Safety Assessment of *Camellia sinensis*-Derived Ingredients as Used in Cosmetics

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ABSTRACT

Cosmetic ingredients derived from *Camellia sinensis* (tea) plant parts function as antioxidants, and skin-conditioning agents – humectant and miscellaneous. The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed relevant animal and human data related to these ingredients. The use of the leaf ingredients in beverages results in larger oral exposures than those from cosmetic uses. Therefore, this safety assessment did not focus on systemic toxicity potential. Because formulations may contain multiple botanical ingredients, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to limit impurities that could be present in botanical ingredients. The Panel concluded that the *C. sinensis* leaf-derived ingredients are safe as used when formulated to be non-sensitizing. The available data are insufficient to determine whether the non-leaf-derived ingredients are safe for use in cosmetics.

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).[1] The 14 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 powder
- hydrolyzed camellia sinensis leaf
- hydrolyzed camellia sinensis seed extract

Camellia sinensis seed oil was included in a 2011 CIR safety assessment of plant based oils with the conclusion that it was safe in the present practices of use and concentration.[2]

Some of the *C. sinensis*-derived ingredients in this safety assessment are used to make tea. Exposures to the leaf-derived ingredients in beverages results in much larger systemic oral exposures than would result from cosmetic uses. Therefore, though oral data are included in this report, the systemic toxicity potential of the leaf-derived cosmetic ingredients is not the focus of this report. While data on the potential for reproductive toxicity, genotoxicity, and carcinogenicity are presented, the primary focus of this report is on the potential for irritation and sensitization.

Due to the nature of plant-derived ingredients, there is variation in the constituent content of these ingredients. For example, the definition of camellia sinensis catechins does not provide any limitations on which catechins or what quantities of catechins are in the ingredient. Therefore, all studies of catechins are listed under the ingredient camellia sinensis catechins.

CHEMISTRY

Definition and Description

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

CAMELLIA SINENSIS

There are 4 varieties of the *C. sinensis* plant: *sinensis*, *assamica*, *pubilimba*, and *dehungensis*. The first 2 are most commonly used to prepare tea for human consumption. The type of tea (ie, 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 the process actually involves an enzymatic oxidation.[3,4]

The *C. sinensis* plant is native to East, South and Southeast Asia.[4-6] However, it is also cultivated in other tropical and subtropical regions. The leaves of this evergreen shrub can be lanceolate to obovate, up to 30 cm long (usually 4-15 cm) and 2-5 cm broad, pubescent, and sometimes become 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. sinensis* are provided in Table 2. The constituent group having the highest concentrations is the flavanols (25.0% dry weight), which is followed by proteins (15.0%) and polysaccharides (13.0%).[4]

Constituent groups found in *C. sinensis* plant parts include:

Amino acids – The most abundant amino acid is one not typically found in proteins, theanine (5-*N*-ethylglutamine).[4,7]

Carotenoids – These are present in low levels in the leaves. They include neoxanthin, violaxanthin, lutein, chlorophylls a and b, and β-carotene.[4,8,9] Seventy-nine pigments, 41 chlorophylls and 38 other carotenoids have been detected.[10]

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

Methylxanthines - Theobromine can range from 0.16%-0.2% of a dry-weight leaf.[4,11,12] 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, flavanols, and glycosides. Flavanols include catechins, which are present in small amounts, and may occur as flavonols and glycosides.[4,13,14] Flavonols reported to be in leaf extract are kaempferol, quercetin, and myricetin.[4,15]

Catechins - These polyphenolic molecules are a subgroup belonging to the flavanol family.[4,16-18] They typically make up 20%-30% of the weight of tea leaves. Catechins are especially concentrated in the leaves of green tea wherein they account for 30%-40% of the dry weight of the leaves. 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).

Minerals elements - Potassium is the most abundant mineral element, present at 40% of the total mineral element content of dry matter of fresh leaves. The leaves are rich in fluoride and they also accumulate aluminum and manganese.[4,19,20] Other elements present in mineral form include calcium, magnesium, sodium, phosphorus, and sulfur. Minor elements include boron, barium, chromium, copper, iron, molybdenum, nickel, rubidium, strontium, and zinc.[19] Trace elements include silver, arsenic, beryllium, bismuth, cadmium, cerium, cobalt, cesium, mercury, indium, lithium, lead, rare earth elements, antimony, selenium, tin, tellurium, thallium, uranium, vanadium, ytterbium, and zirconium.

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.[4,14,21,22]

Constituents reported to be predominately in the seeds of *C. sinensis* include caffeine, glucothea saponin, stearic acid, theasponin, and theobromine.[23]

Climatic conditions during cultivation may affect the content of theanine, common proteinaceous α-amino acids (ie, isoleucine, leucine, valine, alanine, threonine, and glutamine), quinic acid, EC, EGC, EGCG and caffeine levels in *C. sinensis* leaf extract (as green tea).[24,25] Soil conditions and cultivation methods may markedly affect mineral levels.[19]

The presence of minerals and elements in an extract depends on the extent of entrapment in the organic matrix, the degree of solubility/choice of solvent, the duration of extraction, temperature, pH, and agitation.[19] Most elements, especially the metals, are complexed with the flavonols, catechols, tannins, and polyphenols.

CONSTITUENTS OF CONCERN

Linalool and several compounds containing the linalool moiety (ie, *R*-linalool, linalool-oxide-(*cis*-furanoid), linalool-oxide-(*cis*-pyranoid), linalool-oxide-(*trans*-pyranoid), linalool-β-D-glucopyranoside, and linalool-oxides) have been reported in the leaves (6-1984 ppm), leaf essential oil (31800-198 400 ppm), and shoot (600-10300 ppm) of *C. sinensis* (Table 3).[23] The International Fragrance Association (IFRA) limits peroxides in linalool to 20 mmol/L.[26]

Quercetin and several compounds containing quercetin (ie, quercetin-glucosides) have been reported in the leaf (760-10000 ppm), plant, and shoot of *C. sinensis* (Table 3).

SAMPLE ANALYSIS

Constituents in medical grade *C. sinensis* extract include methylxanthines, flavanols (10%-25%), flavonols, flavones, phenolic acids, amino acids (including theanine, 3%), terpene saponins, polysaccharides, proanthocyanidins, vitamins, and minerals (Table 4).[16,27-31]

Analyses of 3 lots of *C. sinensis* catechins (each prepared as a dietary supplement) indicated 27.6%-62.3% total catechin monomers and 30.6%-67.4% polyphenols.[32] Analysis for other components showed: caffeine (≤7%), organic acids (≤10%), protein and amino acids (≤10%), saccharide (≤12%), fiber (≤1%), fat (≤1%), and ash (≤5%).

CHARACTERIZATION

As herbal supplements, extracts are characterized by the drug/extract ratio (DER), which is the ratio of the quantity of herbal substance used in the manufacture of an herbal preparation (given as a range) to the quantity of the herbal preparation obtained in the finished product.[33] The specifications for *C. sinensis folium* as an herbal supplement in the European Union (EU) for the dry extract, purified (DER 45-56:1, extraction solvent: water) corresponds to 55%-72% (-)-epigallocatechin-3-*O*-gallate.[27,33] The decaffeinated dry extract (DER 6:1-10:1, 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.

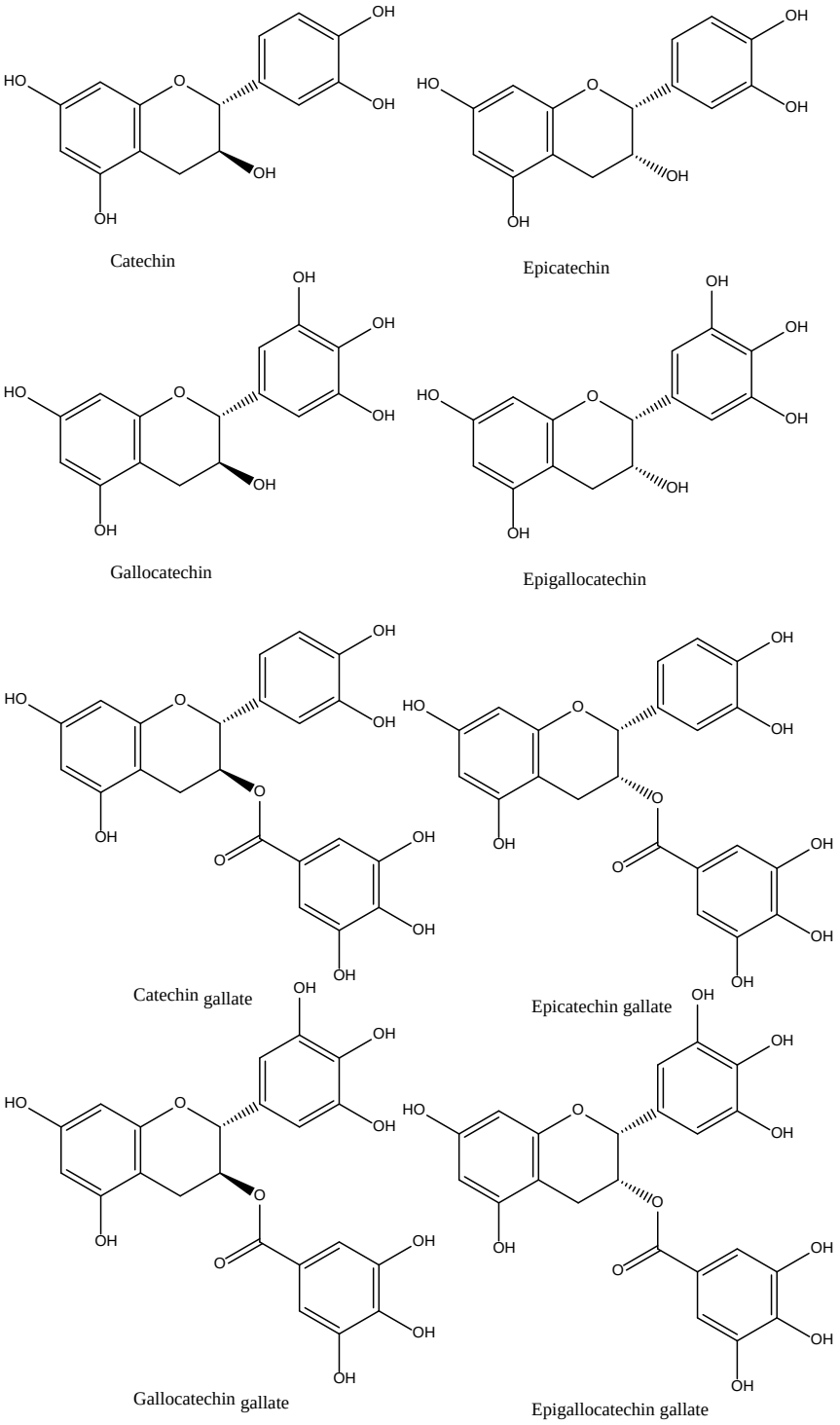

Figure 1. Catechins from *Camellia sinensis*[32]

Impurities

No published data on impurities of these cosmetic ingredients were discovered, and no unpublished data were submitted. The information below applies to impurities found in *C. sinensis* as a food or as food ingredients.

In analyses of twelve *C. sinensis* catechins lots extracted as food ingredients, arsenic (<0.2 ppm), cadmium (<0.1 ppm), lead (0.4 ppm), and tin (not more than 150 ppm) were below levels of detection.[32] Three lots of *C. sinensis* catechins were analyzed and no microbial contamination was detected.

Ten commercial *C. sinensis* teas for drinking were analyzed for metals.[34] 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 asserted that differences in content of the samples were attributable to differences in geographic region of cultivation.

Aflatoxigenic molds and aflatoxins have been reported to be present on *C. sinensis* teas for drinking.[35] In a sampling of 27 commercial black teas (7 branded, 20 nonbranded), aflatoxigenic molds were detected in 1 branded and 6 unbranded (25.9%) tea samples. Only 1 of the samples (nonbranded) had detectable aflatoxins (19.2 µg/kg). In black teas that had been spiked with aflatoxins, most of the aflatoxins residue was still present in the leaves after boiling in water; 30.6% was present in the final beverage.

It was reported that levels of 712-1530, 166-280, 1.7-7.5, and 1.51-2.63 µg/g tea of aluminum, iron, chromium, and lead, respectively, were found in commercial tea samples (n=2) using electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES; Table 5).[36] These samples came in powder or granular forms. For 2 types of green tea, the ranges were 605-620, 1486-1550, 4.5-4.7, and 2.20-2.34 µg/g, respectively. For the infusions of these branded and unbranded teas (exact extraction method not provided), the levels for aluminum, iron, copper, and zinc were 149-367, 7.6-11.0, 0.7-3.2, and 36-43 µg/g, respectively. For green tea infusions, these values were 124-127, 22-23, 0.2-0.5, and 31-32 µg/g, respectively.

Physical and Chemical Properties

C. sinensis catechins have an astringent taste and are soluble in water.[4,16]

Three lots of oolong tea with *C. sinensis* catechins were stable for 18 months in unopened packaging at -20°C.[32] After 6 months of storage at 25°C, total catechin monomers were decreased from 100% to 97% polyethylene terephthalate (PET) bottles and to and 98% in steel cans. At 37° for 2 months, catechin content was decreased to 96% in both types of containers.

A sunscreen with various amounts of *C. sinensis* (2%-5%) in the form of green tea extract was stable for up to 6 months.[37]

Method of Manufacture

The most widely used method for preparing essential oils from plants is associated with steam distillation.[1] The condensate from steam distillation produces 2 distinct fractions that contain the volatile ingredients from the plant. The water insoluble fraction contains the "oil". The water-soluble fraction contains ingredients from the plant that are water soluble. The water insoluble fraction from steam distilled plant materials is identified as "oil" in the International Nomenclature of Cosmetic Ingredients (INCI) name. The water-soluble fraction from the steam distilled plant material is identified as "water" in the INCI name.

No information on the method of manufacture for *C. sinensis*-derived cosmetic ingredients was discovered or submitted. The methods below are general to the processing of *C. sinensis* for food or food ingredients. The makeup of the *C. sinensis* extract will differ with the manufacturing process.

C. sinensis leaf in the form of green tea consists of whole or cut young, unfermented, rapidly-heat-dried leaves.[17,27] The fresh leaves are processed by a method designed to prevent the enzymatic oxidation of catechins. The enzymes are inactivated by heat (pan-frying or steaming).

There are different harvesting and manufacturing processes for white, green, black and oolong teas used for drinking.[17,19,27,38] White tea is made from very young leaves and leaf buds. Green tea is made from new, fully-formed leaves. These 2 types of tea are minimally processed, 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. Phenolic content typically differs substantially between green and black teas (Table 6).

Catechins are isolated through an initial hot water extraction with ethyl acetate, and then separation by chromatography, followed by spray-drying.[39] The spray-dried catechins 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 initial extract is further extracted with water and ethanol, and then filtered through multiple media. The product of the process without tannase is sterilized above 100°C, whereas the product obtained with the tannase treatment is sterilized below 100°C.

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP; Table 7).[40] A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for *C. sinensis*-derived ingredients.[41,42] Data were available from both the VCRP and the Council for the following ingredients:

- Camellia sinensis leaf extract was reported to be used in 1145 leave-on, 785 rinse-off, and 36 bath cosmetic products.[40] There are reported uses in every exposure type (Table 7). Usage of cosmetic ingredients called

"green tea" and "green tea extract" were also reported in the VCRP. Since these are technical names for camellia sinensis leaf extract, the VCRP numbers for these 3 listings were combined. Camellia sinensis leaf extract was reported to be used up to 2% in leave-on products (the highest concentrations in face and neck products) and up to 1% in rinse-off products (the highest concentration in bath soaps and detergents).[41] It is also reported to be used in products diluted in the bath at up to 0.1% (the highest concentration in bubble baths). It is reported to be used up to 0.14% in an ingestible oral hygiene product.

- Camellia sinensis leaf was reported to be used in 38 leave-on, 15 rinse-off, and 1 bath product.[40] Camellia sinensis leaf was reported to be used up to 0.05% in bubble baths.[41] A previously reported product of tea bags for the eyes at 97% is no longer sold.[41,43]
- Camellia sinensis leaf powder was reported to be used in 11 leave-on, 10 rinse-off products, and 1 bath product.[40] Camellia sinensis leaf powder was reported to be used in body and hand products up to 7% and up to 0.01% in rinse-off products (highest concentration in bath soaps and detergents).[41] It is also reported to be used in a professional face and neck product at 50% that is diluted before use.
- Camellia sinensis leaf water was reported to be used in 26 leave-on and 10 rinse-off products.[44] This ingredient was reported to be used up to 30% in mascara.[41]

Data were available only on the frequency of use (VCRP) for camellia sinensis leaf oil. It was reported to be used in 22 leave-on products and 11 rinse-off products, including 1 baby product and 1 lipstick.[40] No concentration of use data were reported by industry.

Data were available only on concentration of use for camellia sinensis seed extract. It was reported to be used in leave-on products up to 0.1% (highest concentration in moisturizing creams and lotions) and in rinse-off products up to 0.0013% (highest concentration in bath soaps and detergents).[41] There were no uses were reported in the VCRP.

There were no frequency of use or concentration of use data reported for:

- Camellia sinensis catechins
- Camellia sinensis flower extract
- Camellia sinensis flower/leaf/stem juice
- Camellia sinensis root extract
- Camellia sinensis seedcoat powder
- Camellia sinensis seed powder
- Hydrolyzed camellia sinensis leaf
- Hydrolyzed camellia sinensis seed extract

Camellia sinensis leaf extract was reported to be used in pump and aerosol sprays. Camellia sinensis leaf extract is reported to be used up to 0.0005% in pump hair sprays, up to 0.0055% in pump deodorant sprays, up to 0.0005% in body and hand sprays, up to 0.22% in foot spray, and up to 0.07% in pump suntan product. In practice, 95%-99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump sprays.[45-48] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[45,47] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[45] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Non-Cosmetic

The essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of tea, under the previous name *Thea sinensis*, are 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.[27] Preparations are also used in cutaneous treatment of external genital and perianal warts (*condylomata acuminata*) in immune-compromised patients.[27,49]

In the United States, green tea products are used as dietary supplements, primarily for purported weight loss and antioxidant properties.[17,18,50-59] 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 antibacterial, antiviral, antifungal, and neuroprotective effects.

Annual tea consumption varies from country to country, ranging from negligible to approximately 3 kg per person.[60] Worldwide average consumption is approximately 0.5 kg per person.

In 2012, over 79 billion servings of tea were consumed in the United States (over 3.60 billion gallons).[61] Of this, Americans consumed approximately 84% black tea, 15% green tea, and the rest oolong and white tea.

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

CAMELLIA SINENSIS CATECHINS

When camellia sinensis leaf extract (as green tea) was administered to full thickness pig ear skin using a Franz cell, there was a dose-dependent transdermal penetration of the catechins EGCG, EGC, and EC (Table 8).[62]

Saturated solutions of camellia sinensis were formulated using water, polyethylene glycol 400, citrate/phosphate buffer (pH 5.5), and a 50:50 mixture of polyethylene glycol 400 and the buffer. The solutions were applied to drug-in-adhesive transdermal patches under occlusion in methanol and applied to the pig skin. The receptor cell was sampled periodically for 48 h. Penetration by the catechins was fastest in the buffer solution and slowest in polyethylene glycol 400 solution.[62]

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, plasma, liver, small intestines, and colon for at least 24 h.[63] The test material was administered once. Over the next 24 h, blood was collected under anesthesia and 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 maximum plasma concentration (C_{max}) at 6 h was 44.5 ± 8.4 ng/mL, the half-life ($t_{1/2}$) was 94.4 ± 13.2 h, and the area under the concentration-time curve ($AUC_{0\rightarrow24}$) was 881.5 ± 123.4 ng/mL/h. The C_{max} at 1 h 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 at 2 h 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 at 2 h 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 at 1 h 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}, $t_{1/2}$ (no time provided), $AUC_{0\rightarrow24}$ for EGCG in the urine were 177 ng/mL, 70.0 h, and 3427.9 ng/mL, respectively.[63]

TOXICOLOGICAL STUDIES

The *C. sinensis*-derived ingredients in this safety assessment are used to make tea and exposure to these ingredients in beverages would result in much greater oral doses than those from oral exposures from the use of cosmetic products. Consequently, their oral toxicity potential is not addressed in this report. Though data are presented on the potential for reproductive toxicity, genotoxicity, and carcinogenicity, the focus in this report is primarily on the potential for irritation and sensitization.

Acute Toxicity

Oral – Non-Human

CAMELLIA SINENSIS LEAF EXTRACT

When camellia sinensis leaf extract (2 g/kg; 1.94 mL/kg) was administered by gavage to Sprague-Dawley (SPF) rats (n=5/sex), the minimum lethal dose was >2 g/kg.[64] The test substance was administered after 16 h on a hydric diet. After administration, the rats were observed for 6 h for clinical signs and then followed for 14 days. There were no effects on weight gains and there were no mortalities. Necropsy was unremarkable. The test substance was a cold extract of green tea using water/propylene glycol (10% dry plant material) and prepared in the same manner as that used to prepare tea for drinking.

The above experiment was repeated with an extract of black tea (2 g) provided to the laboratory as a brown powder with the same conclusion.[65] Decreased motility and ptosis of the eyelids was observed in all rats 1 h after administration. Necropsies were unremarkable.

The oral LD_{50} of a Chinese tea extract (0.85% solids) and a oolong tea extract (1.0% solids) was >2 g/kg for mice, and the oral LD_{50} of a green tea extract (1.6% solids) was >2 L/kg for rats.[66]

Dermal – Non-Human

CAMELLIA SINENSIS CATECHINS

The dermal LD_{50} of EGCG (2000 mg/kg extract; 1860 mg EGCG/kg; 4 mL/kg) was >1860 mg/kg for HanBrl:WIST (SPF) rats (n=5/sex).[39] The acute dermal toxicity test 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 covering the test site 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 signs of systemic toxicity in any of the rats. Slight to moderate erythema was observed in all treated rats after removal of the dressing, which persisted for up to 5 days. No abnormal macroscopic findings were observed at necropsy.[39]

Repeated Dose Toxicity

Inhalation - Human

CAMELLIA SINENSIS CATECHINS

There were no adverse effects observed when subjects (n=36) suffering from cerebrovascular diseases inhaled catechins (3.7 mg/mL in saline; 2 mL; 43% of catechins composed of EGCG) or the vehicle (n=33).[67] The test substance was delivered by a handheld nebulizer 3 times/day for 7 days. The sputum of the subjects all tested positive for methicillin-resistant *Staphylococcus aureus* (MRSA). Total catechin content was 73.0% (31% (-)-EGCG, 21% (-)-EGC, 8.6% (-)-EC, 8.6% (-)-ECG, 2.9% (-)-GCG, and 0.8% (-)-CG).

No adverse effects were observed when tea catechins extract (10 or 20 mg/mL in saline; 2 mL; ≥30% tea polyphenol and ≥10% EGCG; assumed to be *C. sinensis*) was inhaled using a nebulizer by subjects (n=26) being treated for MRSA 3 times per day for 79 days.[68]

No adverse effects were observed when tea catechins extract (10 or 20 mg/mL in saline; 2 mL; ≥38% tea polyphenol and ≥14% EGCG; assumed to be *C. sinensis*) was inhaled using a nebulizer by subjects (n=26) being treated for MRSA 3 times per day for 79 days.[69]

Cytotoxicity and Cellular Effects

CAMELLIA SINENSIS EXTRACT

Camellia sinensis extract (10, 50, 100 μg/mL) was not cytotoxic to rat pheochromocytoma (PC12) cells when exposed in vitro for 24 h.[38] However, at higher concentrations (250, 500, 100 μg/mL), the extract was cytotoxic with <40% viability at the 2 highest concentrations. When the cells were incubated with the extract and hydrogen peroxide (250 μM), hydrogen peroxide poisoning was mitigated by the extract at 50, 100, and 250 μM.

Camellia sinensis water extract (as Korean green tea) had a 50% inhibitory dose (ID_{50}) of 2.05% (0.28 mg/mL dry matter) in the inhibition of protein synthesis in Sprague-Dawley rat hepatic cells.[70] The 25% effective dose (ED_{25}) for 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) for protein synthesis and an ED_{25} for LDH release of 5.11% (0.94 mg/mL).

CAMELLIA SINENSIS LEAF EXTRACT

In a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) test (n=6), camellia sinensis leaf extract (0, 0.00013%, 0.0006%, 0.0032%, 0.016%, 0.08%, 0.4%, 2%, and 10%) was cytotoxic at 2% and 10% to human keratinocytes.[71] Morphological modifications of the cells were observed at 0.4%. The test substance was a cold extract of green tea using water/propylene glycol (10% dry plant material).

When human keratinocytes were incubated in camellia sinensis leaf extract (0.05% and 0.1%) there was a 43% protection against oxidation when the cells were exposed to UV radiation (312 nm; 160 mJ/cm^2) for 45 min.[71]

EGCG induced apoptosis at 400 and 800 μM in neonatal human dermal fibroblasts.[72] At 200 μM 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 of the expression of pNF-κB was concentration dependent but EGCG did not affect NF-κB expression. cDNA microarray analysis revealed that EGCG (200 μM) down-regulated cell cycle-related genes. A/B cyclins and cyclin-dependent kinase 1 was reversibly effected by EGCG (200 μM).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

CAMELLIA SINENSIS LEAF EXTRACT

There were no adverse effects when pregnant Wistar rats (n=6) were administered camellia sinensis leaf extract (0, 84, 167, 501, and 1336 mg/mL/d; in the form of black tea) by gastric intubation.[73] 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 on gestation day 10 (early dose groups) or 15 (mid dose groups). Pups were examined daily until the appearance of hair and the opening of the eyelids. 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 duration, 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 developmental abnormalities observed.

CAMELLIA SINENSIS CATECHINS

Unpublished studies were submitted to the FDA for the approval of a topical ointment as a drug that contains up to 15% camellia sinensis catechins to treat warts (Table 9).[74] In oral studies, there were increased resorptions at 1000 mg/kg/d ointment when administered on gestation days 6-15 in rats and decreased maternal body weights when administered on gestation days 6-18. Rabbit dams had decreased body weights when 1000 mg/kg ointment was orally administered. In

subcutaneous studies using rabbits, 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 4 mg/kg/d. Intravaginal administrations up to 0.15 mL/d yielded no adverse effects in rats.

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.[75] 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 no treatment-related macroscopic findings in the dams. There was no effect to embryo/fetal survival, fetal weights, or sex ratios.

In a 2-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 decreased growth rates, and there was an increase in pup loss. Reduced growth rates were observed among pups at 3600 ppm, but only in the second generation. Both sexes of the F_1 generation in the high-dose group showed decreased absolute kidney and liver weights. The F_1 males had decreased spleen and prostate weights, but the females' spleens were normal. Histological examination revealed no abnormalities. The lowest dose was considered the overall no observed adverse effects level (NOAEL). The authors derived a NOAEL of 200 mg/kg body weight per day EGCG preparation. Because dams consumed twice the amount of feed during the crucial lactation period, during which effects occurred, twice the lowest dose (ie, 2 x 100 mg/kg/d) was estimated to be the NOAEL.[75]

GENOTOXICITY

CAMELLIA SINENSIS CATECHINS

Camellia sinensis catechins were not genotoxic in multiple in vitro and in vivo assays including Ames tests (up to 5000 µg/plate), mouse micronucleus assays (up to 12,600 mg/kg), and other micronucleus assays in rats (up to 8500 mg/kg; Table 10). A polyphenol mixture was lethal at 2000 mg/kg/d to mice. In mouse lymphoma assays at concentrations >100 µg/mL, 3 assays of catechin mixtures were negative; an assay of EGCG was mutagenic at concentrations ≥125 µg/mL with metabolic activation.[74,76,77]

CAMELLIA SINENSIS FLOWER EXTRACT

Camellia sinensis flower extract was not genotoxic in an Ames test up to 5.0 mg/plate, with or without metabolic activation.[78]

CAMELLIA SINENSIS LEAF EXTRACT

Camellia sinensis leaf extract was not genotoxic in 2 Ames tests up to 5000 µg/plate.[66]

CARCINOGENICITY

In 1997, the International Agency of Research on Cancer (IARC) listed green tea in group 3, meaning that it is not classifiable according to its carcinogenicity to humans.[60]

The National Toxicology Program (NTP) has completed a 2-year bioassay in rats and mice of *C. sinensis* as green tea extract with the draft conclusion of no evidence of carcinogenic activity of green tea extract in male and female Wistar Han rats and in male B6C3F1/N mice and recommend the conclusion of no evidence of carcinogenic activity of green tea extract in female B6C3F1/N mice, because the NTP panel disagreed with the NTP conclusion that occurrences of squamous cell neoplasms (squamous cell papilloma or squamous cell carcinoma) of the tongue may have been related to treatment.[79,80] The NTP report has not been finalized at the time of this safety assessment.

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).[74] The mice were treated by gavage daily for 26 weeks. *p*-Cresidine and water served as controls.

The catechins in *C. sinensis*, especially EGCG, have been shown to have preventive and treatment effects in cancer cell lines related to cancers of the prostate, lung, skin, pancreas, breast, and ovaries.[18] There are several reviews regarding the protective effects of green tea extracts and its catechins, especially EGCG, against chemical carcinogens.[52,81-84] According to Yang *et al.*[82], there are more than 133 studies published from 1991 to 2008 on the effectiveness of *C. sinensis* on cancers (Table 11). Inhibitory effects of tea and/or tea constituents on lung, oral, stomach, intestine, dermal, prostate, breast, liver, bladder, pancreas, and thyroid cancers were found.

In a population-based case-control study of residents of southern Arizona (n=238 males, 166 females; mean age 66.6 ± 10), subjects who consumed black tea within the last year had fewer instances of squamous cell carcinoma (SCC) of the skin (odds ratio 0.60) than a control group consisting of residents of Tucson, (n=226 males, 165 females; average age 66.2 ± 11.1 y).[85] Arizona was chosen because it has one of the highest risks of skin SCC worldwide. Variables were controlled for tanning ability, antioxidant intake, education, gender, smoking, and average sun exposure.

When female SKH-l mice (n=28 or 29) were orally administered *C. sinensis* as lyophilized green tea (0.3%, 0.9%; 3,

9 mg of tea solids/mL) in place of drinking water and exposed to UVB (30 mJ/cm^2 for 25-30 s) twice per week for 35 weeks, there was a decrease in the number of tumors per mouse by 35% and 94%, respectively, compared to controls exposed to UVB without *C. sinensis* treatment.[86] The tumor volume per mouse was decreased by 49% and 97%, respectively. The composition of the green tea polyphenol fraction was: EGCG (49.5%), EGC (11.5%), ECG (11.4%), caffeine (7.6%), EC (6.1%), C (0.5%), and gallic acid (0.4%).

When female SKH-1 mice (n=29) were orally administered *C. sinensis* (as green tea for their drinking water; 1.25 g steeped in 100 mL hot water; approximately 4 mg tea solids/mL) UVB-induced complete carcinogenesis was inhibited. This was not the case with decaffeinated green tea. The *C. sinensis* extract was administered for 2 weeks before and concurrently with twice per week treatment with enhanced UVB (280-320 nm; 75%-80% total energy; 30 mJ/cm^2 for 25-30 s) exposure. There were increases in apoptosis in the epidermis observed, but no effect in non-UVB-treated normal epidermis. The authors concluded that administration of green tea and caffeine may inhibit UVB-induced carcinogenesis, at least in part, by enhancing UVB-induced apoptosis.

Oral administration of *C. sinensis* (1.25% as green or black tea leaf extract; 1.25 g of tea leaf steeped in100 mL water; 4.0 or 4.4 mg tea solids/mL) as the drinking water to the UVB-treated mice decreased the number of tumors per mouse by 51% and 41%, respectively. Tumor volume/mouse was decreased by 79% and 70%, respectively. The mice were treated with gradually increasing doses of the test substances for 2 weeks before the start of the twice/week treatment with UVB for 40 weeks. The mice were killed 4 weeks after the end of the UVB administration. Decaffeinated green or black *C. sinensis* leaf extracts (1.25%) containing 3.6 or 3.9 mg of tea solids/mL, respectively, were less effective than regular green or black tea extracts, and decaffeinated black tea was less effective than decaffeinated green tea at inhibiting the formation of skin tumors. Adding 0.36 mg of caffeine/mL to the decaffeinated extracts either fully or partially restored the inhibitory effects on UVB-induced tumorigenesis.[86]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

CAMELLIA SINENSIS LEAF EXTRACT

When camellia sinensis leaf extract (100%; 0.5 mL) was dermally administered to the clipped skin of albino New Zealand rabbits (n=3), there were no signs of irritation.[87] The test substance was a cold extract of green tea using water/propylene glycol (10% dry plant material). It was provided to the laboratory as a brown liquid. The test substance was applied to a 2.5-cm^2 gauze pad, which was then kept in contact with the skin for 24 h using hypoallergenic adhesive tape. The test site was examined within 1 h of removal and at 24 and 72 h after removal.

The above experiment was repeated as stated with an extract of black tea (0.5 g), provided to the laboratory as a brown powder, with a conclusion that the test substance was a slight irritant.[88] A slight to definite erythema was observed on all treated rabbits. Cutaneous dryness and a slight decrease in skin suppleness were observed. The test sites were observed at 1, 25, and 72 h after removing the pad.

Chinese tea extract (10%, 100%; 0.85% solids), green tea extract (100%; 1.6% solids), and oolong tea extract (10%, 100%; 1.0% solids) were not irritating to rabbit skin.[66] No further details were provided.

CAMELLIA SINENSIS CATECHINS

There were no signs of irritation when EGCG was administered to the clipped flanks of male New Zealand White rabbits (n=3) for 4 h under semi-occluded patch.[39] The tests were conducted according to the EC Commission Directive 92/69/EEC, B.4, ‘‘Acute Toxicity—Skin Irritation’’ and OECD guideline number 404 (1992). The dorsal fur of the rabbits was removed with electric clippers 24 h before 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 1 flank using a semi-occlusive patch. After removing the patch, the skin was cleaned with water. Skin reactions and irritation effects were assessed at approximately 1, 24, 48 and 72 h after patch removal. Adjacent areas of untreated skin from each animal served as controls.

In a preliminary study for a guinea pig maximization test, an intradermal injection of 0.09% EGCG was found to be the greatest tolerable dose.[39] 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

When *C. sinensis* preparations (DER ranging from 0.1%->10%) were used in dermal applications of ointments (compositions not provided) to treat genital warts, the following were among the adverse effects: erythema, pruritus, irritation/burning, pain, ulcer, edema, induration, and vesicles.[27] A full list of reported effects is provided in Table 12.

CAMELLIA SINENSIS LEAF EXTRACT

A black tea extract (100%; 1.0% solids) was negative in a patch test (n=100). No further details were provided.[66]

CAMELLIA SINENSIS CATECHINS

In a trial (n=502) of an ointment containing camellia sinensis catechins (10% and 15%) for the treatment of anogenital warts, there was no irritation or other adverse effects reported.[89] The ointment was administered 3 times per day for up to 16 weeks. Observations were made during treatment and during the 12-week follow-up period; and the ointment was reported to be well tolerated.

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 removing the patch.[90] The test substance was administered to the inner side of the upper arm for 24 h using a "pin" chamber.

Mucosal – Non-Human

CAMELLIA SINENSIS CATECHINS

Intravaginal administration of an ointment containing camellia sinensis catechins (15%) to pregnant SD rats (n=25) from gestation day 6 to the end of lactation caused ulceration and erosion of the vaginal mucosa with inflammation for the duration of treatment.[74] The control group (no catechins) did not exhibit damage to the vaginal mucosa. The effects resolved when treatment stopped.

Ocular

CAMELLIA SINENSIS LEAF EXTRACT

Camellia sinensis leaf extract (100%; 0.1 mL) administered to the lower conjunctival sac of the right eye of albino New Zealand rabbits (n=3) was a slight ocular irritant.[91] There was slight irritation of the conjunctiva at 1 h; there were no iris lesions. Two rabbits had a very slight superficial epithelial attack of the cornea. All signs of irritation were resolved within 24 h. The test substance was a cold extract of green tea using water/propylene glycol (10% dry plant material). It was provided to the laboratory as a brown liquid. The eyes were examined 1 h after instillation and 1, 2, and 3 days later.

The above experiment was repeated with an extract of black tea (0.1 g), provided to the laboratory as a brown powder, with the same conclusion.[92] There was a slight irritation of the conjunctiva observed at 1h; there were no lesions of the iris. All rabbits had a slight epithelial attack of the cornea. All signs of irritation were resolved within 48 h.

Green tea extract (100% ;1.6% solids) was not irritating to the eyes of rabbits. No further information was provided.

CAMELLIA SINENSIS CATECHINS

The administration of EGCG preparation (0.093 g EGCG; 0.1 g total) 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.[39] 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. Because EGCG was suspected to be an ocular irritant, a single animal was treated first and observed to recovery. Based on the results from this preliminary study, no additional rabbits were tested.

Sensitization

Dermal – Non-Human

CAMELLIA SINENSIS LEAF EXTRACT

Oolong tea extract (1.0% solids) was not sensitizing to guinea pigs (n not provided).[66] First induction was at 50%; second induction was at 25%. Challenge was at 5% and 10%. No further details were provided.

IFRA reported that in a local lymph node assay (LLNA) reported an EC_3 of >1250 µg/cm^2 for camellia sinensis leaf extract (as tea leaf absolute).[93] Irritation was observed at higher concentrations (not provided) so the actual EC_3 could not be calculated.

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 at challenge (1%, 3%, 5%, and 10%) as well as at a second challenge (0.1%, 0.5%, 1%, 3%, 5% and 10%) 2 weeks later.[39] The skin sensitization assay was performed using a procedure adopted from OECD guideline number 406. During the induction phase of the 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 cm^2 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 after the 13th and 16th administrations, respectively. In the 10% group, a slight erythema was observed in 2 of 6 guinea pigs after the 13th application, with all guinea pigs showing similar signs by the 16th application. For the 5% group, erythema was observed only for 3 days in 1 of 6 guinea pigs. Both EGCG preparations elicited positive effects in the test groups during the challenges.

Control animals showed no response after the first challenge; 1 or 2 of the 6 control guinea pigs had 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 induction doses. There were a greater number of reactions in the 5% induction group (6 at 24 h, 5 at 48 h) than 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 of the test animals were in the same range as those of the controls during the study period.[39]

In a 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.[39] All guinea pigs had grade 3 or 4 erythema following challenge by dermal administration of camellia sinensis catechins (50% in distilled water; 0.15 mL). Grade 1 erythema was observed following the first test challenge in 3 of 10 in the test group and 0 of 5 in the control group. In a second challenge 1 week later, 9 of 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 of the test group were in the same range as those of the controls during the study period.

Dermal – Human

CAMELLIA SINENSIS LEAF EXTRACT

A facial line filler treatment product (150 µL) containing camellia sinensis leaf extract (0.86%; black tea) was not irritating or sensitizing in a human repeated insult patch test (HRIPT).[94,95] The test substance was administered 9 times on a 2 x 2 cm absorbent pad under occlusion. No reactions were observed in any of the 106 to complete the induction phase. No reactions were observed in any of the 101 that completed the challenge phase.

An eye cream containing camellia sinensis leaf extract (0.86%; black tea) was not irritating or sensitizing in an HRIPT (n=638). The test substance was administered under occlusion.[95,96]

A black tea extract (100%; 1.0% solids) was negative in a HRIPT (n=100). No further details were provided.[66]

Camellia sinensis leaf extract (as tea leaf absolute) was reported to have a no observed effect level (NOEL) of 480 µg/cm^2 in an HRIPT.[93]

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.[97]

Phototoxicity

CAMELLIA SINENSIS LEAF EXTRACT

There were no signs of erythema on treated sites on the forearms of subjects (n=6) treated with camellia sinensis leaf extracts (10%; in the form of green or black tea) and exposed to UVA, B, and C.[98] Freeze-dried green and black tea extracts were used to make 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/UVC (UVA=4550 µW/cm^2; UVB=2800 µW/cm^2; UVC=500 µW/cm^2) for 2.5 min. Erythema was observed in the control and carbomer treated sites but not the treatment sites.

Photo Effects

CAMELLIA SINENSIS LEAF EXTRACT

A sunscreen containing various concentrations of camellia sinensis leaf extract (0, 2%, 3%, 4%, 5%; in the form of green tea) protected against photoaging and photoimmunology-related biological measurements in female human subjects (n=20); especially at 3%.[37] The melanoma index decreased in a dose-dependent manner up to 3%; effectiveness decreased at 4% and 5%. The same pattern was observed for the thickness of the stratum corneum and total epidermis measurements. Cytokeratins CK5/6, CK16 were overexpressed on the site irradiated with or without the base cream; the decreased effect followed the same pattern as the other markers. Matrix metalloproteinases MMP-2 and MMP-9 were slightly to moderately expressed on unirradiated skin. Expression of MMP-2 and MMP-9 was decreased on the 2%, 3%, and 4% sites.

The sunscreen was applied 30 min before each irradiation (290–400nm) at 1.5 x each individual's minimal erythemal dose (MED) and 6, 24, and 48 h after the last irradiation. The subjects' backs were irradiated on 4 consecutive days (duration of treatment was not provided). The MED of the subjects ranged from 25 to 40 mJ/cm^2, with an average of 32.46 mJ/cm^2. Punch biopsies were obtained from all the 7 sites 72 h after the last UVR exposure and analyzed. Standardized photographs were taken with a digital camera before each procedure and at the follow-up examinations.[37]

CAMELLIA SINENSIS CATECHINS

Topical treatment with green tea polyphenols (3 mg/2.5 cm^2 in acetone) on human skin decreased the UVB induction of cyclobutane pyrimidine dimer formation and erythema in a dose-dependent manner.[99] The polyphenols consisted of 6% EC, 5% EGC, 65% EGCG, and 24% ECG. 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 MED. The test sites were examined and skin punch biopsies taken 24 h after UVB treatment. Cyclobutane pyrimidine dimers and erythema were decreased in the treated sites exposed to 1.0%, 2.0, and 4.0% of a MED of UVB in a dose-dependent manner.

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.[100] This indicated possible protection of the cells by EGCG from oxidative stress from UVA exposure. 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; duration not provided) 6 h later. Supernatant was collected 24 h after irradiation and analyzed.

The dermal administration of either EGCG (1 mg/cm^2 in a hydrophilic ointment ; >98% pure) or green tea catechins (0.2% in a hydrophilic ointment ; > 86% catechins) to female SKH-1 hairless mice (n=not provided) prevented single and multiple UV (180 mJ/cm^2) exposure-induced depletion of catalase activity and prevented the depletion of antioxidant enzymes (eg, glutathione peroxidase, catalase, and glutathione).[101] Treatment also inhibited UVB-induced oxidative stress when measured in terms of lipid peroxidation and protein oxidation. The test substances were administered to the backs of the mice either once or daily for 10 consecutive days prior to UVB (290-320 nm) and UVA exposure. The mice were killed 24 h after the last UV exposure and the skin was biopsied. The green tea catechins were composed of: EC, 10.4%; EGC, 8.3%; EGCG, 55.8%; GCG, 4.4%; and ECG, 6.9%.

Female SKH-1 hairless mice were administered green tea catechins (0.2% in drinking water) for 10 days before and during UV exposure as described above. Treatment with green tea catechins prevented single or multiple UVB irradiation-induced depletion of antioxidant enzymes, oxidative stress, and phosphorylation of proteins. However, the photoprotective efficacy was less than that of topical treatments of EGCG and green tea catechins. The authors stated that this may be due to less bioavailability in skin target cells.[101]

Green tea catechins at 70 and 140 mg/L were reported to protect human retinal pigment epithelial (RPE) cells, in vitro, from the cytotoxic effects of UVB radiation.[102] The protective effect observed at these concentrations was suggested to be the result of the attenuation of the UVB-induced suppression of survivin gene expression and resultant suppression of mitochondrion-mediated apoptosis. However, 700 and 1400 mg/L appeared to have a toxic rather than protective effect on the UVB-irradiated cells. RPE cells were treated with green tea catechins for 2 h before or after exposure to UVB (100 μw/cm2) for 2 h. Viability of UVB-irradiated RPE cells decreased by 49.2% compared with unirradiated controls. The protective effects of catechin pretreatment were more effective than post-treatment. Viability of RPE cells was assessed by 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Survivin gene expression was examined by real-time PCR analysis. Ultrastructure of RPE cells was examined by transmission electron microscopy. The composition of the catechins was: GC, 44.38; EGC, 85.47; C 14.09; EGCG, 344.73; GCG, 42.49; ECG 103.37; CG 8.80 mg/g.

CLINICAL USE

Case Studies

A 51-year-old man was diagnosed with hypersensitivity pneumonitis (HP) after undergoing catechin inhalation therapy for 1 month.[103] The diagnosis was based on the clinical course, bronchoscopy, and a challenge test. The subject was being treated for tuberculosis and had been administered the catechin inhalation therapy when MRSA was observed in his sputum. He was administered catechin-rich green tea extract solution (2 mL) dissolved in distilled water (50 mg/mL) once or twice daily using a handheld nebulizer. There were no initial symptoms, but the subject later noticed that he coughed frequently during and after inhalation of the extract.

MISCELLANEOUS STUDIES

Antimicrobial Activity

CAMELLIA SINENSIS LEAF EXTRACT

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.[104] The extract was active 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 bacterial 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 intraperitoneally administered to a Swiss strain of white mice (n=20; control=60), the mice were protected from a challenge of a median lethal dose injection of *S. typhimurium*.[104] In the control group (no *C. sinensis*), 48 mice died. In the low-dose group, 4 mice died and no mice died in the high-dose group.

Dermal Effects

CAMELLIA SINENSIS LEAF EXTRACT

There was decreased healing time and no adverse effects in burned rabbits (n=5) administered an aqueous camellia sinensis leaf extract (as green tea; 100%; 0.05 mL) compared to controls.[105] 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 decreased 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 5 weeks, the wounds were almost healed in the treated groups (0.25 ± 0.02 cm^2) while the average size of the wound in the control group was 0.92 ± 0.15 cm^2. Hair growth also began sooner in both of the 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.

Anti-Inflammatory Effects

CAMELLIA SINENSIS EXTRACT

When saponins (0, 50, 100, 200 mg/kg) extracted from *C. sinensis* leaf were orally administered to rats prior to a subcutaneous injection of carrageenan (1%; 0.5 mL) in a rat hind-paw edema assay, edema in response to carrageenan was mitigated in a dose-dependent manner.[106]

OTHER ASSESSMENTS

An IFRA standard for tea leaf absolute (aka camellia sinensis leaf extract; CAS no. 84650-60-2) had the following restrictions for use: lip products, 0.01%; deodorants/antiperspirants, 0.02%; hydroalcoholics for shaved skin, 0.07%; hydroalcoholics for unshaved skin, 0.2%; hand cream, 0.1%; mouthwash, 0.3%; intimate wipes, 0.04%; hair styling aids, 0.5%; and rinse-off hair conditioners, 2.4%.[93] Based on animal data and using the classification system defined by European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)[107], green tea absolute was determined to be a moderate sensitizer. These limits were derived from the application of the exposure-based quantitative risk assessment (QRA) approach for fragrance ingredients; a dose of 480 $\mu g/cm^2$ was the weight of evidence (WoE) no expected sensitization level (NESIL) used to develop the IFRA standard based on a QRA for sensitization.

In a safety assessment of *C. sinensis* as green tea used in dietary supplement products, the U.S. Pharmacopeia Dietary Supplement Information Expert Committee concluded that when supplements containing concentrated green tea extracts are used and formulated appropriately, there are no significant safety issues with the caveat that a caution statement be included in the labeling section.[108] The caution statement warns of the potential of liver damage when concentrated green tea supplements are consumed on an empty stomach. This does not apply to *C. sinensis* as a beverage.

SUMMARY

This is a safety assessment of *Camellia sinensis* (tea)-derived cosmetic ingredients. These ingredients function mostly as antioxidants and skin-conditioning agents – miscellaneous. Though oral data are included in this report, because tea leaves are ingested in food and drink, the systemic toxicity potential of the leaf-derived cosmetic ingredients is not the focus of this report. The primary focus of this report is on the potential for irritation and sensitization.

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

Linalool and several compounds containing the linalool moiety have been reported in the leaves ranging from 6 to 1984 ppm and in the leaf essential oil ranging from 31800 to 198 400 ppm in *C. sinensis* plants. Oxidation products of linalool are dermal sensitizers. Quercetin and several compounds containing quercetin have been reported in the leaf, plant, and shoot ranging from 760 to 10000 ppm. A positive genotoxic effect in an Ames assay has been reported, and genotoxicity was observed in in-vitro tests and in some in-vivo studies of ip exposures, but results were consistently nongenotoxic in oral exposure studies using mice and rats.

Camellia sinensis leaf extract was reported to be used in 1145 leave-on, 785 rinse-off, and 36 bath cosmetic products; it was used up to 2% in leave-on products, 1% in rinse-off products, and up to 0.1% in bath products. Camellia sinensis leaf was reported to be used in 38 leave-on, 15 rinse-off, and 1 bath product; it was used up 0.05% in bubble baths. Camellia sinensis leaf powder was reported to be used in 11 leave-on and 10 rinse-off products; it was used up to 7% in leave-on products, up to 50% in a professional product that is diluted before use, and up to 0.01% in rinse-off products. Camellia sinensis leaf water was reported to be used in 26 leave-on and 10 rinse-off products; it was used up to 30% in mascara. Camellia sinensis leaf oil was reported to be used in 22 leave-on products and 11 rinse-off products. Camellia sinensis seed extract was reported to be used in leave-on products up to 0.1% and in rinse-off products up to 0.0013%. There are no reported uses or concentrations of use for the rest of the ingredients.

The FDA considers *C. sinensis* to be GRAS for use as a food additive.

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

The oral LD_{50} for rats was >2 g/kg for camellia sinensis leaf extract as both green and black tea. The dermal LD_{50}

of EGCG was >1860 mg/kg for rats. There was slight to moderate erythema observed.

There were no adverse effects when up to 20 mg/mL tea catechins (assumed to be *C. sinensis*) were inhaled for up to 79 days by human subjects.

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

There were multiple studies of an ointment that contained up to 15% camellia sinensis catechins. In oral studies, there were increased resorptions at 1000 mg/kg/d ointment when administered on gestation days 6-15 in rats or decreased maternal body weights when administered on gestation days 6-18. Rabbit dams had decreased body weights when 1000 mg/kg ointment was orally administered. 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 4 mg/kg/d. Intravaginal administrations up to 0.15 mL/d yielded no adverse effects in rats.

Camellia sinensis extract had no adverse effects when orally administered to pregnant rats up to 1336 mg/mL/d in drinking water. There were no adverse effects observed when camellia sinensis catechins, up to 14000 ppm in feed, were administered to rats on gestation days 6–20.

In a two-generation study, camellia sinensis catechins up to 3600 ppm in feed caused no clinical signs and no effects to embryo/fetal survival, fetal weights, or sex ratios. The offspring of the 12000 ppm group had decreased growth rates, and there was an increase in pup loss. While there were some decreased organ weights, histological examination revealed no abnormalities. The NOAEL was 200 mg/kg/d EGCG.

Catechins were not genotoxic in multiple in vitro and in vivo assays including Ames tests up to 5000 µg/plate, mouse micronucleus assays up to 12,600 mg/kg, and other micronucleus assays in rats up to 8500 mg/kg. A polyphenol mixture was lethal at 2000 mg/kg/d to mice. In mouse lymphoma assays at concentrations >100 µg/mL, 3 assays of catechin mixtures were negative; an assay of EGCG was mutagenic at concentrations ≥125 µg/mL with metabolic activation.

Camellia sinensis extract at 500 mg/kg/d was not carcinogenic to p53 mice after 26 weeks.

The catechins in *C. sinensis*, especially EGCG, have been shown to have preventive and treatment effects in cancer cell lines related to cancers of the prostate, lung, skin, pancreas, breast, and ovaries.

Camellia sinensis leaf extracts, that contained 10% dry green or black tea, were not dermally irritating to rabbits. Camellia sinensis catechins were not irritating to rabbits with intact skin with a content of 93.4% EGCG.

In a 16-week trial of an ointment containing camellia sinensis catechins at 10% and 15% for the treatment of anogenital warts, there was no irritation or other adverse effects reported.

C. sinensis preparations with >10% plant material in ointments for dermal treatment of genital and perianal warts caused erythema, pruritus, irritation/burning, pain, ulcer, edema, induration, and vesicles in human trials.

There were no adverse effects in a human patch test of mascara containing camellia sinensis leaf water at 30%.

The intravaginal administration of an ointment containing camellia sinensis catechins at 15% caused ulceration and erosion of the vaginal mucosa with inflammation for 4 weeks in rats.

Camellia sinensis leaf extracts from green or black tea were slight ocular irritants. 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 was a sensitizer at 0.1%.

Camellia sinensis leaf extract was not irritating or sensitizing in 2 HIRPTs conducted on 2 cosmetic products that contain this ingredient at 0.86%. A black tea extract was negative in a HRIPT at 100% (1% solids). In an HRIPT of a mascara product containing camellia sinensis leaf water at 30%, there were no signs of irritation or sensitization.

There was no sign of erythema at treatment 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, UVB, and UVC. Topical treatment with green tea polyphenols at 3 mg/2.5 cm^2 to human skin decreased 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. Multiple in vitro and in vivo studies demonstrated UV-protective effects of camellia sinensis catechins.

Camellia sinensis leaf extract exhibited antimicrobial properties towards multiple bacterial species and wound-healing properties.

Camellia sinensis leaf extract at 100% caused no adverse effect to the skin of burned rabbits.

An IFRA standard reported tea leaf absolute (aka camellia sinensis leaf extract) to be a moderate sensitizer based on animal data. The NESIL was 480 µg/cm^2.

DISCUSSION

The essential oils, oleoresins, and natural extractives (including distillates) of tea are considered GRAS by the FDA. The *C. sinensis*-derived ingredients in this safety assessment are from plants that are used extensively in the human diet. The Panel agreed that exposures to these ingredients in beverages result in much larger systemic exposures than from cosmetic uses; thus, potential toxicity from oral exposures is not a primary concern. Reproductive toxicity, genotoxicity, and

carcinogenicity data are presented in the safety assessment; but the primary focus of the assessment is on the potential for irritation and sensitization.

The Panel acknowledged the on-going evaluation of *C. sinensis*-derived green tea by NTP and decided that the current data are sufficient for determining the safety of these ingredients.

Oxidation products of linalool are dermal sensitizers. Linalool and several compounds containing the linalool moiety have been reported in the leaves ranging from 6 to 1984 ppm and in the leaf essential oil ranging from 31800 to 198 400 ppm in *C. sinensis* plants. Also, quercetin and several compounds containing quercetin have been reported in the leaf, plant, and shoot ranging from 760 to 10000 ppm. A positive genotoxic effect in an Ames assay has been reported, and genotoxicity was observed in in-vitro tests and in some in-vivo studies of ip exposures, but results were consistently nongenotoxic in oral exposure studies using mice and rats. The Panel noted that the linalool and quercetin found in *C. sinensis* leaves and essential oil, depending on growing conditions and methods of manufacture, may or may not be found in cosmetic ingredients. Therefore, when formulating products, manufacturers should avoid reaching levels of these plant constituents, and any other constituent, that may cause sensitization or other adverse health effects.

The Panel recognized that every leaf extract would likely be somewhat different and that the compositions of the plant-derived ingredients addressed in this safety assessment are characterized by broad variation. Nonetheless, the available composition data represent what would be found commonly in ingredients prepared in the manner described. The Panel assumes that the manufacturing process is the same in products prepared for oral consumption and for cosmetic uses. The Panel emphasized that the conclusion of this safety assessment applies only to ingredients prepared in a manner that produces a chemical profile similar to that described in this report. Extracts not prepared in a manner that produces similar chemical profiles could be considered safe only if they have similar safety test profiles.

The Panel expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients and they stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit such impurities.

Aflatoxins have been detected in dried *C. sinensis* leaves for drinking. The Panel believes that aflatoxins will not be present at levels of toxicological concern in *C. sinensis*-derived ingredients. The Panel recognizes the United States Department of Agriculture (USDA) designation of ≤15 ppb as corresponding to "negative" aflatoxin content.

Based on several studies that showed photoprotective effects of *C. sinensis*-derived ingredients, the Panel is not concerned that phototoxicity is a problem.

The Panel discussed the issue of incidental inhalation exposure from pump spray deodorants, suntan, and hair sprays and aerosol body and hand, and foot sprays. The limited data available from inhalation studies, including short-term and chronic exposure data, suggest little potential for respiratory effects at relevant doses. The Panel believes that the sizes of a substantial majority of the particles of these ingredients, as manufactured, are larger than the respirable range, and/or aggregate and agglomerate to form much larger particles in formulation. Thus, the adverse effects reported using high doses of respirable particles in the inhalation studies do not indicate risks posed by use in cosmetics. These ingredients are reportedly used at concentrations up to 0.22% in cosmetic products that may be aerosolized and up to 0.0055% in other products that may become airborne. The Panel noted that 95%–99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, these ingredients are not likely to cause any direct toxic effects in the upper respiratory tract, based on the properties of *C. sinensis*-derived ingredients and on data that shows that these ingredients are not irritants

There are insufficient data to come to a conclusion of safety for the ingredients that are not derived from *C. sinensis* leaves. To make a determination of safety of these ingredients, the Panel needs data on:

- method of manufacture
- characterization of the constituents of these ingredients
- concentration of use in cosmetics

Should this data be provided, and if the data are sufficient to change the conclusion, the safety assessment will be reopened and a new safety assessment developed.

CONCLUSION

The CIR Expert Panel concluded that *C. sinensis* leaf-derived ingredients are safe as used in cosmetic products when formulated to be non-sensitizing. These are:

camellia sinensis leaf
camellia sinensis leaf extract
camellia sinensis leaf oil
camellia sinensis leaf powder
camellia sinensis leaf water
camellia sinensis catechins *
hydrolyzed camellia sinensis leaf *

The Panel also concluded that the available data or information are insufficient to make a determination that *C. sinensis* non-leaf derived ingredients are safe for use in cosmetics. These are:

camellia sinensis flower extract
camellia sinensis flower/leaf/stem juice
camellia sinensis root extract
camellia sinensis seedcoat powder
camellia sinensis seed extract
camellia sinensis seed powder
hydrolyzed camellia sinensis seed extract

* Were the ingredient in this group not in current use to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.

TABLES

Table 1. Definitions and functions of *C. sinensis*-derived ingredients in this report.[1]

Ingredient CAS No.	Definition	Function
Camellia Sinensis Leaf Extract 84650-60-2	The extract of the leaves of *C. 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 *C. sinensis.*	Antioxidants
Camellia Sinensis Flower Extract	The extract of the flowers of *C. sinensis.*	Skin-conditioning agents – miscellaneous
Camellia Sinensis Flower/Leaf/Stem Juice 1196791-49-7	The juice expressed from the flowers, leaves and stems of *C. sinensis.*	Antioxidant
Camellia Sinensis Leaf	The leaf of *C. sinensis.*	Skin-conditioning agent – miscellaneous
Camellia Sinensis Leaf Oil 68916-73-4	The oil derived from the leaves of *C. sinensis.* This is an essential oil.	Antioxidant; skin-conditioning agent - miscellaneous
Camellia Sinensis Leaf Powder	A powder derived from the dried, ground leaves of *C. sinensis.*	Exfoliant
Camellia Sinensis Leaf Water	An aqueous solution of the steam distillate obtained from the leaves of *C. sinensis.*	Fragrance ingredient
Camellia Sinensis Root Extract	The extract of the roots of *C.sinensis.*	Skin-conditioning agent – miscellaneous
Camellia Sinensis Seedcoat Powder	The powder obtained from the dried, ground seedcoats of *C. sinensis.*	Skin conditioning agent – miscellaneous
Camellia Sinensis Seed Extract	The extract of the seeds of *C. sinensis.*	Skin-conditioning agent – humectant
Camellia Sinensis Seed Powder	The powder obtained from the dried, ground seeds of *C. sinensis.*	Antioxidant; skin-conditioning agent – miscellaneous
Hydrolyzed Camellia Sinensis Leaf	The hydrolysate of camellia sinensis leaf 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.[4]

Constituent	% of dry weight
Flavanols	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
Monosaccharides	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. Constituents of concern in *C. sinensis.*

Constituent	Effects	Reference
Linalool	Not a dermal sensitizer, but sensitization is thought to be caused by peroxides of linalool. Used safely at up to 4.3% in consumer fragrances. Peroxides of linalool are limited in fragrances to 20 mmol/L.	109 26
Quercetin	Positive genotoxic effect in an Ames assay Consistently genotoxic in in vitro tests and in some in vivo studies of ip exposures, but was consistently nongenotoxic in oral exposure studies using mice and rats.	110 111

Table 4. Constituent groups in medical grade *Camellia sinensis* leaf extract.[16,27-31]

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	
Terpene 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	
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 5. Trace elements in commercial teas and their infusions.[36]

Tea	Na (µg/g)	K (mg/g)	Rb (µg/g)	Ca (mg/g)	Mg (mg/g)	Al (µg/g)	Fe (µg/g)	Mn (µg/g)	Cu (µg/g)	Zn (µg/g)	Cr (µg/g)	Pb (µg/g)
Dried tea leaves												
Unbranded 1	75±5	17±1.6	50±2.3	4.50±0.21	6.23±0.31	757±28	211±20	420±37	32.3±2.1	87±6	5.8±0.4	1.51±0.14
Unbranded 2	84±4	14.5±0.7	41.5±1.8	4.42±0.23	2.34±0.15	712±36	185±15	372±29	21.4±1.7	93±8	3.6±0.1	1.82±0.16
Unbranded 3	65±5	11.3±0.4	43±1.7	6.24±0.35	3.52±0.20	925±44	187±21	738±53	40.3±3.4	97±7	7.5±0.3	2.00±0.13
Red Label	81±6	16.2±0.8	46.7±2.4	5.31±0.38	2.81±0.08	1530±67	280±24	864±47	33.6±2.8	96±10	3.5±0.1	1.58±0.17
Tata Gold	48±4	17.0±1.5	42.8±1.9	2.44±0.08	3.95±0.32	891±51	190±13	1130±96	21.9±2.3	111±8	5.7±0.3	2.63±0.14
Society	39±2	17.4±1.4	43.4±2.1	6.25±0.47	5.76±0.30	713±41	166±9	258±18	29.5±0.8	85±6	1.7±0.1	166±0.20
Tetley Green 1	18±0.8	10.2±0.4	17.2±0.7	3.87±0.28	1.97±0.10	605±29	1550±74	1120±65	8.2±0.2	80±7	4.5±0.2	2.20±0.19
Tetley Green 2	21±1	11.3±0.5	19.3±0.8	3.20±0.31	2.31±0.09	620±38	1486±82	1030±82	7.3±0.3	78±5	4.7±0.2	2.34±0.23
Aqueous infusion (percentage of total leached into the infusion)												
Unbranded 1	68 (90)	11.6 (68)	37 (74)	0.20 (5)	1.31 (21)	196 (26)	8.5 (4.3)	168 (40)	0.7 (2.2)	36 (42)	-	-
Unbranded 2	90 (107)	10.1 (70)	30 (75)	0.18 (4)	0.56 (24)	149 (21)	9.6 (5.2)	122 (33)	1.0 (5)	40 (43)	-	-
Unbranded 3	51 (73)	7.6 (67)	32 (74)	0.37 (6)	1.09 (31)	278 (30)	7.6 (4.1)	273 (37)	3.2 (8)	43 (44)	-	-
Red Label	78 (96)	11.3 (70)	33 (71)	0.27 (5)	0.76 (27)	367 (24)	11.0 (4.7)	259 (30)	2.3 (7)	40 (42)	-	-
Tata Gold	41 (85)	12.4 (73)	32 (75)	0.17 (7)	1.03 (26)	196 (22)	9.3 (4.9)	452 (41)	1.8 (8)	30 (45)	-	-
Society	42 (108)	12.0 (69)	30 (70)	0.31 (5)	1.44 (25)	192 (27)	7.6 (4.6)	80 (31)	1.2 (4)	38 (45)	-	-
Tetley Green 1	14 (77)	6.6 (65)	4.1 (23)	0.12 (3)	0.57 (29)	127 (21)	22 (1.4)	380 (34)	0.2 (3)	32 (41)	-	-
Tetley Green 2	19 (95)	7.5 (66)	4.8 (25)	0.10 (3)	0.72 (31)	124 (20)	23 (1.5)	360 (35)	0.5 (7)	31 (40)	-	-

- Not measured.

Table 6. Phenolic composition of green and black tea from young leaves and leaf buds.[58]

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 7. Frequency and concentration of use according to duration and exposure of *C. sinensis*-derived ingredients.[40-43]

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Camellia sinensis leaf extract		**Camellia sinensis leaf**		**Camellia sinensis leaf oil**		**Camellia sinensis leaf powder**	
Total/range	**1966**	**0.00002-2**	**54**	**0.05**	**33**	**NR**	**22**	**0.005-50**
Duration of use								
Leave-on	1145	0.00002-2	38	NR	22	NR	11	0.005-50
Rinse-off	785	0.00002-1	15	NR	11	NR	10	0.01
Diluted for (bath) use	36	0.0001-0.1	1	0.05	NR	NR	1	NR
Exposure type[a]								
Eye area	138	0.00002-0.87	6	NR	NR	NR	1	0.3
Incidental ingestion	38	0.001-0.14	NR	NR	1	NR	NR	NR
Incidental Inhalation-sprays	719[b]	0.0001-0.005[b]; 0.0005-0.22	31	NR	14[b]	NR	8[b]	NR
Incidental inhalation-powders	628[c]	0.0003-2; 0.0003-0.0037	29	NR	13[c]	NR	6[c]	0.005-7[c]
Dermal contact	1569	0.00002-2	51	0.05	23	NR	22	0.005-50[g]
Deodorant (underarm)	13	0.0055[d]; 0.0055-0.023[e]	NR	NR	NR	NR	NR	NR
Hair-noncoloring	292	0.000055-0.0063	3	NR	9	NR	NR	NR
Hair-coloring	60	0.003-0.006	NR	NR	NR	NR	NR	NR
Nail	1	0.00002-0.53	NR	NR	NR	NR	NR	NR
Mucous Membrane	378	0.0001-1	1	0.05	6	NR	NR	0.01
Baby	13	NR	NR	NR	1	NR	NR	NR

	Camellia sinensis leaf water		**Camellia sinensis seed extract**			
Total/range	**36**	**30**	NR	**0.001-0.1**		
Duration of use						
Leave-on	26	30	NR	0.001-0.1		
Rinse-off	10	NR	NR	0.001-0.0013		
Diluted for (bath) use	NR	NR	NR	NR		
Exposure type						
Eye area	4	30	NR	NR		
Incidental ingestion	NR	NR	NR	NR		
Incidental Inhalation-sprays	21	NR	NR	0.1[b]		
Incidental inhalation-powders	20	NR	NR	0.1[c]		
Dermal contact	36	NR	NR	0.001-0.1		
Deodorant (underarm)	NR	NR	NR	NR		
Hair-noncoloring	NR	NR	NR	NR		
Hair-coloring	NR	NR	NR	NR		
Nail	NR	NR	NR	NR		
Mucous Membrane	NR	NR	NR	0.0013		
Baby	NR	NR	NR	NR		

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

[a] Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[b] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

[d] Spray products.

[e] Not spray products.

[f] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[g] used in a professional face and neck product at 50% that is diluted before use.

Table 8. The permeation of constituents of camellia sinensis leaf extract (as green tea) through full thickness pig ear skin using different vehicles a Franz cell.[62]

Constiuent	Permeated at 24 h ($\mu g/cm^2$)	Permeated at 48 h ($\mu g/cm^2$)
Buffer solution		
EGCG	1.37 ± 0.40	1.88 ± 0.45
EGC	$0.189 \pm 4.10 \times 10^{-2}$	$0.342 \pm 7.48 \times 10^{-2}$
EC	32.4 ± 11.3	71.2 ± 35.2
Caffeine	0.32 ± 0.05	0.49 ± 0.01
Mixed solution		
EGCG	1.27 ± 0.38	1.62 ± 0.18
EGC	$0.128 \pm 1.71 \times 10^{-3}$	0.392 ± 0.004
EC	22.2 ± 17.3	40.2 ± 43.8
Caffeine	173 ± 24.6	368 ± 52.9
Polyethylene glycol 400 solution		
EGCG	Below detection limit	Below detection limit
EGC	Below detection limit	Below detection limit
EC	1.34 ± 0.35	4.96 ± 0.85\
Caffeine	46.8 ± 3.43	88.9 ± 0.08
Water		
EGCG	0.27 ± 0.15	0.66 ± 0.30
EGC	0.06 ± 0.02	0.10 ± 0.03
EC	1.32 ± 0.22	2.34 ± 0.34
Caffeine	28.4 ± 2.46	50.2 ± 1.54

Table 9. Reproductive and developmental studies submitted to the FDA for the approval of an ointment containing 15% catechins from *C. sinensis*.[74]

Species (n); administration	Results
Oral	
Pregnant rats (6-7); 0, 125, 250, 500, 750, 1000 mg/kg ointment in water (assume by gavage) on gestation days 6-15	Complete resorptions in 2/6 dams in the highest dose group. No other treatment related effects.
Sprague-Dawley rats (27); 0, 250, 500, 1000 mg/kg ointment on gestations days 6-18 by gavage	Body weight gains were decreased in all treatment groups compared to controls (14%, 7%, and 10%, respectively). No effects on fertility, embryo/fetal development.
Rabbits (not provided); 0, 62.5, 125, 250, 500, 1000 mg/kg ointment on gestations days 6-18 by gavage	No treatment related effects observed.
White rabbits (not provided); 0, 100, 300, 1000 mg/kg ointment on gestations days 6-18 by gavage	Mortality due to gavage trauma. Body weight gains were decreased in the low- and high-treatment groups (-31%, +10%, -84%, respectively). Feed consumption was decreased in the high-dose group. No effects on fertility, embryo/fetal development.
Subcutaneous	
Rabbits (6); 0, 37.5, 150 mg/kg/d ointment on gestation days 6-19	High-dose group- irritation with severe subcutaneous lesions/necrosis at injection sites. Treatment was discontinued after 6 days. 1 rabbit aborted. There was body weight loss, decreased feed consumption, and embryonic resorptions. 2 fetuses from separate litters had umbilical hernia (one with hyperflexed limb), 1 fetus had a short tail. Low-dose group-Local irritation, decreased 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 ointment on gestation days 6-19	High-dose group-severe local irritation at injection sites, decreased weight gain and feed consumption, decreased fetal weight. Abortions on gestation day 26. Decreased 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- 1 abortion on last day of gestation. 6 fetuses (from 5 litters) were malformed. 1 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- 7 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.

Table 9. Reproductive and developmental studies submitted to the FDA for the approval of an ointment containing 15% catechins from *C. sinensis*.[74]

Species (n); administration	Results
Intravaginal	
Sprague-Dawley rats (25); 0.15 mL ointment 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 ointment 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 decreased 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 ointment 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 Controls-5 stillborn pups from 3 litters. F_1 – No mortalities. 1 male in the mid-dose group was missing the tip of his tail and o1 female had dental abnormalities. No clinical signs, body weight gains, pinna unfolding, incisor eruption, eye opening, surface righting, gripping pupillary and auditory reflex, age of vaginal opening, and balano-preputial separation were normal. Water maze field tests were normal. All mating and fertility parameters were normal.

Table 10. Genotoxicity studies of *C. sinensis* extracts and constituents.

Assay	Ingredient/constituent (concentration)	Results	Reference
	In vitro		
Ames test (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. 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.	76
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.	78
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.	77
Ames test (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A)	Chinese tea extract (0.85% solids) (5000µg/plate)	Negative	66
Ames test (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A)	Oolong tea extract (1.0% solids) (5000µg/plate)	Negative	66
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.	76
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.	76
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.	74
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	77

Table 10. Genotoxicity studies of *C. sinensis* extracts and constituents.

Assay	Ingredient/constituent (concentration)	Results	Reference
	In vivo		
Mouse micronucleus assay (n=5/sex)	Polyphenol mixture containing 51.4% EGCG and 4 other catechins (0-1500 mg/kg) by gavage	Not mutagenic	[76]
Mouse micronucleus assay (n=5/sex)	EGCG (91.9% pure) (500, 1000, 2000 mg/kg) by gavage	Not mutagenic	[77]
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) by gavage. 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.	[76]
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	[77]
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	[77]
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	[74]

Table 11. 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.[82]

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 12. Dermal reactions to *C. sinensis* leaf (aqueous extract or dried leaves) application in ointments for dermal treatment of genital and perianal warts.[27]

Frequency	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. Application site infection, application site pustules, herpes simplex, infection, pyoderma, *staphylococcal* infection, urethritis, vaginal candidiasis, vulvovaginitis and vulvitis.

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