) and vehicle controls were given refined groundnut oil (10 ml/kg); body weights were measured before and after the treatment period; 8 rats/group were sacrificed 24 h after the last dose. Testis, cauda epididymis, and adrenal glands were dissected out and weighed; sperm was obtained from the cauda epididymis; sperm count and mobility, cholesterol, ascorbic acid content, 3β-HSD and G-6-PD activity were measured in the testis.	Statistically significant decreases in the weights of testis, epididymis, and adrenal gland, the rate of body growth, sperm count, and motility were observed in treated rats, compared to controls. The researchers considered the statistically significant decrease in 3β-HSD and G-6-PD activity to possibly be due to inhibition of testicular steroidogenesis.	[20]
Nelumbo nucifera seed extract (petroleum ether)	peanut oil	Female Wistar albino rats (12/group)	0, 2.5, 5, or 7.5 mg/kg bw	orally administered; rats were dosed on alternate days for 15 d. An untreated group received saline (5 mg/kg) and vehicle controls were given refined groundnut oil (10 ml/kg). Body weights were measured before and after the treatment period. Rats were inspected daily for vaginal opening and a daily vaginal lavage was taken to determine the age at first estrus. Eight rats/group were sacrificed 24 h after the last dose. Ovaries and uteri were dissected and weighed; cholesterol, ascorbic acid content, 3β-HSD and G-6-PD activity was measured in the ovaries.	Delayed onset of sexual maturity was indicated by the age of vaginal opening and appearance of first estrus. Statistically significant inhibition of vaginal opening (38%) and first estrus (32%) were observed in 7.5 mg/kg bw rats, compared to vehicle controls. Statistically significant decreases in body weights (16.3%), ovary weights (57.3%), and uterus weights (80.8%) were observed in rats treated with the highest dose, compared to vehicle controls. Ovarian cholesterol content also increased by 99% and ascorbic acid increased by 29% in the 7.5 mg/kg bw group, compared to vehicle controls. The researchers considered that suppressed activity of 3β-HSD (21%) and G-6-PD (23%) in treated rat ovaries may be due to reduced ovarian steroidogenesis.	[20]
Nelumbo nucifera seed extract (50% ethanol)	N/A	Female Wistar albino rats (10/group)	0 or 800 mg/kg bw	orally administered for 40 d; animals were killed on day 41. Body weights were measured at the end of the experiment. Ovaries, uteri, and vaginas were dissected out, weighed and examined; blood was also collected for hematological studies.	Statistically significant decreases in ovary, uterus, and vagina weights were observed in treated animals, compared to controls; changes in body weights of the experimental animals were not significant. Total erythrocyte count, total leucocyte count, hemoglobin, blood sugar, and hematocrit values were within normal range when compared to controls. Statistically significant decreases in serum protein and glycogen levels and an increase in serum cholesterol were observed in treated animals, compared to controls. Prolonged length of the estrous cycle and an increase in the diestrous phase of the cycle in treated animals, compared to controls, was statistically significant.	[21]

Table 12. Reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Nelumbo nucifera seed extract (50% ethanol)	N/A	Male Wistar rats (10/group)	0, 50, 100, or 200 mg/kg bw/d	dosed via gavage for 60 d; initial and final body weights were recorded; blood was collected for hematological analysis; upon necropsy, reproductive and accessory sex organs (testes, epididymis, seminal vesicle, ventral prostate, and vas deferens) along with the liver were weighed; cauda epididymal sperm motility and density was assessed; serum testosterone was measured using ELISA.	No statistically significant changes in body weights, blood sugar and serum levels of protein, cholesterol, triglycerides, and phospholipids were observed, compared to controls. Statistically significant decreases in testes, epididymis, seminal vesicle, and ventral prostate weights were observed in a dose-dependent manner. Reduced sperm motility was statistically significant in all treated groups. Concentrations of testicular and caudal epididymal sperm reduced by 25.04 and 30.70% in the 50 mg/kg group, 56.4 and 71.68% in the 100 mg/kg group, and 63.55 and 84.14% in the 200 mg/kg group, respectively. Fertility reduced up to 100% after treatment with the *Nelumbo nucifera* seed extract. Decreases in serum testosterone were also statistically significant in a dose-dependent manner, compared to controls.	[63]

DAPI – 4',6-diamidino-2-phenylindole, dihydrochloride; ELISA – enzyme linked immunosorbent assay; G-6-PSD – glucose-6-phosphate dehydrogenase; 3β-HSD – 3β-hydroxysteroid dehydrogenase; N/A – not applicable; PI – propidium iodide

Table 13. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
IN VITRO						
trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)	not specified	10 – 100 mg/ml	Balb/c 3T3 fibroblasts	3T3 NRU cytotoxicity assay	IC_{50} = 14.71 mg/ml (14,710 μg/ml). non-toxic	[11]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	none	undiluted	reconstructed human epidermis	skin irritation test (OECD TG 439); additional details not provided	non-irritant	[15]
ANIMAL						
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	10 and 100% doses (effective test concentration: 0.05 – 0.15% and 0.5 – 1.5% Nelumbo Nucifera Germ Extract)	3 rabbits	Details not provided	non-irritant	[15]
HUMAN						
trade name mixture containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate)	mineral oil	25% (effective test concentration: 0.25-1.25% Nelumbo Nucifera Flower Extract) 0.02 ml was applied to a 50 mm^2 area	10 subjects	0.02 ml was applied to a 50 mm^2 area on the back of each subject, Test sites were evaluated 30 min after patch removal, evaluated following a 48-h occlusive application	The primary cutaneous irritation index was 0.20, and cutaneous compatibility was deemed "good."	[10]

Table 13. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/System	Protocol	Results	Reference
trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)	not specified	15% (effective test concentration: 0.075 – 0.15% Nelumbo Nucifera Flower Extract)	11 subjects	0.02 ml was applied to a 50 mm^2 area on the back as a 48-h occlusive patch. Test sites were evaluated 15 min after patch removal	non-irritating	[11]
foundation containing 0.2% Nelumbo Nucifera Flower Water	none	details not provided	30 subjects	28-d use test (details not provided)	very good tolerance, no comedogenicity	[75]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	50% (effective test concentration: 0.25 – 0.75%)	46 subjects	patch test; occlusive patch	negative	[15]
trade name mixture containing a maximum of 0.5 - 1.2% Nelumbo Nucifera Leaf Extract	water	25% (effective test concentration: 0.125 - 0.3% Nelumbo Nucifera Leaf Extract)	11 subjects	patch test (details not provided)	non-irritating	[17]
foundation containing 0.2% Nelumbo Nucifera Root Water	none	details not provided	33 subjects	28-d use test (details not provided)	very good tolerance, no comedogenicity	[76]
Nelumbo nucifera extract solution (1%); *Nelumbo nucifera* leaf, root, seed, and stem extracts		1% of individual extract	20 subjects	Several patch tests were performed to evaluate the irritation potential of each extract. Patches were placed on the forearm using a Haye's test chamber for 24 h; blank patches were used for comparison.	No signs of skin irritation were observed for up to 3 d after patch removal.	[9]
Water cream containing 1% each of *Nelumbo nucifera* flower, leaf, root, and stem extract and 4% combined extract (identity not specified)			20 subjects		no skin irritation observed	[9]
SENSITIZATION						
IN CHEMICO/IN VITRO						
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	none	100 mM	Details not provided	(DPRA	negative	[15]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	not stated	0.04% (effective test concentration: 0.0002 – 0.0006% Nelumbo Nucifera Germ Extract)	Details not provided	KeratinoSens assay performed according to OECD TG 442D	negative	[15]
ANIMAL						
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	The first and second induction concentrations were 50 and 100%, respectively, and challenge was performed at 10 and 100%.	5 guinea pigs/group	skin sensitization study (details not provided)	not a sensitizer	[11]
HUMAN						
trade name mixture containing 0.5 – 1%, Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)	not specified	Tested concentration 15%, (effective test concentration, 0.075 – 0.15% Nelumbo Nucifera Flower Extract; vehicle not specified).	53 subjects	HRIPT was completed. Patches (48-h) were applied 3x/wk for 3 wk. After a 2-wk non-treatment period, one 48-h challenge patch was applied	not an irritant or a sensitizer	[11]

Table 13. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/System	Protocol	Results	Reference
a foundation containing 0.00001% Nelumbo Nucifera Flower Extract	none	0.2 ml tested neat (~0.05 ml/cm^2)	50 subjects	HRIPT; 3 (24-h) occlusive patches applied each wk for 3 wk ; challenge was performed following a 2-wk non-treatment period	not an irritant or sensitizer	[78]
foundation containing 0.2% Nelumbo Nucifera Flower Water	none	20 µl tested neat, (50 mm^2)	100 subjects	HRIPT (details not provided)	not an irritant or sensitizer	[75]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	20% (no other details provided) (effective test concentration – 0.10 – 0.30%)	56 subjects	HRIPT (No other details provided)	not an irritant or sensitizer	[15]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	not specified	30%, (no other details provided) (effective test concentration – 0.15 – 0.45%)	57 subjects	HRIPT (No other details provided)	not an irritant or sensitizer	[15]
Nelumbo Nucifera Germ Extract (emulsion containing 0.0001%)	none	0.1 - 0.15 g of the test material (as received (~25 -38 mg/ cm^2)	52 subjects	HRIPT; 3 (24-h) occlusive patches applied each wk for 3 wk ; challenge was performed following a 2-wk non-treatment period	not an irritant or sensitizer	[77]
trade name mixture containing a maximum of 0.5 - 1.2% Nelumbo Nucifera Leaf Extract	water	25% (effective test concentration: 0.125 - 0.3% Nelumbo Nucifera Leaf Extract)	56 subjects	HRIPT (details not provided)	not an irritant or sensitizer	[17]
foundation containing 0.2% Nelumbo Nucifera Root Water	none	40 µl tested undiluted, (110 mm^2)	103 subjects	HRIPT (details not provided)	not an irritant or sensitizer	[76]
PHOTOTOXICITY						
IN VITRO						
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	not specified	1000 µg/ml	no details provided	OECD TG 432 (3T3 NRU phototoxicity test)	no phototoxicity	[15]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and saccharides)	not specified	50 µg/ml	no details provided	ROS assay (photosafety)	negative	[15]
ANIMAL						
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	10 and 30% (effective test concentration: 0.05 – 0.15% and 0.15 – 0.45%, respectively)	5 guinea pigs/group	phototoxicity study (detail not provided)	negative	[15]
trade name mixture containing 0.5 – 1.5 w/v% Nelumbo Nucifera Germ Extract (tannins and flavonoids)	not specified	photoinduction: 30% (effective test concentration: 0.15 – 0.45%) photochallenge: 3, 6, and 10% (effective test concentrations: 0.015 – 0.045%, 0.03 – 0.135%, and 0.05 – 0.15%, respectively)	5 guinea pigs/group	photosensitization study (detail not provided)	negative	[15]
HUMAN						
foundation containing 0.2% Nelumbo Nucifera Flower Water	none	applied neat; 50 µl over a 110 mm^2 surface	28 subjects	phototoxicity study: UVB and UVA (290 - 390 nm); dose equal to 0.75 MED or with UVA only (315 - 390 nm); dose equal to 20 J/cm^2)	not phototoxic	[75]

Table 13. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/System	Protocol	Results	Reference
foundation containing 0.2% Nelumbo Nucifera Flower Water	none	applied neat; 50 µl over a 110 mm^2 surface	28 subjects	photosensitization study induction: UVB and UVA (290 - 390 nm); immediately after clinical examinations, dose levels equal to 1.5 times the MED challenge: UVA only (315 - 390 nm); dose equal to 5 J/cm^2 UVA	not photosensitizing	[75]
foundation containing 0.2% Nelumbo Nucifera Root Water	none	applied neat; 50 µl over a 110 mm^2 surface	26 subjects	phototoxicity study: UVB and UVA (290 - 390 nm; dose equal to 0.75 MED) or with UVA only (315 - 390 nm); dose equal to 20 J/cm^2	not phototoxic	[76]
foundation containing 0.2% Nelumbo Nucifera Root Water	none	applied neat; 50 µl over a 110 mm^2 surface	26 subjects	photosensitization study induction: UVB and UVA (290 - 390 nm); immediately after clinical examinations, dose levels equal to 1.5 times the MED challenge: UVA only (315 - 390 nm); dose equal to 4 J/cm^2 UVA	not photosensitizing	[76]

Table 14. Ocular Irritation Studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
IN VITRO						
trade name mixture containing 1 - 5% Nelumbo Nucifera Flower Extract (extracted in isostearyl isostearate)	mineral oil	25% in mineral oil (effective test concentration 0.25-1.25%)	Fresh fertile White Leghorn PA12 eggs	HET-CAM assay	non-irritant mean irritation index – 2.3	[10]
trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)	not specified	15% (effective test concentration 0.075-0.15%)	Fresh fertile White Leghorn eggs	HET-CAM assay	slightly irritating the mean irritation index - 2.25	[11]
trade name mixture containing 0.5 – 1% Nelumbo Nucifera Flower Extract (extracted in propanediol and glycerin with 0.5 – 1% Nymphaea Caerulea Flower Extract)	none	tested neat	isolated bovine corneas	BCOP test	very well tolerated corneal score (30 min) – 0.1 corneal score (4) – 0.0	[11]
foundation containing ~0.2% Nelumbo Nucifera Flower Water	not specified	not specified	not specified	Neutral red release assay	negligible cytotoxicity	[75]
foundation containing ~0.2% Nelumbo Nucifera Flower Water	not specified	not specified	not specified	HET-CAM	practically non-irritant	[75]
trade name mixture containing a maximum of 0.5 - 1.2% Nelumbo Nucifera Leaf Extract	none	tested neat	not specified	Neutral red release assay	non-cytotoxic	[17]

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