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Final Report on the Safety Assessment of Candelilla Wax, Carnauba Wax, Japan Wax, and Beeswax

Toxicological test data on four waxes – Candelilla, Carnauba, Japan, and Beeswax, singularly and in combination – are presented. On the basis of the documented animal and clinical test data, it is concluded that these waxes are safe for cosmetic use.

INTRODUCTION

Waxes are water-insoluble, solid mixtures of long-chain hydrocarbons and/or esters of higher fatty acids and alcohols.[1] Waxes become soft, pliable, and liquid when warm, and variably hard when cold. They are found on the skin and fur of animals, the feathers of birds, and on the exoskeleton of many insects. Waxes cover the leaves, stems, petioles, and fruits of higher plants, and are also derived from mineral and fossil sources.[1-4]

People have used wax products since early recorded history.[5] Wax is an ingredient in eye and facial makeup, fragrance, skin care, and personal cleanliness preparations. It lends structure, firmness, gloss, and smoothness to cosmetics, and it also forms creamy emulsions.[6-9] Waxes are used as gellants for various liquid cosmetic ingredients, enabling these cosmetics to retain the shape of their container. Wax-containing formulations melt or soften at specific temperatures, releasing ingredients contained in the wax phase and imparting smooth spread and long wear on skin surfaces.[9]

The waxes reviewed in this report are grouped according to the similarities in their chemistry and derivation. Those listed under the plant waxes include Candelilla, Carnauba, and Japan Wax. The only insect wax reviewed is Beeswax.

PLANT WAXES: INTRODUCTION AND OVERVIEW

Candelilla, Carnauba, and Japan Wax are plant waxes widely used as ingredients in cosmetic products.

The Plants

The plant waxes used in cosmetics are obtained from the Gymnospermae and Angiospermae.[2] The waxes are synthesized in the outer wall of the epidermal cells of leaves, stems, and fruit. This wall is covered with cutin, a fatty, water-impermeable polyester, exuded through the pores and from which the wax is derived. Plants from tropical and arid regions are most highly cutinized to check transpiration and avoid dehydration. Regional and climatic differences produce varying amounts of wax with varying composition within a given species.[2,4]

Synthesis and Composition

The synthesis of plant waxes begins with fat or oils (glycerides) which are built up by reductive processes and carboxylation; these are transformed into high molecular weight alcohols, acids, and hydrocarbons through light, heat, and moisture. Each wax is peculiar to the plant that synthesizes it.[2]

Plant waxes are complex mixtures of chemical compounds which include hydrocarbons, esters, free primary alcohols, aldehydes, free acids, secondary alcohols, ketones, hydroxy ketones, β-diketones and hydroxy- and oxo-β-diketones, free diols, glycerides, and triterpenes.[2] They also contain small quantities of dyes,

TABLE 1. Components of Unsaponified Epicuticular Waxes.[a]

Components	*Common Source*	*Remarks*
η-Alkanes	Most plants	Common, major or minor
η-Alkenes	Rose flower, sugar cane	Uncommon, very minor
Branched Alkanes	Tobacco	Uncommon, very minor
Monoesters	Most plants	Common, major or minor
Diesters of short diols	Wheat, rye	Uncommon, minor
Esters of aromatic acids	Carnauba	Uncommon, minor
Acetates	Lolium species	Uncommon, minor
Hydroxyesters	Ouricuri	Uncommon, minor
Polyester of ω-hydroxy acids	Gymnosperms	Common, major
Primary alcohols	Most plants	Common, major or minor
Aldehydes	Sugar cane	Uncommon, major or minor
Acids	Most plants	Common, minor
Secondary alcohols and ketones	Cabbage, rose flower pea, apples and Gymnosperms	Common, major or minor
Hydroxyketones	Cabbage	Uncommon, very minor
β-Diketones	Eucalyptus, Acacia carnation, wheat, oats, barley, rye, grasses	Common, major or minor
Hydroxy or oxo-β-diketones	Wheat, barley, rye, oats, grasses	Uncommon, major or minor
Diols	Apples, rose flower	Uncommon, minor
Glycerides	Bayberry and Japan waxes	Uncommon, major
Triterpenes	Apple, grape and cranberry fruits	Common, minor

[a] From Ref. 2.

sterols, aromatic, and terpenoid compounds, and their reaction products.[10] Wax hydrocarbons, ketones, and secondary alcohols usually contain an odd number of carbon atoms and the acids and primary alcohols contain an even number. The ketones and alcohols of some waxes consist almost entirely of one chain length. Table 1 lists most of the components of unsaponified epicuticular waxes and their common sources.[2]

The n-alkanes containing an odd number of carbon atoms are present in all plant waxes. Chain lengths range from about C_{21} to C_{37}, with C_{31} and C_{29}, respectively, the most common chain lengths. Alkanes with even number of carbons occur as minor components and the n-alkenes and branched alkanes are minor constituents of waxes.[2]

Long chain acid and alcohol components produce monoesters with chain lengths of C_{32} to C_{64}. The monoesters generally have even-numbered carbon chains, but some odd numbered carbon components have been detected. Diesters, hydroxy esters and polyesters of ω-hydroxy acids are also present.[2]

The free primary alcohols are straight chain compounds usually with even numbered carbons and chain lengths of C_{22} to C_{34}. The aldehydes and free acids also have straight chains with even numbered carbon atoms.[2]

The secondary alcohols, ketones, and hydroxy ketones have a close biosynthetic relationship. The secondary alcohols, always free and with an odd number of carbons, often occur together with their corresponding ketones.[2]

The β-diketones and hydroxy- and oxo-β-diketones are major wax components, with chain lengths that include C_{29}, C_{31}, C_{33}. Free diols are rare, and glycerides are found mainly in Japan Wax and Bayberry Wax. Triterpenes, mainly found in fruit waxes, are also contained in Ouricury Wax at about 5%.[2,4,11-15]

Analysis

Wax can be extracted from the plants mechanically by scraping or flailing, by solvent extraction with hexane, chloroform, or methylene chloride, or by melting in hot water.[2-4]

After the wax is freed from the major impurities, various methods may be used individually or in combination to obtain their chemical composition. Thin layer chromatography (TLC) using silica gel G with benzene-chloroform is a common method, and column chromatography using silicic acid produces good separation. Whole, unsaponified waxes may be analyzed by gas–liquid chromatography (GLC). Ozonation and permanganate-periodate oxidation are common chemical determinations of double bond formation, and saponification is used to identify component acids and alcohols of esters. Aldehydes can be determined by sodium borohydride reduction to alcohols, and ketones and secondary alcohols by chromic acid oxidation. β-Diketones are determined by copper salt formation.[2,4,10-21]

Spectroscopic identification methods are also used in wax analysis. Ultraviolet absorption detects β-diketones, and infrared absorption can identify a number of functional groups. Mass spectrometry and x-ray diffraction are used for determination of structure and nuclear magnetic resonance (NMR) spectroscopy can characterize pure fractions or simple mixtures of wax components.[2,4,11-17,19-22]

CANDELILLA WAX: CHEMICAL AND PHYSICAL PROPERTIES

Structure and Composition

Candelilla Wax is produced by a weed-like plant of the genus *Euphorbia*, which grows on the semisandy soil of North Central Mexico and in the Texas Big Bend area. Harvested from October to June, this is a suffruticose herb with slender single, or simply-branched limbs, covered with a waxy layer.[2,3,23-25] Regional and climatic differences may cause variations in the chemical composition of the wax (Table 2).[23]

Properties

Candelilla Wax is a yellowish-brown hard, brittle, lustrous solid with an aromatic odor. It is soluble in organic solvents and insoluble in water.[2-4,10,23-25] Candelilla Wax absorbs UV radiation at approximately 276 nm.[26] For other specific properties, see Table 3.

Extraction and Analytical Methods

Candelilla Wax is extracted from the plant's surface by submerging whole plants in boiling water to which some sulfuric acid has been added. The wax is collected as it accumulates on top of the water.[23,24]

The refined wax can be separated into its main components by the use of thin layer chromatography and column chromatography. Differential thermal analysis and colorimetry are used to characterize the wax, and x-ray diffraction and melting point tests elucidate the acid and alcohol content.[10,16-19]

Impurities

About 10% of the crude Candelilla Wax consists of soil (~1%), water (3%–8%), and plant residue. The sole criterion for determining degree of purity is color, judged by comparison with the color of a standard wax block.[23]

USE

Noncosmetic Uses

Candelilla Wax is a Generally Recognized As Safe (GRAS) ingredient used in chewing gum and as a surface finishing agent, listed with no limits.[30] It is also

TABLE 2. Composition of Candelillia Wax.

Components	*Amount reported (%)*	*Ref.*
Hydrocarbons	50–53	3,25
Esters of Hydroxylated Wax Acids	35	25
Free Wax Acids: C_{28}–C_{34} (even numbered)	10	3,25
Resins	Trace	3
Lactones	5–6	3,25

TABLE 3. Plant-Derived Waxes Properties and Grades.

Properties	*Candelilla Wax*	*Ref.*	*Carnauba Wax*	*Ref.*	*Japan Wax*	*Ref.*
Specific gravity	0.983	27	0.995 (15°C)	27	0.97–0.99	27
Saponification value	43–65	25,27	72–88	28	217–237	29
Iodine value	19–44	25	7–14	27,28	4–16	29
Melting range	66°–73°C	25,27	72°–86°C	27,28	50°–56°C	27,29
Refractive index	1.4555	27	1.463 at 60°C	—	1.450 at 60°C	3
Acid value	10–24	25	2–9	27,28	6–30	29
Alcohol	—	—	Soluble in boiling	27	Soluble in hot	27
Ether	Soluble in hot	27	—	—	—	—
Chloroform	Soluble	—	—	—	Soluble	—
Benzene	—	—	—	—	Soluble	—
Oils	—	—	—	—	Soluble	—
Waxes	Insoluble	—	Insoluble	—	Insoluble	—
Others	Soluble in CCl_4, turpentine, toluene, trichloroethylene alkalai	—	Soluble in alkalai	—	Soluble in naptha	—
Grades	Crude Refined Powdered	27	Crude Refined Powdered Graded by numbers	27	—	—

approved for use in adhesives and adjuvants and as a component of paperboard.[31] Candelilla Wax is used in candles, as a substitute or diluent for Beeswax and Carnauba Wax, as a hardener for soft waxes and as an ingredient in acid-proofing agents for metal etching. Other applications include: adhesives, polishes, carbon papers, crayons and inks, celluloid compositions, insulation, explosives, insect-proofing material, leather dressings, linoleum, lubricants, metal protecting and molding compositions, paint removers, paper sizing, phonograph recordings, rubber compositions, sealing waxes, varnishes, plastics, and as a coating to extend the storage life of fruit.[3,4,23,24]

Purpose and Use in Cosmetics

Candelilla Wax gives structure to solid and stick-like cosmetic preparations, even at elevated temperatures, and still allows ease of application.[7]

Candelilla Wax is used in facial and eye makeup preparations, fragrance products, hair and skin care preparations, suntan and sunscreen products, and cleansing, moisturizing and wrinkle-removing creams (Table 4).[32]

The cosmetic product formulation computer printout which is made available by the FDA is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the CFR.[31] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concen-

TABLE 4. Product Formulation Data.[a]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)						
			Unreported concentration	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Candelilla Wax									
Eyebrow pencil	145	2	—	—	—	1	1	—	—
Eyeliner	396	18	—	—	—	4	14	—	—
Eye shadow	2582	172	—	—	6	17	126	23	—
Mascara	397	66	—	1	17	28	10	10	—
Other eye makeup preparations	230	18	—	1	1	7	8	1	—
Colognes and toilet waters	1120	1	—	—	—	—	1	—	—
Perfumes	657	3	—	—	2	1	—	—	—
Other fragrance preparations	191	5	—	—	2	1	2	—	—
Hair conditioners	478	1	—	—	—	—	—	1	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	—	3	—
Hair dyes and colors (all types requiring caution statement and patch test)	811	1	—	—	—	—	1	—	—
Blushers (all types)	819	45	—	—	2	14	27	2	—
Makeup foundations	740	20	—	—	—	1	18	1	—
Lipstick	3319	831	—	—	173	405	238	14	1
Makeup bases	831	33	—	—	1	16	15	1	—
Rouges	211	23	—	—	4	16	3	—	—
Makeup fixatives	22	4	—	—	—	—	4	—	—
Other makeup preparations (not eye)	530	22	—	—	1	2	16	3	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	—	—	—	—	2	—	—

Moisturizing skin care preparations	747	7	—	—	1	4	2	—	—
Night skin care preparations	219	2	—	—	—	—	2	—	—
Wrinkle smoothers (removers)	38	5	—	—	—	3	2	—	—
Other skin care preparations	349	1	—	—	1	—	—	—	—
Suntan gels, creams, and liquids	164	4	—	—	—	2	2	—	—
Other suntan preparations	28	1	—	—	—	—	1	—	—
1981 TOTALS		1290	—	2	211	522	495	59	1
Carnauba Wax									
Eyebrow pencil	145	6	—	—	—	1	4	1	—
Eyeliner	396	41	—	—	5	5	31	—	—
Eye shadow	2582	235	—	1	8	18	161	46	1
Eye lotion	13	2	—	—	—	—	1	—	1
Mascara	397	161	—	2	16	46	95	2	—
Other eye makeup preparations	230	24	—	—	—	5	4	12	3
Perfumes	657	1	—	—	—	—	—	1	—
Other fragrance preparations	191	5	—	—	—	2	3	—	—
Hair conditioners	478	1	—	—	—	—	—	1	—
Hair dyes and colors (all types requiring caution statement and patch test)	811	1	—	—	—	—	1	—	—
Other hair coloring preparations	49	4	—	—	—	1	3	—	—
Blushers (all types)	819	68	—	—	1	28	32	6	1
Makeup foundations	740	37	—	—	1	5	31	—	—
Lipstick	3319	731	—	—	5	81	554	74	17
Makeup bases	831	60	—	—	1	3	55	1	—
Rouges	211	15	—	—	1	3	11	—	—
Makeup fixatives	22	11	—	—	—	—	11	—	—
Other makeup preparations (not eye)	530	37	—	—	1	4	25	7	—
Nail creams and lotions	25	1	—	—	—	—	1	—	—
Other manicuring preparations	50	1	—	—	—	—	1	—	—

TABLE 4. (Continued.)

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)						
			Unreported concentration	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	1	—	—	—	1	—	—	—
Depilatories	32	1	—	—	—	—	1	—	—
Moisturizing skin care preparations	747	7	—	—	—	1	5	1	—
Night skin care preparations	219	3	—	—	—	—	3	—	—
Wrinkle smoothers (removers)	38	4	—	—	—	1	3	—	—
Other skin care preparations	349	22	—	—	1	2	19	—	—
Suntan gels, creams, and liquids	164	2	—	—	—	—	1	1	—
Other suntan preparations	28	2	—	—	—	1	1	—	—
1981 TOTALS		1484	—	3	40	208	1057	153	23
Japan Wax									
Eyebrow pencil	145	24	—	12	10	1	1	—	—
Eyeliner	396	24	—	4	14	6	—	—	—
Eye shadow	2582	1	—	—	1	—	—	—	—
Tonics, dressings, and other hair grooming aids	290	2	—	—	—	1	1	—	—
Blushers (all types)	819	1	—	—	—	—	1	—	—
Lipstick	3319	145	—	—	14	15	78	38	—
1981 TOTALS		197	—	16	39	23	81	38	—

[a] From Ref. 32.

tration, the value reported by the cosmetic formulator may not necessarily reflect the concentration found in the finished product; the concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to 10-fold error in the assumed ingredient concentration.

The FDA in 1981 listed Candelilla Wax in 1,290 cosmetics. It is used in concentrations of less than 0.1% to as high as 50%. Formulations containing this wax may come in contact with the body for several hours (fragrance preparations) to several days (hair care preparations).[32]

Candelilla Wax may come in contact with the eye, face, and general body surface, hair, scalp, hands, and the mucous membranes of the lips and mouth.[32]

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute

Oral toxicity

Candelilla Wax, as the ingredient and in formulation, has been tested for acute oral toxicity. These studies are detailed below and summarized in Table 5.

Raw Candelilla Wax was administered in a 5 g/kg dose by oral intubation to five rats. No animals died during the following 14 days, and the ingredient was considered nontoxic.[33]

A 25 g/kg dose of a lipstick formulation containing 10.98% Candelilla Wax was administered by gavage to 10 Sprague–Dawley rats. No animals died and no toxic effects were seen.[34]

A 5 g/kg dose of each of two lipstick formulations containing 12% Candelilla Wax was administered by oral intubation to five Hooded Long Evans Strain Rats. No animals died during the subsequent 14 days and no lesions were found at necropsy.[35,36]

Primary skin irritation

The results of primary skin irritation tests of Candelilla Wax on rabbits are shown in Table 6 and summarized as follows:

TABLE 5. Acute Oral Toxicity: Candelilla Wax.

Wax conc. (%)	*Dose/kg of test material*	*Neat/ Formulation*	*Species and number*	*LD_{50}/kg of material*	*LD_{50}/kg of wax*	*Comments*	*Ref.*
100	5.0 g	Neat	5 rats	>5 g	>5 g	Nontoxic	33
10.98	25 g	Formulation-lipstick	10 Sprague–Dawley rats	—	—	No deaths	34
12	5.0 g	Formulation-lipstick	10 rats	—	—	No abnormalities; no deaths	35,36

TABLE 6. Primary Skin Irritation/Dermal Toxicity: Candelilla Wax.

Wax conc. (%)	*Dose of test substance*	*Neat/ solution/ formulation*	*Species and no.*	*Irritation score* — *Maximum score*	*Contact time*	*Observation time*	*Comments*	*Ref.*
Irritation								
75	—	25% corn oil solution	9 rabbits	0.28/8.0	24 h	—	Potential for minimal irritation	37
100	0.5 ml	Neat	9 rabbits	0.0/8.0	24 h	24, 48 h	No observable irritation	33
100	—	Neat	9 rabbits	0.0/8.0	24 h	—	Single, full strength, closed patch	38
100	—	Neat	9 rabbits	0.0/8.0	24 h	—	Single, full strength, closed patch	38
100	—	Neat	9 rabbits	0.0/8.0	24 h	—	Single, full strength, closed patch	38
10.98	0.5 g	Formulation-lipstick	6 New Zealand rabbits	—	24 h	72 h	At 72 h very slight erythema and edema in 1/6 with intact skin and 1/6 with abraded skin. Well defined edema and erythema at 72 h in 1/6.	34
Toxicity								
3.5	500 mg/kg	Formulation-cream	8 New Zealand rabbits	—	24 h	24 days	No systemic toxicity. Skin irritation included slight erythema and drying and flaking.	39

The primary irritation potential of 75% Candelilla Wax in corn oil was tested on nine albino rabbits. The material was applied to the shaved skin for 24 h in a single closed patch. The Primary Irritation Index (PII) was 0.28 (maximum score 8.0) which suggests that the mixture had no more than a minimal irritation potential.[37]

A 0.5 ml volume of 100% Candelilla Wax was held in contact under occlusion on the clipped skin of the backs of nine female albino rabbits. The patches were removed after 24 h, and the sites were scored for erythema and edema immediately and after 24 h. This compound scored 0.0.[33]

Three separate batches of 100% Candelilla Wax were applied full strength to the skin of albino rabbits under occlusive dressing. The substances were nonirritating.[38]

A lipstick product containing 10.98% Candelilla Wax was tested by the Modified Draize Method on six New Zealand white rabbits. A 0.5 g dose of the material was applied to abraded and intact skin, left uncovered for 24 h after which the material was then removed. After 72 h, one animal with abraded skin and one with intact skin had very slight erythema and edema, and one animal had well defined edema and erythema on abraded skin.[34]

Dermal irritation and systemic toxicity

A cream containing 3.5% Candelilla Wax was tested for dermal irritation and systemic toxicity on eight New Zealand albino rabbits. Another group of eight rabbits served as the untreated control. A dose of 500 mg/kg of the cream was applied to the clipped, intact and abraded skin of the back, five times per week for four weeks. Twenty-four days after the first application, the rabbits were fasted for 16 h and blood samples were obtained. No changes in erythrocyte count, white blood cell count, hemoglobin, and hematocrit value, serum urea nitrogen, fasting serum glucose, and activities of serum alkaline phosphatase, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminse were found. Slight erythema and drying and flaking occurred at the application sites, but body weight, general appearance, behavior, relative organ weights and gross and histopathologic examination of gonads, heart, intestine, lung, pancreas, stomach, and bone marrow were normal. No animals died from the treatment[39] (see Table 6).

Ocular irritation

In an acute eye irritation study, a 0.1 ml volume of 75% Candelilla Wax in corn oil was instilled into one eye of each of six albino rabbits; the untreated eye served as the control. No irritation was observed during the subsequent seven days.[37]

A 0.1 g dose of a formulation containing 10.98% Candelilla Wax, instilled into one eye of each of 6 rabbits, caused no irritation after 24, 48, and 72 h.[34]

Special Studies

Teratogenicity/embryotoxicity

A cream formulation containing 3.5% Candelilla Wax was tested for teratogenicity and embryotoxicity in 24 pregnant Charles River rats. A 2 g/kg dose of the formulation was applied daily to the clipped skin of 12 rats from Day 6 of gestation to Day 16. Twelve untreated pregnant rats were used as controls.

Observations were made daily for pharmacologic and toxicologic effects. Animals were weighed on Days 2, 6, and 20 of gestation and were sacrificed on Day 20. The skin of nine test animals had slight to moderate desquamation at the test site. Body weights, corpora lutea, number of implantation sites, fetuses and resorption sites, sex ratios, fetal length, and weights were all normal. There were no teratogenic or embryotoxic effects.[40]

Clinical Assessment of Safety

The results of Clinical Studies are described below and summarized in Table 7.

Skin Irritation/Sensitization

Patch testing

In a single insult patch test, 25% Candelilla Wax (wt/wt) in castor oil applied to the upper back and arm of 20 subjects caused no irritation.[41]

A rouge containing 3.5% Candelilla Wax was tested for skin irritation on 20 human volunteers. A single 24 h insult patch test of the undiluted product under occlusive patching was negative for irritation.[42]

Repeated insult patch test

In a repeated insult patch test, a cream formulation containing 3.5% Candelilla Wax caused no reactions in 108 men and women.[43]

A Modified Draize-Shelanski-Jordan repeated insult patch test was conducted on 200 subjects with an undiluted lipstick formulation containing 10.98% Candelilla Wax. Patches impregnated with the formulation were applied for 24 h to the upper backs of 108 men and women on each Monday, Wednesday, and Friday for three consecutive weeks. After each 24 h period, the patches were removed and the sites were read 24 h later on the next patch replacement day. After a two-week rest period, two consecutive 48 h challenge patches were applied to sites adjacent to the original patch site. After 48 and 96 h, no irritation or sensitization occurred.[34]

Cumulative irritation tests

A cumulative irritancy test was conducted on two lipstick formulations containing 12% Candelilla Wax. Patches impregnated with 0.2 ml of the materials were applied to the backs of 12 panelists for 21 consecutive 23 h applications. One hour after patch removal, the sites were scored on a maximum irritation score scale of 756. The composite total score for one formulation was 31 and for the other, it was 2; the two products were essentially nonirritating.[44]

Controled use test

In a 4 week controled-use test, a lipstick containing 10.98% Candelilla Wax produced no reactions in 25 subjects.[34]

CARNAUBA WAX: CHEMICAL AND PHYSICAL PROPERTIES

Structure/Composition

Carnauba Wax is obtained from the leaves of the Brazilian tropical palm tree, *Copernica cerifera*. During dry months, the leaves and petioles exude a wax

TABLE 7. Clinical Data: Candelilla Wax.

TEST Wax conc. (%)	Dose	*Solution/ formulation*	*No. of subjects*	*Days on test*	*Irritation score* Max. score	*Comments*	*Ref.*
24 h patch test							
2.5	—	Solution	20	1	0/4	No irritation	41
3.5	—	Formulation-rouge	20	1	0/4	No irritation	42
Repeat insult patch test							
3.5	—	Formulation-cream	108	—	—	No irritation or sensitization	43
10.98	—	Formulation-lipstick	200	See comments	—	Three-week induction phase; 2-week rest period; 2-48 h challenges. No irritation or sensitization	34
21-day cumulative irritancy test							
12	0.2 ml	Formulation-lipstick	12	21	31/756; 2/756	Both products essentially nonirritating	44
Controlled use test							
10.98	—	Formulation-lipstick	25	28	—	No adverse reactions	34

TABLE 8. Composition of Carnauba Wax.

Component	Amount reported (%)	Ref.
Aliphatic esters	38–40	13,14,20
ω-hydroxy aliphatic esters	12–14	
p-hydroxycinnamic aliphatic diesters	20–23	
uncombined alcohols (C_{30}, C_{32}, C_{34})	10–12	
p-methoxycinnamic aliphatic diesters	5–7	
hydrocarbons (C_{27}, C_{29}, C_{31})	0.3–1	
triterpene-type diol	0.5	
free acids and unknowns	5–7	

which, when dry, is removed by flailing. The crude wax is melted in boiling water, and skimmed from the surface; it can also be melted in clay vessels or open iron pots and then sieved.[2-4,28]

For a list of the components of Carnauba Wax, see Table 8.

Properties

Carnauba Wax is the hardest of the commercial vegetable waxes with the highest melting point of 83–86°C. It is tough, amorphous, lustrous, has a pleasing odor, and breaks with a clean fracture. Carnauba Wax may vary from dirty yellow, brown, or green to white.[3,4,8,27,28,45] For other specific properties, see Table 3.

Reactivity

Cumarone resins increase the toughness of Carnauba Wax and make it stringy when melted. One percent Beeswax increases its melting point by 3°C, and 2% Beeswax, Microcrystalline Wax or oleic acid increases its flexibility. The precipitation temperature of Carnauba Wax in n-butyl alcohol and n-heptane is much higher than that of other waxes. Carnauba Wax mixes in all proportions with all animal, mineral, or vegetable oils, if the oil does not exceed 70% of the mixture. Resins and ethyl and nitrocellulose are miscible with, but not soluble, in, the wax.[3]

Analytical Methods

Column chromatography separates Carnauba Wax into constituent hydrocarbons, esters, alcohols, and acids. Alkaline hydrolysis, gas–liquid chromatography and thin layer chromatography are also used for analysis. Infrared spectroscopy and lithium aluminum hydride reduction demonstrate lesser components.[10,14,16,17,20]

Impurities

Carnauba Wax contains water and unspecified inorganic materials as impurities. Contamination of the wax can be detected by the Cleveland open-cup flash point test.[3]

USE

Noncosmetic Uses

Carnauba Wax is a GRAS food additive under 21 CFR 175.105 for adhesives, 175.320 for resinous and polymeric coatings for polyolefin films, and 176.181 for components of paper and paper board in contact with dry food. The FDA has proposed affirmation under 184.1976 as a GRAS lubricant and surface finishing agent at levels not to exceed good manufacturing practice in hard candy and chewing gum. It is also used in candles, polishes, lubricants, greases, floor and automobile waxes, insulating material, carbon paper, chalks, matches, soaps, salves, phonograph records, plastics, enteric capsules, coating ointment bases, and as a citrus fruit dip. Carnauba Wax is added to other waxes to increase their melting points, hardness, and lusters and to decrease their stickiness, plasticity and crystallizing tendencies.[3,4,46,47]

Purpose and Use in Cosmetics

In eye makeup preparations, Carnauba Wax forms a water repellent film, counteracts the solubilizing action of soaps, and imparts a luster to the dried application. This wax is also used in the formulation of stick and solid cosmetics for ease of application without breakdown at elevated temperatures.[6,7]

Carnauba Wax is used in face and eye makeup preparations, fragrance preparations, hair coloring and conditioning preparations, and manicuring, skin care, and suntan preparations.[32]

Carnauba Wax was listed as being used in 1,484 formulations by the FDA in 1981.[32] It is used at concentrations of less than 0.1% to 50%. Carnauba Wax may come in contact with the mucous membranes of the eyes, nose, and mouth, as well as the skin of the face, hands, and scalp, for periods ranging from several hours, as in fragrance and eye makeup preparations, to several days, as in hair conditioners and hair coloring preparations.[32] See Table 4.

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute

Oral toxicity

A 20 g/kg dose of a lipstick formulation containing 5.6% Carnauba Wax was administered by gavage to 10 Sprague–Dawley rats. This represented a dose of 1.1 g/kg of the Carnauba Wax. There were no deaths and no toxic effects.[48]

A blush formulation containing 10% Carnauba Wax was diluted to 33.3% in corn oil, making the wax concentration 3.33%. None of the animals died when a 15 g/kg dose (0.5 g/kg of wax) was administered to five rats by oral intubation.[49]

Eye irritation

A 0.1 ml volume of a lipstick formulation containing 5.6% Carnauba Wax was instilled into one eye of each of nine New Zealand white rabbits. No irritation occurred during the seven observation days.[48]

A blush product containing 10% Carnauba Wax was instilled full strength into one eye of each of six rabbits. No irritation occurred during the following seven days (score = 0).[49]

Skin irritation

The primary skin irritation tests of Carnauba Wax are detailed below and summarized in Table 9.

A 0.1 ml volume of each of three samples of Carnauba Wax (100%) was applied under occlusion to the clipped skin of the backs of nine albino rats. The samples were removed after 24 h and sites were scored after 24 and 72 h. All three samples produced no irritation (PIIs of 0.0).[50]

Three open patch applications of 0.5 g of a pink lipstick product containing 5.61% Carnauba were applied at 24 h intervals to six New Zealand white rabbits. After 72 h, five out of six rabbits showed pink, uniform coloration over most of the test site from the lipstick color.[48]

A blush formulation containing 10% Carnauba Wax was tested for primary skin irritation on six rabbits. A single closed patch application caused no irritation (PII = 0.0).[49]

Subchronic

Oral toxicity

FDRL Wistar rats and purebred beagle dogs were fed Carnauba Wax as 0%, 0.1%, 0.3%, and 1.0% (w/w) of their diets. The rats were kept on the test material through one full generation (F_1A) and through the full gestation period. It was fed to the pups born of these dams through the time of weaning to their maturity. F_1B litters were used to determine the teratogenic potential of the wax. Hematological and biochemical tests, body weights, food consumption, reproductive indices, and gross and microscopic examinations of tissues were used to evaluate the toxicity of Carnauba Wax. The F_1A rats had no significant toxicological effects. There was no effect on reproductive indices in either the first or second generation. The dogs consumed the test diet for six months and were tested in all the above parameters except the reproductive index. There were no toxicologically significant effects at any of the doses.[51]

TABLE 9. Primary Skin Irritation: Carnauba Wax.

Conc. (%)	*Dose*	*No. of rabbits*	*Irritation score/8.0*	*Contact time*	*Observ. time*	*Comments*	*Ref.*
100	0.1 ml	9	0.0	24 h	72 h	No irritation	50
100	0.1 ml	9	0.0	24 h	72 h	No irritation	50
100	0.1 ml	9	0.0	24 h	72 h	No irritation	50
5.6 (lipstick)	0.5 g	6	See comments	72 h	72 h	5/6 rabbits showed pink, uniform erythema over most of test site; practically nonirritating to rabbit skin	48
10 (blusher)	—	6	0/8	24 h	—	No irritation	49

A diet containing 10% Carnauba Wax was fed to Wistar rats for 13 weeks (approximately 8.8 and 10.2 g/kg body weight/day in males and females, respectively). There were no treatment related differences in body weight, hematological values, serum enzyme activities, organ weights, or histological findings.[52]

Special Studies

Mutagenicity

Carnauba Wax did not induce mutagenesis in assays with *Salmonella typhimurium* strains TA-1535, TA-1537, and TA-1538 and *Saccharomyces cerevisiae* strain D4 with and without activation. Dimethylsulfoxide (DMSO) was used to dissolve the wax in this study.[53] DMSO has been reported as a mutagen when tested using yeast and cultures of hamster lung cells.[54]

Clinical Assessment of Safety

Skin Irritation/Sensitization

Single insult patch test

A blush formulation containing 10% Carnauba Wax was tested for human skin irritation in a single 24 h insult patch test. No irritation was observed when the test material was applied undiluted under occlusion to 17 subjects (score = 0.0).[55]

Repeated insult patch test

A Modified Draize–Shelanski–Jordan patch test for irritation/sensitization was conducted on a lipstick formulation containing 5.61% Carnauba Wax. Samples were applied under occlusion to 151 men and women for nine days. One person had faint, macular erythema over 25% of the test area and one person had moderate erythema, with or without induration, over 25% of the area. It was concluded that the test product produced neither sensitivity nor clinically significant irritation.[48]

Photosensitization

A total of 23 of 28 subjects completed an occluded photosensitivity patch test of 25% Carnauba Wax dissolved in heavy mineral oil. The material (0.1 ml) was applied to the backs of the subjects on Webril occlusive test patches for three consecutive weeks. A total of nine applications were used during the induction phase which was followed by a challenge patch during the sixth week. During the induction phase 14 of the subjects were exposed for 15 min to long wavelength ultraviolet light (UVA, 320–400 nm) from 4-F40 BL fluorescent tubes which had an output of approximately 4.4 $\mu W/cm^2$. Nine separate subjects received 5 min of UVA followed by short ultraviolet exposure (UVB, 280–320 nm) at twice the predetermined Minimal Erythemal Dose. A 150 W Xenon Arc Solar Simulator was to provide the UVB radiation. Each test site and its adjacent nonirradiated control site was scored prior to the next patch application. There was no evidence that Carnauba Wax was either a phototoxic or photosensitizing agent.[56]

JAPAN WAX: CHEMICAL AND ?HYSICAL PROPERTIES

Structure/Composition

Japan Wax is a fatty material obtained from the berries of the sumac *Rhus succedania,* found in Japan and China. This is a tree of the *Rhus* family, a relative of poison ivy which grows up to thirty feet and begins to produce berries after fifteen years. The berries are dried and crushed, and the wax is removed by steam stripping.[3,4,22,29] See Table 10 for the specific composition of Japan Wax.

Properties

Japan Wax is a tough, malleable, and sticky substance with a tallowy odor. The purified form is white, opaque, and gloss-free. It becomes rancid, and effloresces and darkens on aging. When melted and allowed to solidify, its specific gravity and melting point are lowered, but these constants tend to return to normal after a period of time. The specific gravity of solid Japan Wax at 15°C is 0.990, and that of the molten wax (at 98°C) is between 0.875 and 0.877.[3,4,8,10,29,45] From UV absorbance tests the peak absorbance of UV light by Japan Wax occurs in the wavelength range of 275–280 nm.[26] See Table 3 for other specific properties.

Analytical Methods

Thin layer chromatography, mass spectroscopy, methanolysis, and gas–liquid chromatography are common analytical methods for Japan Wax.[4,12,13]

Impurities

Crude Japan Wax contains varying amounts of vegetable matter, ash, and moisture. The refined grades are free from impurities.[3]

USE

Noncosmetic Uses

Japan Wax is a GRAS substance listed with no specific limits used in cotton fabrics for dryfood packaging (21 CFR 121.101). It is also used as a component in paper and paperboard in producing, manufacturing, treating, packaging, transporting, or holding aqueous or fatty foods (21 CFR 121.2526). Japan Wax is

TABLE 10. Composition of Japan Wax.

Component	*Amount reported (%)*	*Ref.*
Palmitin, palmitic acid, tripalmitin	77	3,4,12,14,29
Stearin	5	
Japanic acid triglycerine	6	
Olein	12	
Linoleic acid	1	
Unsaponifiables		

used in the manufacture of matches, polishes, creams, special soaps, laundry glazes, textile finishes, metal lubricants, and as a substitute for Beeswax.[3,4,10]

Cosmetic Uses

The 1981 FDA data listed Japan Wax as an ingredient in 197 formulations.[32]

Japan Wax is used primarily in eye and facial makeup preparations at concentrations of 0.1%–50%. These products may be applied several times per day, perhaps for several hours' duration. The ingredient may come in contact with the mucous membranes of the eye, nose, and mouth, and the skin of the face and hands (see Table 4).[32]

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute

Oral toxicity

No animals died and no toxic signs were observed when 5 g/kg of wax was introduced by rigid stomach tube to each of ten Charles River rats.[57]

Eye irritation

Two products each containing 35% Japan Wax were tested for ocular irritation. Nine albino rabbits were used in each modified Draize irritation study. One eye of each rabbit was instilled with 0.1 g of the product and the other eye served as the control. In each of the two studies, the eyes of three rabbits were irrigated immediately with 20 ml of water. Eyes were evaluated at 24, 48, and 72 h after instillation. One product caused no irritation in either washed or unwashed eyes and was considered a nonirritant.[58] The second product produced minimal irritation in unwashed eyes (score = 0.3) and no irritation in washed eyes. This product was likewise considered a nonirritant.[59]

A sample of "Natural Japan Wax" was tested for acute ocular irritation using the Draize Method. A 0.1 ml sample of the wax was instilled into the right eye of each of three albino rabbits with no further treatment. The untreated left eye served as the control and all eyes were examined after one, two, three, four, and seven days. The instillation of Japan Wax produced no irritation in any eye.[60]

Skin irritation

A 0.5 ml sample of the Japan Wax was applied under occlusion to the clipped, abraded and intact back skin of three albino rabbits. Evaluations made at 24 and 72 h were negative for irritation.[61]

Special Studies

Mutagenic Activity

Japan Wax was tested in vitro for mutagenic activity using *Saccharomyces cerevisiae,* strain D4, and *Salmonella typhimurium* strains TA-1536, TA-1537, and TA-1538. In assays with and without metabolic activation with liver, lung, kidney,

or testis homogenates from mice, rats, or monkeys, the wax did not induce mutagenesis. Dimethylsulfoxide (DMSO), a known mutagen with this species of yeast, was used as the solvent.[62]

Clinical Assessment of Safety

Skin Irritation/Sensitization

A modified Draize-Shelanski-Jordan patch test was conducted using 50% Japan Wax in petrolatum and on three products containing up to 36% wax. In each study, the test material was applied to the cleansed upper backs of each panelist for ten 24 h periods and evaluations were made for each application. A 13-day nontreatment period followed the last induction patch, after which a challenge 48 h patch was applied to each subject. A second 48 h patch was applied seven days later; these sites were read 48 and 72 h after application. In the test of the 50% pure wax in petrolatum, no irritation or allergic reactions were observed in any of the 56 women tested.[63] No irritation or allergic reaction occurred in the 53 women testing the product containing 2.5% Japan Wax.[64] The two separate products containing 36% Japan Wax were tested on 50 women and three men, and 56 women, respectively. No allergic reactions or irritation were produced by either product.[65,66]

BEESWAX: INTRODUCTION AND OVERVIEW

The cuticle (surface) of some insects is covered with a waxy layer which restricts the movement of water across the surface and keeps the insect from becoming dessicated.[2-4] The biosynthetic sites of cuticular lipids are probably the oenocytes, located in the peripheral fat body of the insect or associated with the cuticle. Hydrocarbons and esters are believed to be synthesized by the oenocytes, whereas esters are synthesized by the fat body cells.[2] Some insects, such as honey bees, also secrete large amounts of waxes in addition to cuticular lipids.[4] The bees of the genus *Apis* produce the greatest quantity of commercial Beeswax. *Apis dorsata, Apis indica,* and *Apis florea,* and the domesticated bee *Apis mellifera* all produce wax.[2-4]

CHEMICAL AND PHYSICAL PROPERTIES

Structure/Composition

Beeswax is a complex mixture of several chemical entities, each with its own chemical and physical properties. Beeswax is snythesized from even numbered alcohols ranging from C_{14} to C_{32}. The alcohols are oxidized and combine with higher alcohols to form esters. Mixed dimers may be formed by the combination of certain acids and hydrocarbons by decarboxylation of esters.[2-4,67-72] For the specific composition of Beeswax, see Table 11.

Properties

Fresh Beeswax is white, but pollens and other impurities give it a light yellow to deep brown color. The light colored crude waxes can be bleached by natural

TABLE 11. Composition of Beeswax.

Component	*Amount reported (%)*	*Ref.*
Hydrocarbons	14	70,71
Esters	73	70,71
Monoesters	35	
Diesters	14	
Triesters	3	
Hydroxymonoesters	4	
Hydroxypolyesters	8	
Acid Monoesters	2	
Acid Polyesters	7	
Specifically-lignoceryl myristate myricyl palmitate		69
Free Acids	12	70,71
Myristic Acid		69
Hydroxy Acids	unreported	70,71
15-hydroxy hexadecanoic	unreported	
14-hydroxy hexadecanoic	unreported	
16-hydroxy octadecanoic	unreported	
17-hydroxy octadecanoic	unreported	
19-hydroxy eicosonoic	unreported	
21-hydroxy docosonoic	unreported	
23-hydroxy tetracosonoic	unreported	
Diols	unreported	70,71

processes. Bleached wax is shiny, diaphanous in thin layers, odorless, and soft.[2-4,8,10,45,67-72] UV absorbance data show that peak absorbance occurs in the 280 nm range.[73] For specific properties of Beeswax, see Table 12.

TABLE 12. Properties of Beeswax.

Properties		*Ref.*
Specific gravity	0.95	8,27
Color	Brown to white (refined)	4,27,45,68
Saponification value	80–100	4,8,68,69,71
Cloud point	65°C max	71,76
Iodine value	5–15	4,8,69
Ester value	72–79	69,76
Melting range	62°–65°C	8,27,45,68
Refractive index	1.438–1.4527	3
Acid value	17–24	8,68,69,76
Solubility:		
Alcohol	Slightly soluble	27
Chloroform	Soluble	
Ether	Soluble	
Oils	Soluble	
Water	Insoluble	
Grades:	Technical	27
	Crude	27
	Refined	27

Extraction and Analytical Methods

After the Beeswax and honey are separated, the wax is melted with hot water, by high or low pressure steam, or under solar heat. In hot water, the molten wax floats and is strained through cloth to remove particulate matter.[3,4]

Beeswax may be bleached with sunlight or with sulfuric acid and hydrogen peroxide. Fuller's earth, animal charcoal, permanganate, zinc oxide, and benzoyl peroxide are also used as bleaching agents.[3,4,70]

The constituents can be separated by thin layer chromatography and the fractions saponified and subjected to infrared spectroscopy. The constituents may also be fractionated by dry distillation and separated by thin-layer chromatography.[67-69] Investigators have used gas–liquid chromatography and nuclear magnetic resonance spectroscopy.[70]

Impurities

Natural impurities include resins, pollens, and insect and plant matter, all of which are removed in the refining process.[3,4] Refined Beeswax may contain a number of additives such as tallow, paraffin, ceresin, and vegetable waxes.[67,71]

USES

Noncosmetic Uses

Beeswax is listed as a GRAS substance as a miscellaneous and/or general purpose food additive (21 CFR 121.101). It is also used in candle making, adhesive compositions, dressing and polishing leather, artistic modeling media, wax polishes and finishes, transparent papers, pomades, plaster base, engraving, lithography, sizing and finishing textiles, chewing gums, and as a vehicle for drug administration and medicinal experimentations. Beeswax has been used in cigarette filters, as a lubricant and as a water repellant in packaging films.[3-5,74,75]

Purpose and Use in Cosmetics

Beeswax is used primarily in the production of emulsions for cosmetics that require a creamy consistency, and in the formulation of solid or stick-like cosmetics. In eye makeup, Beeswax stiffens but does not harden the preparation, and its flexibility and plasticity facilitate application. In lipsticks, it gives structure to the stick, maintains the oil phase, and keeps it solid even when ambient temperatures reach 50°C. The wax allows smooth, easy application of color with a minimum of pressure against the lips.[6-8,45]

Beeswax is used in baby products and bath preparations, eye and facial makeup preparations, fragrance products, coloring and noncoloring hair preparations, manicuring and skin care products, personal cleanliness preparations, and shaving and suntan products[32] (see Table 13).

Table 13 lists the FDA data on the use of Beeswax in cosmetic formulations. In 1981, Beeswax was used in 1,909 products. Its concentration ranges from less than 0.1% to 50%. Beeswax is found in cosmetics that may be applied from several times per day (fragrance preparations) to a few times per month (hair coloring preparations). They may remain on the body for several hours (eye and facial makeup) or several days (hair conditioners).[32]

TABLE 13. Product Formulation Data.[a]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)						
			Unreported concentration	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Beeswax									
Baby lotions, oils, powders, and creams	56	2	—	—	—	1	—	1	—
Eyebrow pencil	145	7	—	—	3	3	1	—	—
Eyeliner	396	25	—	—	4	4	17	—	—
Eye shadow	2582	298	—	—	8	18	200	72	—
Eye lotion	13	4	—	—	—	1	3	—	—
Eye makeup remover	81	2	—	—	—	1	1	—	—
Mascara	397	150	—	10	50	47	43	—	—
Other eye makeup preparations	230	36	—	1	4	12	15	4	—
Colognes and toilet waters	1120	2	—	—	2	—	—	—	—
Perfumes	657	26	—	4	22	—	—	—	—
Sachets	119	25	—	6	6	—	5	8	—
Other fragrance preparations	191	9	—	1	4	3	—	1	—
Hair conditioners	478	13	—	—	1	3	2	7	—
Hair straighteners	64	2	—	—	2	—	—	—	—
Hair rinses (noncoloring)	158	1	—	—	—	—	—	1	—
Hair shampoos (noncoloring)	909	1	—	—	—	—	—	1	—
Tonics, dressings, and other hair grooming aids	290	24	—	—	5	5	8	5	1
Wave sets	180	1	—	—	—	—	—	1	—
Other hair preparations (noncoloring)	177	1	—	—	—	1	—	—	—
Hair dyes and colors (all types requiring caution statement and patch test)	811	4	—	—	—	1	—	3	—
Other hair coloring preparations	49	8	—	—	—	—	8	—	—
Blushers (all types)	819	55	—	—	1	15	21	17	1
Face powders	555	17	—	—	—	—	—	17	—
Makeup foundations	740	65	—	—	—	2	36	27	—
Lipstick	3319	511	—	1	20	125	284	80	1
Makeup bases	831	80	—	—	—	2	76	2	—

TABLE 13. (Continued.)

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)						
			Unreported concentration	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Rouges	211	22	—	—	—	6	9	7	—
Makeup fixatives	22	6	—	—	1	—	5	—	—
Other makeup preparations (not eye)	530	54	—	4	8	14	20	8	—
Cuticle softeners	32	5	—	—	—	3	2	—	—
Nail creams and lotions	25	4	—	—	1	1	2	—	—
Bath soaps and detergents	148	4	—	—	—	—	—	1	3
Deodorants (underarm)	239	1	—	—	—	—	1	—	—
Other personal cleanliness products	227	1	—	—	—	1	—	—	—
Other shaving preparation products	29	2	—	—	—	—	—	2	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	143	—	—	24	59	46	10	4
Depilatories	32	5	—	1	1	—	3	—	—
Face, body, and hand skin care preparations (excluding shaving preparations)	832	81	—	—	8	18	44	10	1
Hormone skin care preparations	10	2	—	—	1	—	1	—	—
Moisturizing skin care preparations	747	77	—	—	7	22	31	17	—
Night skin care preparations	219	70	—	—	5	29	31	5	—
Paste masks (mud packs)	171	1	—	—	—	—	1	—	—
Skin lighteners	44	1	—	1	—	—	—	—	—
Wrinkle smoothers (removers)	38	13	—	—	—	3	10	—	—
Other skin care preparations	349	36	—	—	8	5	21	2	—
Suntan gels, creams, and liquids	164	10	—	—	—	5	5	—	—
Other suntan preparations	28	2	—	—	—	—	1	1	—
1981 TOTALS		1909	—	29	196	410	953	310	11

[a] From Ref. 32.

Cosmetics containing Beeswax are applied to the skin of all parts of the body, including the lips, nails, scalp, and hair. The ingredient may enter the body through the mucous membranes and by inadvertent ingestion.[32]

Potential Interactions with Other Ingredients

Beeswax reacts with Borax to produce a white, highly lustrous, and grainless emulsion. No other interactions have been reported.[6-8,45]

BIOLOGICAL PROPERTIES

Animal Studies

Acute

Oral

The acute oral toxicity tests of Beeswax are detailed below and summarized in Table 14.

Undiluted Beeswax was administered orally at a single 5 g/kg dose to 10 rats. Four animals died on the second of the 14 observation days, and depression and ataxia were observed in surviving animals. The oral LD_{50} was not reached.[77]

A cold cream product containing 0.3% Beeswax was administered by gavage to five albino rats in doses up to 5.0 g/kg. The LD_{50} was not determined.[78]

Two lipstick formulations containing 6.4% Beeswax were not toxic when intubated orally into five albino rats. One sample was tested undiluted and the second was diluted to a concentration of 33.3% (2.13% Beeswax) in corn oil.[79,80]

Three cleansing cream formulations containing 13% Beeswax were each

TABLE 14. Acute Oral Toxicity: Beeswax.

Beeswax conc. (%)	*Ingredient or formulation*	*Dose of test material/kg*	*No. of rats*	*Comments*	*Ref.*
—	Ingredient	5 g	10	4 deaths in 2 days	77
0.3	Formulation-cold cream	5 g	5	No toxicity	78
2.1	Formulation-lipstick	15 g	5	No toxicity	79
6.4	Formulation-lipstick	15 g	5	No toxicity	80
13.0	Formulation-cleansing cream	5 g	5	—	81
13.0	Formulation-cleansing cream	10 g	5	—	82
13.0	Formulation-cleansing cream	15 g	5	—	83

tested for acute oral toxicity in five albino rats. Oral intubation of 5 g, 10 g, and 15 g/kg, did not allow calculation of an LD_{50}.[81-83]

Primary irritation

Beeswax, in solution and in formulation, was tested for primary skin irritation. Studies are detailed below and summarized in Table 15.

A primary skin irritation test of a mineral oil solution of 50% Beeswax was conducted on nine rabbits. The single closed patch was applied for 24 h and produced minimal irritation with a PII of 0.50.[84]

Undiluted Beeswax was not irritating to the backs of hairless mice and swine[73] and caused no irritation to intact or abraded rabbit skin when applied under occlusion for 24 h.[77]

A closed patch test using 100% Beeswax on nine rabbits for 24 h caused no irritation.[38] Two samples of 100% Beeswax caused mild acute skin irritation when applied for three consecutive 24 h periods to the skin of rabbits.[85,86]

A cold cream product containing 0.3% Beeswax was applied full strength under occlusion to the clipped back skin of nine rabbits for 24 h. After 23 and 72 h, minimal irritation was observed and the PII was 0.83.[87]

Two lipstick formulations containing 6.4% Beeswax caused no irritation when each was applied full strength under closed patch to nine albino rabbits for 24 h.[79,80]

Three cleansing cream formulations containing 13% Beeswax were applied full strength under occlusion for 24 h to nine albino rabbits. The compounds were mildly irritating with PIIs of 0.78, 1.55, and 0.33.[81,83]

Dermal toxicity

A corn oil solution of 50% Beeswax was tested on the skin of 10 New Zealand white rabbits. The abdomens were clipped and abrasions were made in the exposed skin of five rabbits. A dose of 2.0 g/kg test material was applied to the clipped, abraded or intact areas under 24 h occlusion. When the patches were removed, the sites were evaluated by the Draize scoring method and the animals were observed daily for 14 days for pharmacological effects. No animals died during the study, but the investigators observed some signs of lethargy, ptosis, diarrhea, and yellow and white nasal discharge from Days 4 to 14. No controls were reported[88] (see Table 15).

Ocular irritation

Ocular irritation studies of Beeswax are detailed below and summarized in Table 16.

A 0.1 ml volume of 50% Beeswax solution in mineral oil was applied to one eye of each of six albino rabbits according to the Draize Method, and the other eye was used as a control. Observations were made up to seven days or until no irritation was present. On Day 1, the solution produced a maximum irritation score of 2.0. The signs of irritation resolved during the period of observation. This solution was practically nonirritating.[84] Two other 50% solutions of Beeswax in oil caused no irritation when similarly tested.[85,86]

A cold cream containing 0.3% Beeswax was instilled full strength in a 0.1 ml volume to one eye of each of six albino rabbits. Mild irritation occurred on Day 1 (score = 1) and all irritation had disappeared by Day 2.[78]

TABLE 15. Acute Skin Irritation/Dermal Toxicity: Beeswax.

Wax conc. (%)	*Ingredient or formulation*	*N/S/F*[a]	*No. and species*	*PII*[b] / *8.0*	*Contact time*	*Observ. time*	*Comments*	*Ref.*
Irritation								
50	Ingredient	S	9 rabbits	0.50	24 h	72 h	Minimal irritation	84
100	Ingredient	N	Mice, swine	—	—	—	No irritation	73
100	Ingredient	N	rabbits	—	24 h	—	No irritation	77
100	Ingredient	N	9 rabbits	0.0	24 h	72 h	No irritation	38
100	Ingredient	N	9 rabbits	—	3 24-h applications	3 days	Mild irritation to Day 3	85
100	Ingredient	N	9 rabbits	—	3 24-h applications	3 days	Mild irritation to Day 3	86
0.3	Formulation-cold cream	F	9 rabbits	0.83	24 h	72 h	Minimal irritation	87
6.4	Formulation lipstick	F	9 rabbits	0.0	24 h	72 h	No irritation	79
6.4	Formulation-lipstick	F	9 rabbits	0.0	24 h	72 h	No irritation	80
13	Formulation-cleansing cream	F	9 rabbits	0.78	24 h	72 h	Minimal irritation	82
13	Formulation-cleansing cream	F	9 rabbits	1.55	24 h	72 h	Minimal irritation	81
13	Formulation-cleansing cream	F	9 rabbits	0.33	24 h	72 h	Minimal irritation	83
Toxicity (%)	*dose*							
50 (in corn oil)	2 g	S	10 New Zealand rabbits	—	24 h	14 days	No deaths; some lethagy, diarrhea, ptosis, nasal discharge.	88

[a] N/S/F = Neat/Solution/Formulation.
[b] PII = Primary Irritation Index; Maximum score = 8.0.

TABLE 16. Ocular Irritation: Beeswax.

Conc. (%)	Dose (ml)	Solution/ Formulation	No. of Albino rabbits	Observation days	Comments[a]	Ref.
50	0.1	Solution in mineral oil	6	7	No irritation (max. score = 2.0)	84
50	0.1	Solution in mineral oil	6	7	No irritation (score = 0)	85
50	0.1	Solution in mineral oil	6	7	No irritation (score = 0)	86
0.3	0.1	Formulation-cold cream	6	7	Mild irritation on Day 1 (score = 1.0) which cleared by Day 2 (score = 0)	78
6.4	0.1	Formulation-lipstick	6	7	No irritation (score = 0)	79
6.4	0.1	Formulation-lipstick	6	7	No irritation (score = 0)	55
13	0.1	Formulation-cleansing cream	6	7	Mild irritation (score = 3)	82
13	0.1	Formulation-cleansing cream	6	7	Mild irritation (score = 2)	81
13	0.1	Formulation-cleansing cream	6	7	Mild irritation (score = 1)	83

[a]Maximum Irritation = 110.

Two lipstick formulations each containing 6.4% Beeswax produced no irritation when tested for ocular irritation on six albino rabbits.[79,80]

Three cleansing cream formulations containing 13% Beeswax were tested for ocular irritation in six rabbits. Mild irritation (scores of 3, 2, and 1) occurred on Day 1 and disappeared by Day 2.[81-83]

Phototoxicity

Beeswax was not phototoxic when tested on hairless mice and swine. This is consistent with the finding that greatest UV absorbance occurred in the 280 nm range.[72]

Subchronic Dermal

A cold cream containing 13% Beeswax was tested in a four-week dermal toxicity study in rabbits. The backs of each of nine New Zealand strain albino rabbits were clipped and the skin of three was abraded. The test material was applied to the skin in a dose of 2.0 g/kg, for five days per week, for a total of 20 applications. No compound-related abnormalities were found during the study. Necropsy, body weight, and histopathologic examinations were negative for compound related abnormalities.[89]

A cleansing cream formulation containing 13% Beeswax was used in a six-week dermal toxicity study using 20 rats. A 0.3 g/kg dose of the test material was applied to the shaved skin of each of 10 rats, once per day, five days per week,

for a total of 30 applications. Ten other rats were used as untreated controls. Observations were made throughout the test period for general appearance, behavior, and toxicologic signs. Body weights were obtained weekly and blood samples were taken before the end of the study. All animals survived the study, and had normal appearance and behavior. External appearance of the skin at the site of application did not differ from the skin of untreated controls. Gross and microscopic examinations detected no abnormalities, and it was concluded that the formulation produced no systemic toxic effects.[90]

Carcinogenesis Studies

Threads impregnated with Beeswax (controls) or 3-methylcholanthrene (MC) plus Beeswax were implanted in the cervices of six-week-old virgin female C34/HeJ mice. Epithelialization of the cervical canal occurred after two and four weeks with both Beeswax and Beeswax plus MC. Invasive carcinoma developed in mice treated with Beeswax plus MC after four weeks of treatment, but not in the control (Beeswax-treated) animals.[91]

In a similar experiment, Beeswax and Beeswax plus MC-impregnated thread were implanted into the cervices of mice. In Beeswax-treated mice, and invasive squamous carcinoma was found in one of the 35 mice tested after four and six weeks; MC plus Beeswax induced 58% carcinoma of the cervix in treated mice.[92]

Mutagenic Activity

Beeswax was not mutagenic in tests using *Salmonella typhimurium* or *Saccharomyces cerevisiae* with and without metabolic activation.[93]

Clinical Assessment of Safety

Clinical testing of Beeswax is summarized in Table 17 and detailed below.

Skin Irritation/Sensitization

Single 24 hour patch test

A 24 h occlusive patch test of 100% Beeswax was conducted using 20 human volunteers. A 0.5 g sample of the wax was applied under occlusion to the upper back or forearm. Nineteen subjects had no irritation, and one had mild irritation; the PII was 0.03 out of a 4.0 maximum.[94] Another similar test with raw ingredient Beeswax (100%), minimal irritation was observed in one of 20 panelists and the PII was 0.03.[95]

Patch test of a lipstick formulation containing 6.4% Beeswax were negative for irritation in 20 people.[96]

Three cleansing cream formulations containing 13% Beeswax were each patch-tested on 20 subjects; a fourth containing 13% Beeswax was also applied to 19 individuals. The first three formulations were practically nonirritating and had PII scores of 0.03, 0.05, and 0.06.[97-99] The fourth formulation produced no irritation in the 19 people tested.[100]

Schwartz–Peck prophetic patch test

A Schwartz–Peck prophetic patch test was performed on a mascara formulation containing 3% Beeswax. A closed 48 h patch was applied to the backs of

TABLE 17. Human Clinical Data: Beeswax.

TEST		Neat/formulation solution	No. of subjects	Irritation score	Comments	Ref.
Conc. (%)	Dose			Maximum score		
24 h Occlusion Patch Test						
100	0.5 g	Neat	20	0.03/4.0	19 scored 0; one scored ½; barely perceptible erythema	94
100	0.5 g	Neat	20	0.03/4.0	19 scored 0; one scored ½; barely perceptible erythema	95
6.4	—	Formulation-lipstick	20	0/4.0	No irritation	96
13	—	Formulation-cleansing cream	20	0.03/4.0	Minimal irritation	97
13	—	Formulation-cleansing cream	20	0.05/4.0	Minimal irritation	98
13	—	Formulation-cleansing cream	20	0.06/4.0	Minimal irritation	99
13	—	Formulation-cleansing cream	19	0.0/4.0	No irritation	100
Schwartz-Peck Prophetic Patch Test						
3	—	Formulation-mascara	386	0/+3	No irritation, sensitization, or photosensitivity	101
21-day Cumulative Irritancy Test						
13	—	Formulation-cold cream	7	0/4.0	No irritation	102
Repeated Insult Patch Test/Contact Allergy Test				*No. Sensitized*		
3.0	—	Formulation-mascara	192	0	No irritation, sensitization, or photosensitization	101
6.4	—	Formulation-lipstick	200	0	No irritation	103
10	—	Formulation-mascara	1595	0	No irritation	104
13	—	Formulation-cold cream	100	0	No irritation	105
Photosensitization Test						
10	—	Formulation-mascara	68	0	No irritation	104
Maximization Test						
40	—	Petrolatum solution	22	—	No sensitization	106
Controled Use Test						
3	—	Formulation-mascara	52	0	No irritation	107

each of the 386 subjects and a simultaneous open patch was applied to the upper arm. Sites were read immediately on patch removal. A 14-day nontreatment period followed and challenge open and closed patches were applied to the same sites and read 48 h later. After the second closed patch was read, the sites were exposed to UV light including a wavelength of 360 nm at a distance of 12 in

for 1 min in order to evaluate light sensitivity. No irritation or sensitization occurred under open or closed conditions. No reactions indicating photosensitivity were found.[101]

21-Day cumulative irritancy

In a 21-day cumulative irritancy test, a cold cream product containing 13% Beeswax was applied under patches for 48 h, three times per week to seven subjects. Sites scored on patch removal had no irritation (score = 0).[102]

Repeated insult patch test/contact allergenicity

A Shelanski and Shelanski Repeated Insult patch test was conducted on a mascara formulation containing 3% Beeswax. Ten 24 h occlusive patches were applied to the backs of 192 subjects. After a two-week rest, a closed 48 h challenge patch was applied to some areas to assess sensitivity. In order to test UV-light sensitization, the sites were irradiated with a UV source after removal of the first, fourth, seventh, and eleventh patches. Under closed patch conditions, one person had mild irritation after patches 7–10. No sensitivity or UV sensitization occurred. The product was nonirritating, nonsensitizing, and nonphotosensitizing.[101]

A lipstick formulation containing 6.4% Beeswax was tested for contact allergenicity in a repeated insult patch test. During the induction phase, the material was applied 10–15 times over a two to three week period to the arm or upper backs of 200 persons. After a nontreatment period of 10–14 days, a single challenge was applied to the same and/or to an adjacent site. The formulation produced no sensitization.[103]

A repeated insult patch test of a mascara formulation containing 10.0% Beeswax was negative for allergic responses in the 1,595 subjects.[104]

One hundred panelists, half of whom were "cosmetic reactive" or "sensitive subjects", were negative for sensitization when tested with a cold cream containing 13% Beeswax.[105]

Photosensitivity

A mascara formulation containing 10.0% Beeswax was tested for photosensitivity on 68 panelists. Applications of 0.1 ml/cm^2 were made for 24 h, evaluated for irritation, and then irradiated with a 150 W quartz bulb lamp with a continuous emission of 300–370 nm at a distance of 12 in from the test site. Scoring of the irradiated sites were made after 48 h, and the process was repeated six times. After a 10-day nontreatment period, a challenge application was applied to an adjacent, untreated site for 24 h. This new site was then irradiated. After 72 h, none of the 68 subjects developed photosensitivity.[104]

Skin sensitization/maximization

The sensitizing potential of Beeswax (concentration unreported) was assessed on 22 subjects. The Beeswax was applied under occlusion to the same site on the forearms for five alternate-day, 48 h periods. The initial occlusive patch site was pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. After a 10- to 14-day nontreatment period, an occlusive 48 h challenge patch was applied to a fresh site on the back. The challenge was preceded by a 30 min application of 2% aqueous SLS under occlusion to the back. The Beeswax produced no reactions that were considered allergic.[106]

The nonantigenic property of 4% unfiltered crude and filtered Beeswax was evaluated in scratch tests on 12 patients with varying degrees of sensitivity to grass pollen. There were no reactions in either test. Intracutaneous tests on 31 patients with 0.2 ml 4% Beeswax in corn oil were negative.[108]

Controled use study

A mascara containing 3% Beeswax caused no irritation when used by 52 persons in a four-week controled use study.[107]

BEESWAX/CARNAUBA WAX COMPOSITE

Some waxes are used in combination with others to produce certain physical characteristics in a cosmetic product. The following are studies on a mixture of Beeswax and Carnauba Wax included as supportive to the safety of the individual waxes.

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute

Eye irritation

Two mascara formulations, each containing 16.0% Beeswax and 5.0% Carnauba Wax, were tested for acute eye irritation in each of nine albino New Zealand rabbits. A 0.1 mg volume of each product was instilled into one eye and the untreated eye served as a control. The eyes of three rabbits from each group were washed with 20 ml of deionized water 30 sec after instillation. Observations were made 24, 48, and 72 h and four and seven days after treatment. The unwashed eyes of three of six rabbits instilled with the first sample had mild corneal stippling, minimal to moderate conjunctival redness, chemosis, and discharge for 24–48 h. The washed eyes of two of six animals had corneal stippling, redness, minimal chemosis, and discharge to 48 h.[109]

The second sample, when not washed from the eye, caused corneal stippling, minimal to moderate redness, minimal chemosis, and minimal discharge for at least 24 h. Two of six rabbits with irrigated eyes showed corneal stippling, minimal to moderate conjunctival redness, minimal to moderate chemosis, and minimal discharge from 24 to 48 h.[110]

Clinical Assessment of Safety

Skin Irritation/Sensitization

21-day cumulative irritancy test

Two formulations containing 16% Beeswax and 5% Carnauba Wax were tested for 21-day cumulative irritancy using 12 subjects. Patches were impregnated with 0.2 ml of the formulations and applied daily for 21 days to the backs of each panelist. After 21 days, the cumulative total score (CTS) which is

calculated on a basis of 10 patients, was 36.6 for the first product and 44.0 for the second product (maximum = 633).[111]

Contact sensitization

The contact sensitization potential of two products each containing 16% Beeswax and 5% Carnauba Wax was tested on 25 subjects. The materials were applied under occlusion to the same sites on the volar surface of the forearm or to the upper back for five alternate 48 h periods. The sites were pretreated with sodium lauryl sulfate (SLS) 24 h before the product was applied. After a 10-day nontreatment period, challenge patches of the formulations were applied to adjacent sites for 48 h under occlusion. Each site was pretreated with SLS 1 h prior to challenge. Observations were made immediately and 24 h after patch removal. Neither formulation caused irritation.[112,113]

CANDELILLA, PARAFFIN, OZOKERITE, AND CARNAUBA WAX MIXTURE

In order to obtain certain desirable physical properties, some cosmetic formulations may contain mixtures of waxes rather than just one wax. A mixture of 4% Candelilla Wax, 5% Ozokerite Wax, 2.5% Paraffin Wax, and 3% Carnauba Wax in a lipstick formulation designated (M) is included here to support the safety of individual waxes.

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute

Oral toxicity

The lipstick product (M) containing this mixture of waxes was tested for acute oral toxicity in 10 rats. Administration of 5 g/kg of the substance produced no toxicity.[114]

Dermal toxicity

The acute dermal toxicity of 2 g/kg of the lipstick product (M) containing the same mixture was tested on 10 rabbits. A percutaneous LD_{50} of the material was not reached.[114]

Primary skin irritation

The lipstick (M) containing the wax mixture was tested for primary skin irritation on six rabbits. The substance was minimally irritating with a PII of 0.92 (maximum score = 8.0).[114]

Ocular irritation

The acute ocular irritation of the formulation (M) containing this wax mixture was tested on six rabbits. The undiluted material produced no ocular irritation at 24, 48, and 72 h.[114]

Clinical Assessment of Safety

Skin Irritation/Sensitization

Patch testing

The wax mixture formulation (M) was tested in a Schwartz–Peck prophetic patch test and a Draize–Shelanski patch test on 212 subjects. The formulation was impregnated into patches which were then applied to the cleansed skin of the upper back for 24 h. Results were read 48 h later. After a two-week nontreatment period, open and closed patches of the formulation were applied to the test site. No reactions occurred.[114]

In a Draize–Shelanski patch test, patches impregnated with the formulation (M) were applied to the cleansed upper back skin of 100 subjects for 48 h. A simultaneous open patch was applied to the elbow. This procedure was repeated every Monday, Wednesday, and Friday for three and one-half weeks for a total of 10 insults. After a two-week nontreatment period, an eleventh open and closed patch was applied for 48 h. To evaluate light sensitization, the first, fourth, seventh, tenth, and eleventh insults were irradiated after their initial reading. These irradiated sites were then read 48 h later. No reactions occurred from this test procedure in the 100 subjects.[114]

Controled Use

Fifty-four women used the wax mixture in the lipstick formulation (M) at least three times daily for 28 days. No product-related irritation resulted from the test.[114]

SUMMARY

Waxes are water-insoluble, solid mixtures of long-chain hydrocarbons and/or esters of higher fatty acids and alcohols. Some may contain small percentages of other agents. Waxes become soft, pliable, and liquid when heated and harden when cooled.

Candelilla Wax is produced by a plant of the genus *Euphorbia*. The wax is composed of hydrocarbons, resins, acids, alcohols, and lactones. It is a hard, brittle, lustrous solid with an aromatic odor. Candelilla Wax is soluble in organic solvents, has a low melting point, and a specific gravity of 0.983. Candelilla Wax is a GRAS food substance. In cosmetic formulations, Candelilla Wax is used in facial and eye makeup preparations, in fragrance, hair and skin preparations, and in sun-protection products. Concentrations of use range from less than 0.1% to as much as 25%.

The acute oral toxicity of Candelilla Wax was tested both as a raw ingredient and in formulation. The LD_{50} of a sample of the wax in rats was greater than 5.0 g/kg. Formulations containing Candelilla Wax in concentrations of 11% and 12% were not lethal in rats in doses of 25 g/kg and 5 g/kg, respectively.

Primary skin irritation tests of 100% Candelilla Wax caused no irritation. A 75% solution of the wax in corn oil caused minimal irritation. One product containing 11% Candelilla Wax caused slight to well defined erythema and edema. A dermal irritation and systemic toxicity test of 3.5% Candelilla Wax in a formula-

tion on rabbits produced no systemic toxicity but there was slight erythema and drying of the skin at the application site.

A 75% concentration of Candelilla Wax in corn oil caused no eye irritation in rabbits. A formulation containing 11% Candelilla Wax was nonirritating to the rabbit eye.

A formulation containing 3.5% Candelilla Wax was neither teratogenic nor embryotoxic when dermally administered for 10 days to pregnant rats.

In clinical studies, a patch test of 25% Candelilla Wax in castor oil caused no irritation in 20 subjects. Also, a formulation containing 3.5% Candelilla Wax produced no irritation.

Repeated insult patch tests of formulations containing 3.5% and 11% Candelilla Wax caused no irritation, and in a cumulative irritation test, 12% Candelilla Wax in a formulation was nonirritating. In a controled use test, 25 women who used a formulation containing 11% Candelilla Wax had no irritation.

Carnauba Wax is obtained from the Brazilian tropical palm *Copernica cerifera,* and it is composed of hydrocarbons, esters, diesters, acids, and alcohols. This is the hardest plant wax, has the highest melting point (83°–86°C), and is soluble in hot organic solvents. It is a tough, brown to white lustrous material with a pleasing odor. Column, gas–liquid, and thin-layer chromatography, alkaline hydrolysis and I-R spectroscopy are used in its analysis.

Carnauba Wax is a GRAS food ingredient. Cosmetic uses of Carnauba Wax included face and eye makeup, fragrance preparations, and hair, skin, and nail preparations in concentrations of less than 0.1% to 50%.

Carnauba Wax produced no mutagenic activity when tested on microorganisms with and without metabolic activation.

In animal toxicology tests, formulations containing 5.6% and 10.0% Carnauba Wax, in doses of 1.0 and 0.5 g/kg, respectively, produced no toxicity in rats. Two formulations, one containing 5.6% and the other 10.0% Carnauba Wax, produced no ocular irritation in rabbits. In primary skin irritation studies in rabbits, three samples of 100% Carnauba Wax caused no irritation. A pink lipstick formulation containing 5.6% Carnauba Wax induced pink, uniform coloration after three days of contact with the skin. No irritation was produced by 10% Carnauba Wax in formulation.

Subchronic oral toxicity tests of Carnauba Wax were conducted on rats and dogs. Concentrations of 0%, 0.1%, 0.3%, and 1.0% wax in the diet were fed to pregnant rats and their offspring through one full generation, with no effects. Dogs consumed the same concentration for six months and had no abnormalities.

A single insult patch test of 10% Carnauba Wax in humans produced no irritation in 17 test subjects, and a repeated insult patch test of a formulation containing 5.5% wax was also nonirritating. There was no evidence of phototoxicity or photosensitivity when Carnauba Wax was tested at a concentration of 25%.

Japan Wax is a fat extracted from the berries of a small sumac, *Rhus succedania.* Japan Wax is composed of palmitin, palmitic, and other high molecular weight fatty acids. The purified wax is white and gloss-free, melts between 48°C and 55°C, and is soluble in organic solvents. Peak U-V absorption occurs between 275 and 280 nm. Japan Wax is a GRAS food substance. It is used in eye and facial cosmetics in concentrations between 0.1% and 25%

A dose of 5 g/kg Japan Wax produced no toxicity in a group of 10 rats. In acute eye irritation studies using rabbits, Japan Wax in products at concentrations of 36% caused no irritation. A 0.1 ml sample of the pure wax caused no irritation when instilled into one eye of each of three albino rabbits. A 0.5 ml sample of Japan Wax was not irritating to the clipped, abraded and intact skin of albino rabbits. Japan Wax was not mutagenic to several species of *Sacaharomyces* and *Salmonella*. Japan Wax was not mutagenic to microorganisms when they were exposed to the wax both with and without metabolic activation.

Japan Wax is not a sensitizer or irritant. A modified Draize-Shelanski-Jordan human skin irritation and sensitization study on 50% Japan Wax in petrolatum caused no irritation or allergic reactions in the 56 women tested. Similar studies on products containing 2.5% and 36% Japan Wax caused no irritation or allergy. No data on phototoxicity or photosensitivity were available for review; however, photospectrometric investigations show minimal absorbance in the UVB range. Photoreactions are not likely to occur and no clinical observations of such reactions have been reported.

Beeswax is secreted by the wax glands of bees of the species *Apis dorsata, A. indica, A. florea* and *A. mellifera*. The wax is composed of alcohols, acids, esters, and hydrocarbons; it is analyzed by various chromatographic and spectroscopic methods.

Beeswax is a GRAS food ingredient. In cosmetics, it is used in baby products, bath and fragrance preparations, eye and facial makeup, hair, skin, nail, shaving and suntan preparations, at concentrations of less than 0.1% to greater than 50%.

Beeswax was not mutagenic when tested with and without metabolic activation on microorganisms.

Acute oral doses of 5 g/kg Beeswax produced no toxicity in rats. Products containing 0.3%, 6.4%, and 13.0% Beeswax produced no toxicity in rats at doses up to 15 g/kg.

In a primary skin irritation test on rabbits, 50% Beeswax in mineral oil caused mild irritation and a PII of 0.50. One sample of 100% Beeswax caused no irritation, and two 100% samples caused mild irritation in rabbits. Formulations containing Beeswax in 0.3% and 13% concentrations resulted in minimal irritation, and two formulations containing 6.4% caused no irritation. The dermal toxicity of 50% Beeswax in corn oil was tested on the shaved, abraded abdomens of rabbits for 14 days. Abnormal responses included lethargy, ptosis, diarrhea, and nasal discharge, but no deaths.

Fifty percent Beeswax in mineral oil was practically nonirritating, and two other 50% samples caused no irritation to the eyes of rabbits. Two formulations, one containing 0.3% and the other containing 6.4% Beeswax, produced minimal irritation. No irritation was produced by two other formulations containing 6.4% Beeswax. Beeswax caused no phototoxic reactions in hairless mice and swine. Two formulations containing 13% Beeswax were tested for subchronic dermal toxicity in rabbits and rats with no topical or systemic toxic effects.

In clinical studies, patch tests of 100% Beeswax caused mild irritation in one of 20 subjects. No irritation resulted from a patch test of a formulation containing 6.4% Beeswax. Three formulations containing 13% Beeswax had a potential for minimal irritation, and a fourth containing 13% wax caused no irritation. No irritation was produced by 13% Beeswax in a formulation in a 21-day cumulative patch test.

In human subjects, repeated insult patch tests of formulations containing

6.4%, 10%, and 13% Beeswax caused no irritation. A phototoxicity test of a formulation containing 10% Beeswax and a maximization test of one with 4% wax both caused no reactions. Scratch tests and intracutaneous tests of Beeswax in peanut oil caused no reactions in tested individuals.

Formulations containing 16% Beeswax and 5% Carnauba Wax were tested for biological activity. Ocular irritation of a mascara containing the mixture was assayed in rabbits. Samples were instilled into eyes both with and without a rinse. Corneal involvement, redness, and discharge occurred up to 72 h in some animals.

In clinical studies, a 21-day cumulative irritancy test of a formulation containing 16% Beeswax and 5% Carnauba Wax caused an irritation score of 44 out of a possible 633. Two formulations containing the mixture caused no contact sensitization or irritation.

A formulation containing 4% Candelilla Wax, 5% Ozokerite Wax, 2.5% Paraffin Wax, and 3% Carnauba Wax, was tested for oral and dermal toxicity and ocular and primary skin irritation. An oral dose of 5 g/kg in rats and a dermal dose in rabbits of greater than 2 g/kg produced no toxic effects. No ocular irritation was produced in rabbits, and a PII of 0.92 indicated minimal skin irritation. A human clinical patch test and controled use test with the same formulation produced neither reactions nor irritation.

CONCLUSION

The Panel concludes that Candelilla Wax, Carnauba Wax, Japan Wax, and Beeswax are safe as used in cosmetics under present practices of concentration and use.

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