Safety Assessment of Camellia Sinensis-Derived Ingredients as Used in Cosmetics

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All interested persons are provided 60 days from the above date to comment on this Draft Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 Director, Lillian J. Gill D.P.A.

The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

1101 17th Street, NW, Suite 412 ∀ Washington, DC 20036-4702 ∀ ph 202.331.0651 ∀ fax 202.331.0088 ∀ cirinfo@cir-safety.org

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INTRODUCTION

This is a safety assessment of cosmetic ingredients derived from *Camellia sinensis* (tea) plant parts. The functions of these ingredients include: antifungal agent; antimicrobial agent; antioxidant; cosmetic astringent; fragrance ingredient; light stabilizer; oral care agent; skin protectant; skin-conditioning agent – emollient; skin-conditioning agent – humectant; and skin-conditioning agent – miscellaneous (Table 1). The 15 ingredients in this report are:

- Camellia sinensis leaf extract,
- Camellia sinensis catechins,
- Camellia sinensis flower extract,
- Camellia sinensis flower/leaf/stem juice,
- Camellia sinensis leaf,
- Camellia sinensis leaf oil,
- Camellia sinensis leaf powder,
- Camellia sinensis leaf water,
- Camellia sinensis root extract,
- Camellia sinensis seedcoat powder,
- Camellia sinensis seed extract,
- Camellia sinensis seed oil,
- Camellia sinensis seed powder,
- Hydrolyzed camellia sinensis leaf, and
- Hydrolyzed camellia sinensis seed extract.

It is unclear if camellia sinensis leaf water is solely used as a fragrance ingredient. If so, it will not be included in this report as it will be the purview of the Research Institute for Fragrance Materials (RIFM).

The *C. sinensis*-derived ingredients in this safety assessment are from edible plant sources and exposure to these ingredients from beverages results in a much larger systemic dose than that resulting from use in cosmetic products. Therefore, oral toxicity potential of these cosmetic ingredients will not be addressed in this report. The focus is on the potential for reproductive toxicity, genotoxicity, carcinogenicity, irritation, and sensitization.

CHEMISTRY

Definition and Description

The definitions and functions of *Camellia sinensis*-derived ingredients are provided in Table 1.

CAMELLIA SINENSIS

There are four varieties of the *C. sinesis* plant: *sinesis*, *assamica*, *pubilimba*, and *dehungensis*. The first two are most commonly used to prepare tea for human consumption. The type of tea used as a beverage (white, green, oolong, black) depends on time of year harvested, age of leaves when harvested, location/soil/climate, and processing after harvest. The processing of tea for a beverage is referred to as fermentation, because it was originally believed that the leaves were fermented, but, it has been discovered since that it is actually an enzymatically catalyzed oxidation process.[1,2] It is not known which of these teas or which process are used in cosmetics.

The *C. sinensis* is native to East, South and Southeast Asia.[2-4] However, it is also cultivated in various other tropical and subtropical regions. The plant is an evergreen shrub or small tree that may grow to 16 m tall if not pruned, but, is usually trimmed to below 2 m when cultivated for its leaves. The leaves are variable. They are lanceolate to obovate, up to 30 cm long (usually 4-15) and 2-5 cm broad, pubescent, sometimes becoming glabrous, serrate, acute, or acuminate. The plant has a strong taproot. The 3 – 5 cm, yellow/white flowers are globular and have a delicate fragrance.

These plants are not the source of nor are they related to tea tree oil, which is derived from *Malaleuca alternifolia*.

CONSTITUENTS

The constituent groups of fresh green leaf *C. sinsensis* are provided in Table 2. The highest concentration group of constituents is the flavanols (25.0% dry weight), followed by proteins (15.0%) and polysaccharides (13.0%).[2]

The constituent groups include:

Amino acids – The most abundant amino acid is one not typically found in proteins, theanine (5-*N*-ethylglutamine).[2,5]

Carotenoids – These are present in low levels in the leaf. They include neoxanthin, violaxanthin, lutein, chlorophylls a and b, and β-carotene.[2,6,7] Seventy-nine pigment species, 41 chlorophylls and other 38 carotenoids have been detected.[8]

Enzymes – Fresh *C. sinsensis* leaves contain high levels the enzyme of polyphenol oxidase.

Methylxanthines - Theobormine can range from 0.16% - 0.2% of a dry-weight leaf.[2,9,10] Dried leaves contain not less than 2% caffeine (dried weight). Increased use of nitrogen fertilizer can increase caffeine content by up to 40%. Theophylline is present at < 0.04% dry leaf weight.

Flavonoids – These include flavonols, floaonols, and glycosides. Flavanols include catechins. These are present is small amounts and may occur as flavonols and glycosides.[2,11,12] The leaf extract is reported to contain the flavonols kaempferol, quercetin and myricetin.[2,13]

Catechins - These polyphenolic molecules are a subgroup belonging to the flavenol family.[2,14,15] They make up 20% - 30% of the weight of a tea leaf. The most abundant type of catechin in green tea is epigallocatechin gallate (EGCG; 12%).

The other catechins are catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), catechin gallate (CG), gallocatechin gallate (GCG), and epicatechin gallate (ECG) (Figure 1).

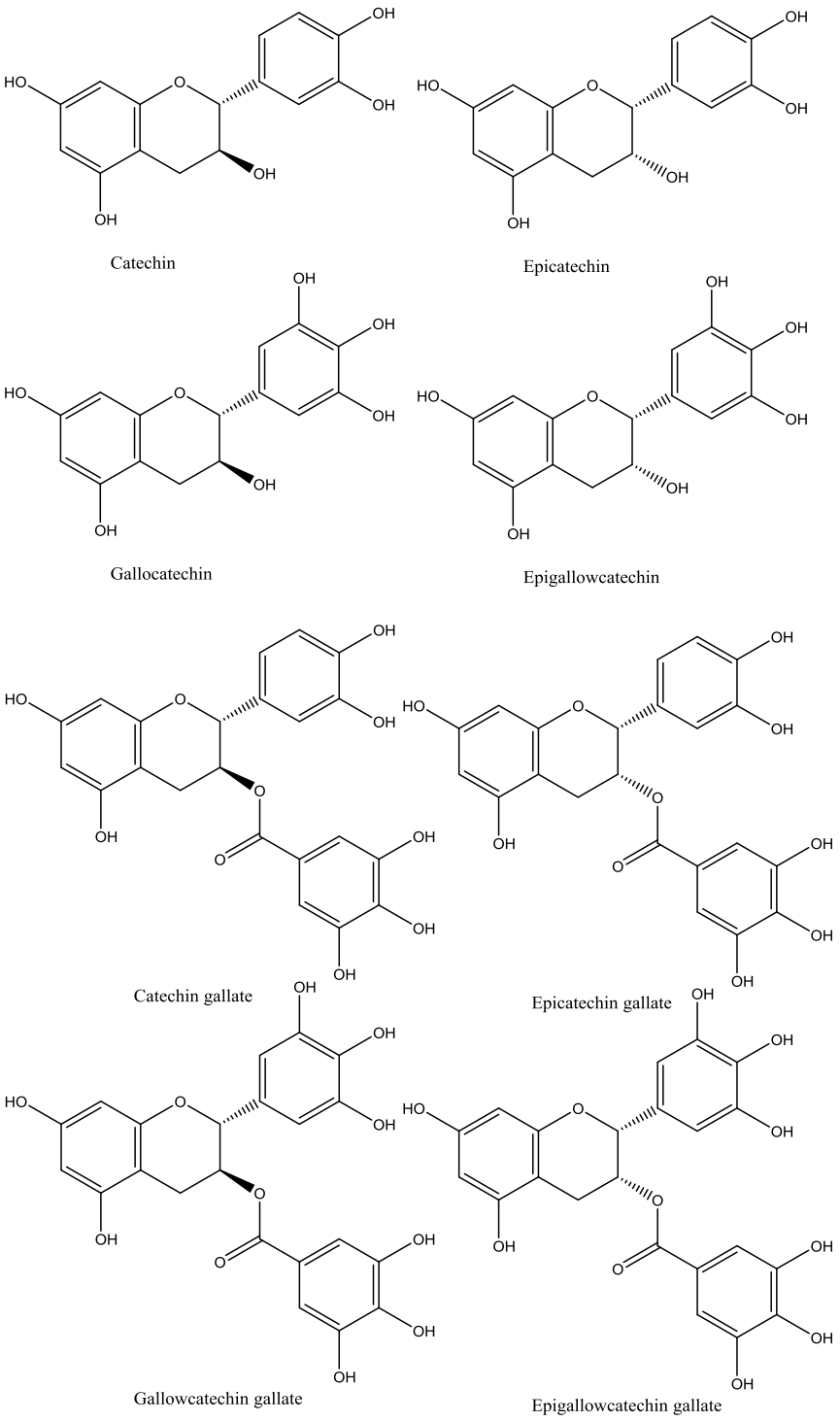

Figure 1. Catechins from *Camellia sinensis*

Minerals and elements - Potassium is the greatest mineral present at 40% of the total mineral content. The leaves are rich in fluoride and they accumulate aluminum and manganese.[2,16,17] Other minerals present include calcium, magnesium,

sodium, phosphorus, and sulfur. Minor elements include boron, barium, chromium, copper, iron, molybdenum, nickel, rubidium, strontium, and zinc.[16] Trace elements include silver, arsenic, beryllium, bismuth, cadmium, cerium, cobalt, cesium, mercury, indium, lithium, lead, rare earth elements, antimony, selenium, tin, tellunium thallium uranium, vanadium, ytterbium, and zircon.

Volatiles – There are a large number of volatile constituents in fresh leaves. *Trans*-2-hexenal and *cis*-3-hexenol are present in the greatest amounts.[2,12,18,19]

Climatic conditions during cultivation may affect the composition of theanine, standard α-amino acids (i.e., isoleucine, leucine, valine, alanine, threonine, glutamine), quinic acid, EC, EGC, EGCG and caffeine levels in green tea.[20,21] Soil conditions and cultivation methods affect mineral levels.[16]

SAMPLE ANALYSIS

Constituents present in medical grade *C. sinensis folium* extract include methylxanthines, flavanols (10% - 25%), flavonols, flavones, phenolic acids, amino acids (including theanine, 3%), therpene saponins, polysaccharides, proanthocyanidins, vitamins, and minerals (Table 3).[14,22-26]

Analyses of three lots of *C. sinensis* catechins (each prepared as a food additive) had 28% - 90% total catechin monomers and 37% - 100% polyphenols.[27]

CHARACTERIZATION

As an herbal supplement, extracts are characterized by the drug/extract ratio (DER) which is the ratio between the quantity of herbal substance used in the manufacture of an herbal preparation (given as the actual range) and the quantity of the herbal preparation obtained.[28] The parameters for *C. sinensis folium* as an herbal supplement in the EU are: dry extract, purified (DER 45-56:1, extraction solvent: water) which corresponds to 55-72% (-) epigallocatechin-3-*O*-gallate.[22,28] Dry extract (decaffeinated; DER 6:1 to 10:1, solvents such as alcohol, methanol, acetone, or water or mixtures of these solvents) contains not less than 60% of polyphenols, calculated as (-)-epigallocatechin-3-*O*-gallate, not less than 40% of (-)-epigallocatechin-3-O-gallate, and not more than 0.1% of caffeine, calculated on the anhydrous basis.

Physical and Chemical Properties

C. sinensis catechins taste astringent and are soluble in water.[2,14]

Three lots of oolong tea with *C. sinensis* catechins were stable for 18 months in unopened packaging at -20°C.[27] Total catechin monomers were reduced from 100% to 97% and 98% after 6 months of storage at 25°C in polyethylene terephthalate (PET) bottles and steel cans, respectively. At 37° for 2 months, catechin content was reduced to 96% for both containers.

No other information on the physical and chemical properties of C. *sinensis*-derived ingredients were discovered or submitted.

Method of Manufacture

No information on the method of manufacture for camellia sinensis-derived cosmetic ingredients was discovered or submitted. The methods below are general processes for food or food ingredients and it is unknown if they apply to cosmetic ingredients. The makeup of the *C. sinensis* extract will differ with manufacturing process.

Camellia sinensis leaf in the form of green tea consists of whole or cut young, unfermented, rapidly hot dried leaves.[15,22] The fresh leaves are processed by a method designed to prevent the enzymatic oxidation of catechins. The enzymes are inactivated by heat (steam or pan-fried).

There are different harvesting and manufacturing processes for white, green, black and oolong teas for drinking.[15,16,22,29] White tea is made from very young leaves and buds. Green tea is made from new, fully formed leaves. These two teas are minimally processed and steamed and dried. Black tea and oolong tea are made from older, fully formed leaves. Oolong tea is withered, and rolled during "fermentation", then fired and dried. Black tea is withered, crushed, and rolled during "fermentation" then fired and dried. The differences in the phenolic content between green and black tea are presented in Table 4.

Catechins are isolated in an initial hot water extract with ethyl acetate and then separated by chromatography followed by spray drying.[30] The spray-dried EGCG may be recrystallized.

Two other processes for the extraction of catechins from *C. sinensis* leaves are conducted with or without enzymatic treatment with tannase. The extract is then extracted with water and ethanol, and then filtered through multiple mediums. The process without tannase is sterilized above 100°C and the process with the tannase is below 100°C.

The presence of minerals and elements in an extract is dependent on the extent of entrapment in the organic matrix, the degree of solubility/choice of solvent, the length of time of extraction, temperature, pH, and agitaion.[16] Most elements, especially the metals, are complexed by the flavonols, catechols, tannins, and polyphenols.

Impurities

No published data on impurities of these cosmetic ingredients were discovered and no unpublished data were

submitted. The information below applies to food or food ingredients.

Analysis of twelve *C. sinensis* catechins lots extracted for food ingredients showed that arsenic, cadmium, lead, and tin were below levels of detection.[27] Three lots of *C. sinensis* catechins were analyzed for other components: caffeine (≤7%), organic acids (≤10%), protein and amino acids (≤10%), saccharide (≤12%), fiber (≤1%), fat (≤1%), and ash (≤5%). No microbial contamination was detected.

Ten commercial *C. sinensis* teas for drinking were analyzed for metals.[31] The ranges for metal content were: zinc 1.05 – 3.21 mg/kg; iron, 5.47 - 8.41 mg/kg; manganese, 1.27 – 2.73 mg/kg; copper, 0.01 – 0.93 mg/kg; nickel, 0.01 – 0.64 mg/kg; lead, 0.26 – 1.25 mg/kg; and cadmium, 0.01 – 0.05 mg.kg. The authors concluded that the differences in content were due to differences in geographic region of cultivation.

Aflatoxigenic molds and aflatoxins have been reported to be present on *C. sinensis* teas for drinking.[32]

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP; Table 5).[33] A survey is being conducted by the Personal Care Products Council (Council) of the maximum use concentrations for these ingredients.

Camellia sinensis leaf extract is reported to be used in 1011 leave-on, 710 rinse-off, and 35 bath cosmetic products. There are reported uses for every exposure type. Usage of "green tea" and "green tea extract" were also listed in the VCRP. Since these are technical names for the ingredient camellia sinensis leaf extract, those uses reported in the VCRP were combined with this ingredient.

Camellia sinensis leaf was reported to be used in 38 leave-on, 14 rinse-off, and 1 bath product.

Camellia sinensis leaf oil was reported to be used in 24 leave-on products and 9 rinse-off products.

Camellia sinensis leaf powder was reported to be used in 7 leave-on and 8 rinse-off products.

Camellia sinensis leaf water was reported to be used in 26 leave-on and 11 rinse-off products.

Camellia sinensis seed oil was reported to be used in 33 leave-on and 6 rinse-of products.

Non-Cosmetic

Tea, under the previous name *Thea sinensis*, is generally regarded as safe (GRAS) by the FDA. (21 CFR 582.20)

In Europe, *C. sinensis* preparations are used to treat asthenia and as an adjuvant treatment in weight loss/control.[22]

Preparations are also used in cutaneous treatment of external genital and perianal warts (condylomata acuminata) in immune-compromised patients.[22,34]

In the United States, green tea products are used as dietary supplements (nutraceuticals), primarily for purported weight loss and antioxidant properties.[15,35-45] Other health benefits attributed to green tea include prevention and/or control of atherosclerosis, hypertension, coronary heart disease, diabetes, metabolic syndrome, obesity, and cancer as well as having antibacterial, antiviral, antifungal, and neuroprotective properties.

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

When camellia sinensis leaf extract (0.32, 0.68, 1.03, 1.35 mg/cm in methanol; green tea) was applied to full thickness pig ear skin using a Franz cell, there was a dose-dependent penetration of the catechins EGCG, EGC, and EC.[46] Saturated solutions of camellia sinensis were formulated using water, PEG-400, citrate/phosphate buffer (pH 5.5), and a 50:50 mixture of PEG-400 and the buffer. The solutions were applied to drug-in-adhesive patches under occlusion in methanol and applied to the pig skin for 48 h.

Penetration by the catechins was fastest in the buffer solution and slowest in PEG-400 solution. In the buffer solution, EGCG permeated the skin at 1.37 ± 0.40 and 1.88 ± 0.45 $\mu g/cm^{-2}$ at 24 and 48 h; EGC permeated at 0.189 ± 4.10 x 10^{-2} and 0.342 ± 7.48 x 10^{-2} $\mu g/cm^{-2}$; EC permeated at 32.4 ± 11.3 and 71.2 ± 35.2 $\mu g/cm^{-2}$, respectively. In the mixed solution, EGCG permeated the skin at 1.27 ± 0.38 and 1.62 ± 0.18 $\mu g/cm^{-2}$ at 24 and 48 h; EGC permeated at 0.128 ± 1.71 x 10^{-3} and 0.392 ± 0.004 [sic] $\mu g/cm^{-2}$; EC permeated at 22.2 ± 17.3 and 40.2 ± 43.8 $\mu g/cm^{-2}$, respectively. In PEG-400 solution, EGCG permeated the skin at 1.37 ± 0.40 and 1.88 ± 0.45 $\mu g/cm^{-2}$ at 24 and 48 h; EGC permeated at 0.189 ± 4.10 x 10^{-2} and 0.342 ± 7.48 x 10^{-2} $\mu g/cm^{-2}$; EC permeated at 32.4 ± 11.3 and 71.2 ± 35.2 $\mu g/cm^{-2}$, respectively. In water, EGCG permeated the skin at 0.27 ± 0.15 and 0.66 ± 0.30 $\mu g/cm^{-2}$ at 24 and 48 h; EGC permeated at 0.06 ± 0.02 and 0.10 ± 0.03 $\mu g/cm^{-2}$; EC permeated at 1.32 ± 0.22 and 2.34 ± 0.34 $\mu g/cm^{-2}$, respectively.

Caffeine in the buffer solution permeated at 0.32 ± 0.05 and 0.49 ± 0.01 $\mu g/cm^{-2}$ at 24 and 48 h; 173 ± 24.6 and 368 ± 52.9 $\mu g/cm^{-2}$ in the mixed solution; 46.8 ± 3.43 and 88.9 ± 0.08 $\mu g/cm^{-2}$ in the PEG-400 solution; and 28.4 ± 2.46 and 50.2 ± 1.54 $\mu g/cm^{-2}$ in water, respectively.[46]

When EGCG was dermally applied in a transdermal gel (50 mg/kg; 28.6 $\mu g/cm^2$) to female SKH-1 mice (n = 4, 5, or 6), EGCG was detected in the skin, the plasma, the liver, small intestines, and color for at least 24 h.[47] The test material was

administered once. Over the next 24 h, blood was collected under anesthesia and the dorsal skin was removed, fractioned into epidermis and dermis, and analyzed. Liver, small intestine, and colon tissues were removed and analyzed.

In the total plasma, the C_{max} was 44.5 ± 8.4 ng/mL, the $t_{1/2}$ was 94.4 ± 13.2 h, and the $AUC_{0\rightarrow24}$ was 881.5 ± 123.4 ng/mL/h. The C_{max} for the epidermis and dermis were 1365.7 ± 613.8 ng/mL and 411.2 ± 21.5 ng/mL, respectively; the $AUC_{0\rightarrow24}$ was 5978.3 ± 2779.9 and 1729.5 ± 259.4 ng/g/h, respectively. The $t_{1/2}$ was 9.3 ± 4.3 and 10.9 ± 1.6 h, respectively.

The C_{max} of EGCG in the liver was 164.8 ± 83.0 ng/g with a $t_{1/2}$ of 74.6 ± 20.1 h and an $AUC_{0\rightarrow24}$ 2494.8 ± 673.6 ng/g/h. The C_{max} in the small intestine was 203.1 ± 64.0 ng/g with a $t_{1/2}$ of 26.8 ± 5.6 h and an $AUC_{0\rightarrow24}$ 2802.8 ± 588.5 ng/g/h. The C_{max} in the colon was 77.0 ± 22.4 ng/g with a $t_{1/2}$ of 21.3 ± 3.2 h and an $AUC_{0\rightarrow24}$ 715.0 ± 107.3 ng/g/h. The C_{max}, $t_{1/2}$, $AUC_{0\rightarrow24}$ for EGCG in the urine were 177 ng/mL, 3427.9 ng/mL/h, and 70.0 h, respectively.[47]

Inhalation

No published inhalation data on these cosmetic ingredients were discovered and no unpublished data were submitted.

Antimicrobial Activity

Constituents

SAPONINS

The decaffeinated methanolic extract of the leaves of *C. sinensis* exhibited in vitro antimicrobial properties against 111 bacteria comprising 2 genera of Gram positive and 7 genera of Gram negative bacteria.[48] The activity of the extract was in the range of 10 - 50 μg/ml. A few strains were sensitive at lower concentrations (5 μg/ml). In decreasing order of sensitivity, the bacteria groups were: *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Bacillus* spp., *Klebsiella* spp. and *Pseudomonas aeruginosa*.

When the above extract (30, 60 μg/mouse) was orally administered to a Swiss strain of white mice, the mice were protected from a challenge with *S. typhimurium*.[48]

Anti-Inflammatory Effects

When saponins (0, 50, 100, 200 mg/kg) extracted from *C. sinensis* leaf were orally administered to rats prior to treatment with carrageenan (1%; 0.5 mL) in a rat hind paw edema assay, edema in response to carrageenan was mitigated in a dose-dependent manner.[49]

Cytotoxicity and Cellular Effects

Camellia sinensis extract (10, 50, 100 μg/mL) was not cytotoxic to rat pheochromocytoma (PC12) cells when exposed for 24 h.[29] However, at higher concentrations (250, 500, 100 μg/mL), the extract was cytotoxic with < 40% viability at the two highest concentrations. When the cells were incubated with the extract and hydrogen peroxide (250 μM), hydrogen peroxide poisoning was mitigated by the extract at 5, 100, and 250 μM.

Camellia sinensis water extract (as Korean green tea) had an ID_{50} of 2.05% (0.28 mg/mL dry matter) in the inhibition of protein synthesis in Sprague-Dawley rat hepatic cells.[50] The ED_{25} of lactate dehydrogenase (LDH) release was 1.84% (0.25 mg/mL). Camellia sinensis extract (in the form of black tea) had an ID_{50} of 2.50% (0.46 mg/mL) and an ED_{25} of LDH release of 5.11% (0.94 mg/mL).

EGCG induced apoptosis at 400 and 800 μmol/L to neonatal human dermal fibroblasts.[51] At 200 μmol/L EGCG, a decrease in the proportion of cells in the S and G_2/M phases of the cell cycle and an increase in the proportion of cells in the G_0/G_1 phase was observed. Regulation on the expression of pNF-κB was concentration dependent but did not affect NF-κB expression. cDNA microarray analysis revealed that EGCG (200 μmol/L) down-regulated cell cycle-related genes. A/B cyclins and cyclin-dependent kinase 1 was reversibly regulated by EGCG (200 μmol/L).

TOXICOLOGICAL STUDIES

The *C. sinensis*-derived ingredients in this safety assessment are from edible sources and exposure to these ingredients from beverages would result in a much larger systemic dose than that resulting from use in cosmetic products. Consequently, their oral toxicity potential is not addressed in this report which is focused on the potential for reproductive toxicity, genotoxicity, carcinogenicity, irritation and sensitization.

Acute Toxicity

Dermal – Non-Human

CAMELLIA SINENSIS CATECHINS

The dermal LD_{50} of EGCG (2000 mg/kg extract; 1860 mg EGCG/kg; 93% in distilled water; 4 mL/kg) was > 1860 mg/kg for HanBrl:WIST (SPF) rats (n = 5/sex).[30] The acute dermal toxicity was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) guideline number 402 under semi occlusion. The day before the study, the backs of the rats were clipped with an electric clipper exposing approximately 10% of the total body

surface. The dressing was removed at 24 h and flushed with lukewarm tap water and dried. The rats were observed twice daily for 15 days. Macroscopic examination of all animals was performed at day 15.

There were no systemic signs of toxicity observed in any of the rats. A slight to moderate erythema was observed in all treated rats after removal of the dressing which persisted for up to 5 days. Body weights were within standard range for this strain and age of rat. No abnormal macroscopic findings were observed at necropsy.[30]

Inhalation

No published inhalation data on these cosmetic ingredients were discovered and no unpublished data were submitted.

Repeated Dose Toxicity

Inhalation

No published inhalation data on these cosmetic ingredients were discovered and no unpublished data were submitted.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Unpublished studies were submitted to the FDA for the drug approval of a topical ointment that contains up to 15% camellia sinensis catechins to treat warts. These are summarized in Table 6. In oral studies, there were increased resorptions at 1000 mg/k/d in rats. In subcutaneous studies, the test substance was not well tolerated; subcutaneous lesions with necrosis developed. There were spontaneous abortions, increased resorptions, and increased fetal malformations at doses as low as 12 mg/kg/d. Intravaginal administrations up to 0.15 ml/d yielded fewer adverse effects.

There were no adverse effects when pregnant Wistar rats (n = 6) were orally administered camellia sinensis extract (0, 84, 167, 501, and 1336 mg/mL/d; in the form of black tea).[52] The caffeine content was 4.14% (865 mg for the highest dose). The test material was administered either on gestation days 1 – 7, 8 – 14, or 15 – 21. Internal examinations of the dams were conducted by laparoscopy under anesthesia. Pups were examined daily until developmental signs were observed. The test doses were calculated to be equivalent to 1.5, 3, 9, and 24 cups of tea. There were no mortalities. There were no differences in number of pregnancies, number of uterine implants, number of viable implants, implantation index, pre-implantation loss, post-implantation loss, gestation index, number of pups born, litter index, live birth index and viability index compared to controls. There were no differences in length of the implants/fetus, gestation length, cranial length, cranial diameter and tail length of pups. There were no differences in time taken to open eyes, eruption of incisors and appearance of fur. There were no gross morphological birth abnormalities observed.

When camellia sinensis catechins (1400, 4200, 14000 ppm in feed; EGCG 90%, ECG ≤3.01%, GCG ≤0.12%, other catechins ≤0.54%) were administered to pregnant Wistar (SPF) rats (n = 25) on gestation days 6 - 20, there were no adverse effects observed.[53] All rats survived treatment and there were no clinical signs. There was a transient reduction in feed consumption in the high-dose group and an increase in water consumption in the mid- and high-dose groups. There were not treatment-related macroscopic findings in the dams. There was no effect to embryo/fetal survival, fetal weights, or sex ratios.

In a two generation study of camellia sinensis catechins (1200, 3600, 12000 ppm in feed) using Sprague-Dawley rats (n = 30/sex), there were no adverse effects in either generation. The rats were treated for 10 weeks and then paired for mating. The diet continued through gestation until after weaning. The dams were killed and necropsied after weaning. The pups were culled to 25/sex and the above treatment repeated with mating taking place after 8 weeks.

The offspring of the high-dose group had reduced growth rates, and there was an increase in pup loss. A growth effect among pups was also observed at 3600 ppm, but only in the second generation. Both sexes of the F_1 generation in the high-dose group showed reduced absolute kidney and liver weights. The F1 males had reduced spleen and prostate weights, but the females' spleens were normal. Histological examination revealed no abnormalities. The lowest dose was considered the overall adverse NOAEL. The authors derived a NOAEL equivalent to 200 mg/kg body weight per day EGCG preparation. Because dams consumed twice the amount of feed during the crucial lactation period, in which effects occurred, twice the lowest dose, which would otherwise have been 100 mg/kg body weight per day was calculated as the NOAEL.[53]

GENOTOXICITY

In Vitro

Catechins were not mutagenic in multiple in vitro and in vivo assays including Ames test (up to 5000 µg/plate), mouse micronucleus assays (up to 2000 mg/kg), and micronucleus assays (Table 7). A polyphenol mixture was lethal at 2000 mg/kg/d to mice. Mixed results were reported in a mouse lymphoma assay at concentrations > 100 µg/mL.

CARCINOGENICITY

In 1997. the International Agency of Research in Cancer (IARC) listed green tea in group 3, meaning that it is not classifiable according to its carcinogenicity to humans.[54]

Camellia sinensis extract (125, 250, 500 mg/kg/d; as green tea; 85% - 95% catechins w/w, 55% EGCG) did not

increase the incidence of neoplastic or non-neoplastic lesions in the organs and tissues of p53 transgenic heterozygous mice (n = 25).[55] The mice were treated daily for 26 weeks. *p*-Cresidine and water served as controls.

Carcinogenicity Reviews

There are several reviews regarding the protective effects of green tea extracts and its catechins, especially EGCG, against chemical carcinogens.[37,56-59]

According to Yang *et al*.[57], there are more than 133 published studies 1991-2008 on this topic (Table 8). Inhibitory effects of tea and/or tea constituents were found in lung, oral, stomach, intestine, dermal, prostrate, breast, liver, bladder, pancreas, and thyroid cancers.

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

CAMELLIA SINENSIS LEAF EXTRACT

There were no adverse effects and reduced healing time in burned rabbits (n = 5) administered camellia sinensis leaf water extract (100%; 0.05 mL) compared to controls.[60] The rabbits were burned with a heated glass rod applied to shaved skin then the extract, antibiotic, or nothing was administered. The rabbits were observed for 5 weeks. The size of the wounds reduced faster with the extract and the antibiotic compared to controls. Closure time for the treatment groups was 8 – 10 days for antibiotics and 7 – 9 days for the extract. At five weeks, the wounds were almost healed in the treated groups while the control was 0.92 ± 0.15 cm^2. Hair growth also began sooner in both of treated groups. Microscopic examination showed skin with a more normal appearance in the camellia sinensis leaf extract group compared to the antibiotic and controls groups.

CAMELLIA SINENSIS CATECHINS

There were no signs of irritation observed when EGCG (0.47 g in 3 ml distilled water) was administered to the clipped flanks of male New Zealand White rabbits (n = 3) for 4 h under semi-occluded patch.[30] The tests were conducted according to the EC Commission Directive 92/69/EEC, B.4, ‘‘Acute Toxicity—Skin Irritation’’ and OECD guideline number 404 (OECD, 1992a). The dorsal fur of three male rabbits was removed with electric clippers 24 h prior to the administration of the test material. Each rabbit was treated with 0.5 g of EGCG preparation (93.4% EGCG) dissolved in 0.3 ml distilled water and applied to the skin of one flank using a semi-occlusive patch. After removal, the skin was cleaned with water. Skin reactions and irritation effects were assessed at approximately 1, 24, 48 and 72 h after removal. Adjacent areas of untreated skin from each animal served as controls. There were no signs of toxicity observed.

In a preliminary study for a guinea pig maximization test, an intradermal injection of 0.09% EGCG was the greatest tolerable dose.[30] A grade 3 erythema was produced, but not necrosis. At 48 h of dermal exposure, there was no reaction in the preliminary test at concentrations up to 50%.

Dermal – Human

CAMELLIA SINENSIS LEAF WATER

In a patch test (n = 10) of a mascara containing camellia sinensis leaf water (30%), there were no signs of irritation at 30 min, and 24 and 48 h after the removal of the patch.[61] The test substance was administered to the inner side of the upper arm for 24 h.

In a trial of an ointment containing camellia sinensis catechins (10% and 15%) for the treatment of anogenital warts, there were no adverse effects reported.[62] The ointment was administered three times per day for up to 16 weeks. No adverse effects were reported during treatment, or the 12-week follow-up, and the ointment was reported to be well tolerated.

When *C. sinensis* preparations (DER ranging from 1/1000 - ≥1/10; 0.1% - >10%) are used to in dermal applications, the following were among the adverse effects: erythema, pruritus, irritation/burning, pain, ulcer, edema, induration, and vesicles. A full list of reported effects is provided in Table 9.

Mucosal

Intravaginal administration of an ointment containing camellia sinensis catechins (15%) caused ulceration and erosion of the vaginal mucosa with inflammation for four weeks.[55] The control group (no catechins) did not cause damage to the vaginal mucosa. The effects resolved when treatment stopped.

Ocular

The administration of EGCG preparation (0.093 g EGCG; 0.1 g) into the eye of a single female New Zealand White rabbit resulted in moderate to severe irritation including reddened conjunctivae and sclera, discharge and chemosis.[30] A slight to moderate corneal opacity affecting the whole area of the cornea was observed up to 72 h after administration of the test material. No damage to the iris, and no corrosion or staining of the eye by EGCG was observed throughout this study. The test was done in compliance with OECD guideline number 405. Both eyes of the rabbits were examined at the beginning

of the study. The lids were briefly held together after administration; the eyes were not rinsed. The animal was observed for ocular irritancy for 17 days. As it was suspected that EGCG might be an ocular irritant, a single animal was treated first and observed to recovery. Due to the results from this preliminary study, more rabbits were not tested.

Sensitization

Dermal – Non-Human

CAMELLIA SINENSIS CATECHINS

In a sensitization assay using female GOHI (SPF) guinea pigs (n = 6), camellia sinensis catechins (5%, 10%, 30% in ethanol; 100 µL/8 cm^2; 4%, 8%, 24% EGCG) was sensitizing when challenged (0%, 1%, 3%, 5%, and 10%) as well as at a second challenge (0%, 0.1%, 0.5%, 1%, 3%, 5% and 10%) two weeks later.[30] The skin sensitization assay was performed according to a procedure adopted from OECD guideline number 406 (OECD, 1992b). During the induction phase of this assay, an EGCG preparation (80% EGCG) was applied to the shaved right flanks of the guinea pigs 5 days/week for 4 weeks. Control animals were treated with ethanol. Treatment sites were left open between applications. During induction, new treatment sites were chosen whenever the irritation became considerable. Immediately following the induction period, the guinea pigs were challenged with EGCG (25 µL/2 cm2 on the left flank. During the induction period the guinea pigs were observed for signs of erythema and edema on each test site. Challenge reactions were assessed at 24 and 48 h after application.

Irritation responses increased throughout the induction period starting with the fifth application; the 30% group had the greatest frequency of reactions. Erythema became evident in the 10% and 5% groups at the 13th and 16th administrations, respectively. In the 10% group, a slight erythema was observed in 2/6 guinea pigs on the 13th application with all guinea pigs showing similar signs by the 16th application. For the 5% group, erythema was only observed for 3 days in 1/6 guinea pigs. Both EGCG preparation challenges elicited positive effects in the test groups.

Control animals showed no response after the first challenge; one or two of the six control guinea pigs did have slight or well defined erythema after the second challenge with 0.8% or higher EGCG. Although there was a positive dose–response effect for the challenge, it did not clearly correlate to the doses. There were more reactions in the 5% induction group (6 at 24h, 5 at 48 h) than did those in the 30% induction group (2 at 24 h, 1 at 48 h). No mortalities or symptoms of systemic toxicity were observed in any of the guinea pigs and body weights for test animals were in the same range as controls during the study period.[30]

In a guinea pig maximization test using female Himalayan strain albino guinea pigs (n = 10; control n = 5), camellia sinensis catechins (0.1% in distilled water; 0.1 ml; 90% EGCG) was a sensitizer.[30] All guinea pigs had grade 3 or 4 erythema following the intradermal injection of the test material and/or Freund's Complete Adjuvant. Grade 1 erythema was observed following the first test challenge in 3/10 in the test group and 0/5 in the control group. In a second challenge 1 week later, 9/10 in the test group showed stronger, grade 2, erythema. No mortalities or signs of systemic toxicity were observed in any of the guinea pigs and body weights for the test group were in the same range as controls during the study period.

Dermal – Human

CAMELLIA SINENSIS LEAF WATER

In an HRIPT (n = 110) of a mascara containing camellia sinensis leaf water (30%), there were no signs of irritation or sensitization.[63]

Phototoxicity

In Vivo

There were no signs of erythema on treated sites on the forearms of subjects (n = 6) treated with camellia sinensis leaf extract (10%) in the form of green or black tea then exposed to UVA and B.[64] Freeze-dried green and black tea extracts were used to make a gels with 1% carbomer solution and sodium hydroxide. These were administered to a 4 cm^2 area. The controls were an untreated area and an area treated with just the gel. The arms were then exposed to UVA/UVB for 2.5 min. Erythema was observed in the control and carbomer treated sites.

Topical treatment with green tea polyphenols (3 mg/2.5 cm^2 in acetone) on human skin reduced the UVB induction of cyclobutane pyrimidine dimer formation and erythema in a dose-dependent manner.[65] The polyphenols consisted of EC at 6%, EGC at 5%, EGCG at 65%, and ECG at 24%. Green tea polyphenols were administered to the buttocks of Caucasian subjects (n = 6) 20 min before the skin was exposed to 0.5%, 1.0%, 2.0%, or 4.0% of the previously established minimal erythema dose. The test sites were examined and skin punch biopsies taken 24 h after UVB treatment. Cyclobutane pyrimidine dimers and erythema were reduced in the treated sites exposed to 1.0%, 2.0, and 4.0% of a minimal erythema dose of UVB in a dose-dependent manner.

In Vitro

Metalloproteinase activity in cultured fibroblasts and keratinocytes decreased when incubated in EGCG (0.01, 0.1 µM in propylene glycol:ethanol 3:7) for 24 h before exposure to UVA radiation.[66] An artificial skin was prepared using

human keratinocytes and dermal fibroblasts on a lattice of bovine type I collagen. The skin was incubated in EGCG for 24 h and washed. The skin was exposed to UVA (340 – 400 nm; 20 J/cm^2; time not provided) 6 h later. Supernatant was collected 24 h after irradiation and analyzed.

SUMMARY

This is a safety assessment of *Camellia sinensis* (tea)-derived cosmetic ingredients. These ingredients function mostly as antioxidants and skin-conditioning agents – miscellaneous. Because tea is ingested in food and drink, this safety assessment does not address oral toxicity but is focused on other end points.

The constituents of *C. sinensis* include amino acids, carotenoids, catechins, enzymes, flavonoids (including flavanols and flavonols), and glycosides. The concentration of these constituents in plant parts is influenced by growing conditions, location, soil conditions, and processing.

Camellia sinensis leaf was reported to be used in 38 leave-on, 14 rinse-off, and 1 bath product. Camellia sinensis leaf oil was reported to be used in 24 leave-on products and 9 rinse-off products. Camellia sinensis leaf powder was reported to be used in 7 leave-on and 8 rinse-off products. Camellia sinensis leaf water was reported to be used in 26 leave-on and 11 rinse-off products. Camellia sinensis seed oil was reported to be used in 33 leave-on and 6 rinse-of products.

The FDA considers *C. sinensis* to be GRAS.

Catechins from camellia sinensis leaf extract penetrated pig ear skin as did caffeine. EGCG penetrated mouse skin.

Camellia sinensis leaf extract exhibited antimicrobial properties towards multiple bacterial species.

Camellia sinensis extract was not cytotoxic to rat pheochromocytoma cells up to 100 µg/mL but induced apoptosis at 400 and 800 µmol/L to neonatal human dermal fibroblasts.

The dermal LD_{50} of EGCG was > 1860 mg/kg for rats. There was slight to moderate erythema observed.

Reproduction and developmental studies of an ointment that contained up to 15% camellia sinensis catechins were conducted. In oral studies, there were increased resorptions at 1000 mg/k/d in rats. In subcutaneous studies, the test substance was not well tolerated; subcutaneous lesions with necrosis developed. There were spontaneous abortions, increased resorptions, and increased fetal malformation as low as 12 mg/kg/d. Intravaginal administration up to 0.15 ml/d had fewer adverse effects.

Camellia sinensis extract had no adverse effects when orally administered to pregnant rats up to 1336 mg/mL/d in drinking water. In a two-generation study, camellia sinensis catechins up to 12000 ppm in feed caused no clinical signs and no effects to embryo/fetal survival, fetal weights, or sex ratios. The offspring of the high-dose group had reduced the growth rates, and there was an increase in pup loss. While there were some reduced organ weights, histological examination revealed no abnormalities. The NOAEL was 200 mg/kg/d EGCG.

Catechins were not mutagenic in multiple in vitro and in vivo assays including Ames test (up to 5000 µg/plate), mouse micronucleus assays (up to 2000 mg/kg), and micronucleus assays. A polyphenol mixture was lethal at 2000 mg/kg/d to mice. Mixed results were reported in a mouse lymphoma assay at concentrations > 100 µg/mL.

Camellia sinensis extract at 500 mg/kg/d was not carcinogenic to mice after 26 weeks.

Camellia sinensis leaf extract at 100% caused no adverse effect to the skin of burned rabbits. Camellia sinensis catechins were not irritating to rabbits with intact skin at 0.47 g.

There were no adverse effects in a patch test of a mascara containing camellia sinensis leaf water at 30%. There were no adverse effects in a trial of an ointment containing camellia sinensis catechins at 10% and 15%.

C. sinensis preparations with > 10% plant material caused erythema, pruritus, irritation/burning, pain, ulcer, edema, induration, and vesicles in dermal tests.

The intravaginal administration of an ointment containing camellia sinensis catechins at 15% caused ulceration and erosion of the vaginal mucosa with inflammation for four weeks.

The administration of a preparation containing 0.093 EGCG into the eye of a single rabbit resulted in moderate to severe irritation including reddened conjunctivae and sclera, discharge and chemosis.

Camellia sinensis catechins were sensitizing to guinea pigs at 5%. In another guinea pig test, camellia sinensis catechins were a sensitizer at 0.1%.

In an HRIPT of a mascara containing camellia sinensis leaf water at 30%, there were no signs of irritation or sensitization

There was no signs of erythema on treated sites on the forearms of subjects treated with 10% camellia sinensis leaf extract in the form of green or black tea then exposed to UVA and B. Topical treatment with green tea polyphenols at 3 mg/2.5 cm^2 to human skin reduced the UVB induction of cyclobutane pyrimidine dimer formation and erythema in a dose-dependent manner. Metalloproteinase activity in cultured fibroblasts and keratinocytes decreased when incubated in EGCG at 0.01 and 0.1 µM for 24 h before exposure to UVA radiation.

DATA NEEDS

None of the available data cover the stem, flowers, leaf oil, root, seed, or seed coat parts of *C. sinensis*. The CIR staff requests data on these plant parts as well as the following data:

- Confirmation that camellia leaf water is only used as a fragrance ingredient.
- Inhalation studies.
- Certificates of analysis of the cosmetic grade of these ingredients.
- What are the manufacturing processes? Do the manufacturing processes include fermentation?
- Physical and chemical properties.

TABLES AND FIGURES

Table 1. Definitions and functions of *Camellia s.*-derived ingredients in this report.

Ingredient CAS No.	Definition	Function
Camellia Sinensis Leaf Extract 84650-60-2	The extract of the leaves of *Camellia sinensis.*	Antifungal agent; antimicrobial agent; antioxidant; cosmetic astringent; fragrance ingredient; light stabilizer; oral care agent; skin protectant; skin-conditioning agent – emollient; skin-conditioning agent – humectant; skin-conditioning agent - miscellaneous
Camellia Sinensis Catechins	A mixture of catechins obtained from the leaves of *Camellia sinensis.*	Antioxidants
Camellia Sinensis Flower Extract	The extract of the flowers of *Camellia sinensis.*	Skin-conditioning agents – miscellaneous
Camellia Sinensis Flower/Leaf/Stem Juice 1196791-49-7	The juice expressed from the flowers, leaves and stems of *Camellia sinensis.*	Antioxidant
Camellia Sinensis Leaf	The leaf of *Camellia sinensis.*	Skin-conditioning agent – miscellaneous
Camellia Sinensis Leaf Oil 68916-73-4	The oil derived from the leaves of *Camellia sinensis.*	Antioxidant; skin-conditioning agent - miscellaneous
Camellia Sinensis Leaf Powder	A powder derived from the dried, ground leaves of *Camellia sinensis.*	Exfoliant
Camellia Sinensis Leaf Water	An aqueous solution of the steam distillate obtained from the leaves of *Camellia sinensis.*	Fragrance ingredient
Camellia Sinensis Root Extract	The extract of the roots of *Camellia sinensis.*	Skin-conditioning agent – miscellaneous
Camellia Sinensis Seedcoat Powder	The powder obtained from the dried, ground seedcoats of *Camellia sinensis.*	Skin conditioning agent – miscellaneous
Camellia Sinensis Seed Extract	The extract of the seeds of *Camellia sinensis.*	Skin-conditioning agent – humectant
Camellia Sinensis Seed Oil	The oil expressed from the seeds of *Camellia sinensis.*	Skin-conditioning agent – humectant; skin-conditioning agent; occlusive
Camellia Sinensis Seed Powder	The powder obtained from the dried, ground seeds of *Camellia sinensis.*	Antioxidant; skin-conditioning agent – miscellaneous
Hydrolyzed Camellia Sinensis Leaf	The hydrolysate of Camellia Sinensis Leaf (q.v.) derived by acid, enzyme or other method of hydrolysis.	Skin-conditioning agent – humectant
Hydrolyzed Camellia Sinensis Seed Extract	The hydrolysate of Camellia Sinensis Seed Extract derived by acid, enzyme or other method of hydrolysis.	Antioxidant; skin protectant; skin-conditioning agent - miscellaneous

Table 2. Constituent groups of fresh green *C. sinensis* leaf.[2]

Constituent	% of dry weight
Flavonols	25.0
Flavonols and flavonol glycosides	3.0
Polyphenolic acids and depsides	5.0
Other polyphenols	3.0
Caffeine	3.0
Theobromine	0.2
Amino acids	4.0
Organic acids	0.5
Monsaccharides	4.0
Polysaccharides	13.0
Cellulose	7.0
Protein	15.0
Lignin	6.0
Lipids	3.0
Chlorophyll and other pigments	0.5
Ash	5.0
Volatiles	0.1

Table 3. Constituent groups in medical grade *Camellia sinensis* extract.[14,22-26]

Constituent group	Constituent	Concentration (%)
Methylxanthines	Caffeine	2.5-4.2
	Theophylline	0.02-0.04
	Theobromine	0.15-0.2
Flavanols (flavan-3-ols)		10-25
	Monomers (catechins)	
	(-)-epicatechin (EC)	
	(-)-epicatechin-3-*O*-gallate (ECG)	
	(-)-epigallocatechin (EGC)	
	(-)-epigallocatechin-3-*O*-gallate (EGCG)	
	Dimers (theaflavins)	
	Theaflavin	
	Theaflavin 3-gallate	
	Theaflavin 3-*O*-gallate	
	Theaflavin3,3-*O*-digallate	
Flavonols		
	Quercetin (and its glycosides)	
	Kaempferol (and its glycosides)	
	Myricetin (and its glycosides)	
Flavones		
	Apigenin	
	Luteolin	
Phenolic acids		
	Chlorogenic acid	
	Gallic acid	
	Theogallin	
Amino acids	Theanine (5-N-ethyl glutamine)	3
	18 other amino acids	
Therpene saponins (theafolia saponins)	Aglycones	
	Barringtogenol C	
	R1-barringenol	
	And others	
Polysaccharides		13
Proanthocyanidins (tannins)		
Vitamins		
	Ascorbic acid	
	α-Tocopherol	
Other compounds		
	Fluoride	
	Chlorophyll	
	Organic acids	
Constituent group	**Constituent**	**Concentration (ppm)**
Elements		
	Copper	270
	Iron	13040
	Nickel	1340
	Sodium	1.800
	Potassium	262
	Magnesium	30,800
	Calcium	13,750
	Zinc	630.0
	Chromium	10.0

Table 4. Phenolic composition of green and black tea.[44]

Constituent	Green tea (%w/w)	Black tea (%w/w)
Catechins	30-42	3-10
Flavonols	5-10	6-8
Other flavonoids	2-4	-
Theagallin	2-3	-
Gallic acid	0.5	-
Quinic acid	2.0	-
Theanine	4-6	-
Methylxanthines	7-9	8-11
Theaflavins	-	3-6
Thearubigins	-	12-18

Table 5. Frequency of use according to duration and exposure of *C. sinensis*-derived ingredients.[33] The Council is conducting a survey or the concentration of use for the ingredients added to this report.

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Camellia sinensis leaf extract[1]		**Camellia sinensis leaf**		**Camellia sinensis leaf oil**		**Camellia sinensis leaf powder**	
Total/range	**1756**	**NS**	53	**NS**	33	**NS**	16	**NS**
Duration of use								
Leave-on	1011	**NS**	38	**NS**	24	**NS**	7	**NS**
Rinse-off	710	**NS**	14	**NS**	9	**NS**	8	**NS**
Diluted for (bath) use	35	**NS**	1	**NS**	NR	**NS**	NR	**NS**
Exposure type								
Eye area	129	**NS**	6	**NS**	NR	**NS**	1	**NS**
Incidental ingestion	31	**NS**	NR	**NS**	5	**NS**	NR	**NS**
Incidental Inhalation-sprays	76	**NS**	1	**NS**	NR	**NS**	1	**NS**
Incidental inhalation-powders	10	**NS**	NR	**NS**	1	**NS**	NR	**NS**
Dermal contact	1412	**NS**	51	**NS**	20	**NS**	16	**NS**
Deodorant (underarm)	9	**NS**	NR	**NS**	NR	**NS**	NR	**NS**
Hair-noncoloring	263	**NS**	2	**NS**	8	**NS**	NR	**NS**
Hair-coloring	42	**NS**	NR	**NS**	NR	**NS**	NR	**NS**
Nail	1	**NS**	NR	**NS**	NR	**NS**	NR	**NS**
Mucous Membrane	364	**NS**	1	**NS**	10	**NS**	9	**NS**
Baby	5	**NS**	NR	**NS**	1	**NS**	NR	**NS**

	Camellia sinensis leaf water		**Camellia sinensis seed oil**			
Total/range	**37**	**NS**	**39**	**NS**		
Duration of use						
Leave-on	26	**NS**	33	**NS**		
Rinse-off	11	**NS**	6	**NS**		
Diluted for (bath) use	NR	**NS**	NR	**NS**		
Exposure type						
Eye area	5	**NS**	10	**NS**		
Incidental ingestion	NR	**NS**	3	**NS**		
Incidental Inhalation-sprays	NR	**NS**	NR	**NS**		
Incidental inhalation-powders	NR	**NS**	1	**NS**		
Dermal contact	36	**NS**	34	**NS**		
Deodorant (underarm)	NR	**NS**	NR	**NS**		
Hair-noncoloring	1	**NS**	2	**NS**		
Hair-coloring	NR	**NS**	NR	**NS**		
Nail	NR	**NS**	NR	**NS**		
Mucous Membrane	NR	**NS**	5	**NS**		
Baby	NR	**NS**	NR	**NS**		

[1] "Green tea" and "green tea extract" are not INCI names of cosmetic ingredients but were listed in the VCRP. Since these are technical names for camellia sinensis leaf extract, these total were combined with this ingredient.

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 6. Reproductive and developmental studies submitted to the FDA for the approval of an ointment containing 15% polyphenols.[55]

Species (n); administration	Results
Oral	
Pregnant rats (6-7); 0, 125, 250, 500, 750, 1000 mg/kg in water on gestation days 6-15	Complete resorptions in 2/6 dams in the highest dose group. No other treatment related effects.
Sprague-Dawley (27); 0, 250, 500, 1000 mg/kg on gestations days 6-18 by gavage	Body weight gains were reduced in all treatment groups compared to controls (14%, 7%, 10%, respectively). No effects on fertility, embryo/fetal development.
Rabbits (not provided); 0, 62.5, 125, 250, 500, 1000 mg/kg on gestations days 6-18 by gavage	No treatment related effects observed.
White rabbits (not provided); 0, 100, 300, 1000 mg/kg on gestations days 6-18 by gavage	Mortality due to gavage trauma. Body weight gains were reduced in the low- and high-treatment groups (-31%, +10%, 84%, respectively). Feed consumption was reduced in the high-dose group. No effects on fertility, embryo/fetal development.
Subcutaneous	
Rabbits (6); 0, 37.5, 150 mg/kg/d on gestation days 6-19	High-dose group- irritation with severe subcutaneous lesions/necrosis at injection sites. Treatment was discontinued after 6 days. One rabbit aborted. There was body weight loss, reduced feed consumption, and embryonic resorptions. Two fetuses from separate litters had umbilical hernia (one with hyperflexed limb), one fetus had a short tail. Low-dose group-Local irritation, reduced body weight gain. Increased early and late resorptions, Decreased corpora lutea, implants, litter size. No effect to fetal weights.
Rabbits (at least 6); 0,4, 12, 36 mg/kg/d on gestation days 6-19	High-dose group-severe local irritation at injection sites, reduced weight gain and feed consumption, reduced fetal weight. Abortions on gestation day 26. Reduced fetal weights. There were 3 malformed fetuses from 2 litters. Number of corpora lutea, pre-implantation loss, number of implantations, and sex ratios were similar to controls. Mid-dose group- one abortion on last day of gestation. 6 fetuses (from 5 litters) were malformed. One aborted fetus had a domed head. Number of corpora lutea, pre-implantation loss, number of implantations, and sex ratios were similar to controls. Low-dose group- Seven fetuses (from 4 litters) were malformed. Control group had 3 malformed fetuses from 2 litters. Blood tests show no accumulation of EGCG in the plasma during treatment.
Intravaginal	
Sprague-Dawley rats (25); 0.15 ml administered 4 days before mating through gestation day 17	No adverse effect on reproductive ability or embryo/fetal development. There were no mortalities. There were no differences in feed consumption.
Rats (25); 0.05, 0.10, 0.15 mL/d administered gestation day 6 - weaning	4 rats in the high-dose group and 3 in the mid-dose groups died possibly due to parturition complications. Dam in high-dose group killed after both pups died. There were no clinical signs observed. High-dose group-Increased stillborn pups (23 from 6 dams). There was reduced litter size and live birth index. There were no other treatment-related effects on pre- and –postnatal development. Controls-5 stillborn pups from 3 dams
Rats (25); 0, 0.05. 0.10, 0.15 mL/rat/d administered gestation day 6 – weaning. F1 generation were paired (25) and were mated untreated	F_0 - High-dose group-4 dams killed due to possible parturition complications. 20 dams delivered successfully with 23 stillborn pups from 2 litters. Mid-dose group-3 dams killed due to possible parturition complications. 22 dams delivered successfully with 9 stillborn pups from 7 litters Low-dose group-22 dams delivered successfully with Controls-5 stillborn pups from 3 litters. F_1 – No mortalities. One male in the mid-dose group was missing the tip of his tail and one female had dental abnormalities. No clinical signs, bode weight gains, pinna unfolding, incisor eruption, eye opening, surface righting, gripping pupillary and auditory reflex, age of vaginal opening, and balano-preptial separation were normal. Water maze field tests were normal. All mating and fertility parameters were normal.

Table 7. Mutagenicity studies of *C. sinensis* extracts and constituents.

Assay	Ingredient/constituent (concentration)	Results	Reference
	In vitro		
Ames test (*Samonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Eschieria coli* WP2*uvr*A);	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0-5000 µg/plate in sterile water); Metabolic activation at 4% and 10%	Not mutagenic with or without metabolic activation. Not cytotoxic.	67
Ames test (*S. typhimurium* strains TA97, TA98, TA100, TA102)	Camellia sinensis flower extract (0.008, 0.04, 0.1, 1.0, 5.0 mg/plate; water extract) with and without metabolic activation	Not mutagenic with or without metabolic activation.	68
Ames test (*S. typhimurium* strains TA97, TA98, TA100, TA102, TA1535)	EGCG (88.1%-95% pure) (50-5000µg/plate) with and without metabolic activation	Not mutagenic with or without metabolic activation.	69
Mouse lymphoma assay	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0-625 µg/mL in sterile water)	Not mutagenic with or without metabolic activation. Cytotoxic at ≥375 µg/mL.	67
Mouse lymphoma assay	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0-500 µg/mL without; 0-625 with metabolic activation in sterile water)	Mutagenic at ≥164 µg/mL without metabolic activation; mutagenic at ≥375 µg/mL with metabolic activation. Cytotoxic at ≥500 µg/mL.	67
Mouse lymphoma assay	EGCG (77% pure) with and without metabolic activation	Not mutagenic without metabolic activation up to 100 µg/mL; mutagenic ≥ 125 µg/mL with metabolic activation	69
Mouse lymphoma assay	Polyphenol mixture (0, 87, 155, 276, 492, 878, 1568, 2800, 5000 µg/mL) with and without metabolic activation	Not mutagenic with or without metabolic activation.	55
	In vivo		
Mouse micronucleus assay (n = 5/sex)	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0-1500 mg/kg)	Not mutagenic	67
Mouse micronucleus assay (n = 5/sex)	EGCG (91.9% pure) (500, 1000, 2000 mg/kg)	Not mutagenic	69
Big blue mutation assay Swiss-Webster mice (n = 7/sex)	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0, 500, 1000, 2000 mg/kg/d for 28 d). Necropsied 28 days after final dose. Tissues analyzed for mutations.	No increase in cII mutant frequencies in the livers, lungs, and spleen tissues at 500 and 2000 mg/kg. Mice died during treatment in the 2000 mg/kg group and were not analyzed.	67
Micronucleus assay diet study using CD-1 mice (6/sex)	EGCG (80% pure) (4200, 8400, 12600 ppm in feed)	No increase in frequency of micronucleated polychromatic erythrocytes	69
Micronucleus assay intravenous study using Wistar rats (5/sex)	EGCG (92.6% pure) (15, 25, 50 mg/kg/d intravenously for 2 days	No increase in frequency of micronucleate polychromatic erythrocytes	69
Micronucleus assay intraperitoneal study using Sprague-Dawley rats (7/sex)	Polyphenol mixture (0, 8500 mg/kg). Bone marrow sampled 24 and 48 h after treatment	No increase in frequency of micronucleate polychromatic erythrocytes	55

Table 8. The number of published studies discovered in a PubMed search (1965-2008) for the carcinogenicity inhibitory effect of green tea extracts and its catechins in animal models.[57]

Organ/tissue	Inhibitory effect (xenograft studies)	No inhibitory effect
Lung	20 (1)	2
Oral cavity	6	0
Esophagus	4	0
Stomach	9	0
Small intestine	8	1
Colon	11 (3)	6
Skin	27 (1)	0
Prostate	4 (5)	0
Breast	10 (8)	0

Liver	7	1
Bladder	3 (1)	0
Pancreas	2 (2)	0
Thyroid	1	0

Table 9. Dermal reactions to *C. sinensis* application in ointments for dermal treatment of genital and perianal warts.[22]

Dose (DER)	Very common	Common	Uncommon
≥ 1/10	Local reactions at the application site including erythema, pruritus, irritation/burning, pain, ulcer, edema, induration and vesicles		
≥1/100 – 1/10		Local reactions at the application site including exfoliation, discharge, bleeding and swelling	
≥1/1,000 - ≤100			Local reactions at the application site including discoloration, discomfort, dryness, erosion, fissure, hyperesthesia, anesthesia, scar, nodule, dermatitis, hypersensitivity, local necrosis, papules, and eczema
≥1/1,000 - ≤100			Application site infection, application site pustules, herpes simplex, infection, pyoderma, staphylococcal infection, urethritis, vaginal candidiasis, vulvovaginitis and vulvitis

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