Final Report on the Safety Assessment of Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate[1]

Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate are related ingredients that function as antioxidants in cosmetic formulations. Ascorbyl Palmitate, Ascorbyl Dipalmitate, and Ascorbyl Stearate are esters and diesters of ascorbic acid with long-chain fatty acids. Erythorbic Acid is a stereoisomer of ascorbic acid and Sodium Erythorbate is the sodium salt of Erythorbic Acid. Although all of these ingredients are used, uses of Ascorbyl Palmitate and Erythorbic Acid predominate, with combined uses in over a thousand cosmetic formulations at low concentrations. Ascorbyl Palmitate is used at concentrations between 0.01 and 0.2%, and Erythorbic Acid is used at concentrations of 0.5–1%. Ascorbyl Palmitate has vitamin C activity approximately equal to that of L-ascorbic acid, whereas Erythorbic Acid has only 5% activity. The esters are likely to penetrate the skin readily, but the acid and its salt are not likely to penetrate. These ingredients exhibit low acute oral toxicity in animals. In chronic feeding studies, decreased body weight gain, the formation of oxalate stones in the bladder, and hyperplasia were seen in rats fed high levels of Ascorbyl Palmitate. Ascorbyl Palmitate (10%) and Ascorbyl Dipalmitate (100%) were not irritating to the intact skin of albino rabbits. Ascorbic Acid (30%) itself caused barely perceptible erythema and Sodium Erythorbate powder caused no irritation to the intact and abraded skin of rabbits. In animal studies, Ascorbic acid was not a sensitizer, and Erythorbic Acid (10%) applied topically to porcine skin reduced ultraviolet B (UVB)-induced phototoxicity. In clinical studies, Ascorbyl Palmitate caused no dermal irritation or sensitization. These ingredients are minimally irritating to the eye. Sodium Erythorbate did not cause fetal or maternal toxicity or developmental toxicity in rats and mice fed high levels. Although these ingredients were generally negative in a wide range of genotoxicity tests, Erythorbic Acid and Sodium Erythorbate did produce isolated positive genotoxicity test results. As antioxidants, these ingredients have been studied in animals after initiation with various carcinogens. In some cases reductions in tumor incidence were seen, in others no effect was noted. In no case did treatment with these ingredients increase tumor incidence. The highest use concentrations of Erythorbic Acid and Sodium Erythorbate are in oxidative hair dyes, where they are completely consumed in the chemical reaction that takes place at mixing. The fatty acid esters of ascorbic acid are used at lower concentrations in leave-on formulations. In consideration of these uses and based on the available safety test data, Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate are safe for use as cosmetic ingredients in the present practices of use.

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INTRODUCTION

Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate function as antioxidants in cosmetic formulations. Ascorbyl Palmitate and Ascorbyl Dipalmitate are the ester and diester of ascorbic acid and palmitic acid, respectively. Ascorbyl Stearate is the ester of ascorbic acid and stearic acid. Erythorbic Acid is a stereoisomer of ascorbic acid. This report reviews safety test and other data relevant to the assessment of Ascorbyl Palmitate, Erythorbic Acid, and Sodium Erythorbate as used in cosmetic products.

CHEMISTRY

Definition and Structure

Ascorbyl Palmitate

Ascorbyl Palmitate (CAS No. 137-66-6) generally conforms to the formula shown in Figure 1a (Wenninger and McEwen 1997), although it may contain Ascorbyl Stearate, shown in Figure 1c (Scientific Committee on Cosmetology [SCC] 1993; Food and Agriculture Organization of the United Nations/World Health Organization [FAO/WHO] 1996). Other names for this chemical include: L-Ascorbic Acid, 6-Hexadecanoate (Wenninger and McEwen 1997; Hazardous Substances Data Base [HSDB] 1994); 6-Hexadecanoyl-L-Ascorbic Acid; 6-Monopalmitoyl-L-Ascorbate; 6-*O*-Palmitoylascorbic Acid; 6-Palmitoylascorbic Acid; Ascorbic Acid Palmitate; Ascorbic Palmitate; Ascorbyl Monopalmitate; Ascorbylpalmitic Acid; Cetyl Ascorbate; L-Ascorbic Acid, 6-Palmitate; L-Ascorbyl 6-Palmitate; L-Ascorbyl Monopalmitate; L-Ascorbyl Palmitate, Ondascora, Palmitoyl L-Ascorbic Acid; Quicifal (HSDB 1994); 6-Palmitoyl-3-Keto-L-Gulofuranolactone; 2,3-Dehydro-L-Threo-Hexono-1,4-Lactone-6-Palmitate; and Vitamin C Palmitate (FAO/WHO 1996).

Ascorbyl Dipalmitate

Ascorbyl Dipalmitate (CAS No. 28474-90-0) is the diester of ascorbic acid and palmitic acid that conforms generally to the formula shown in Figure 1b. Another name for Ascorbyl Dipalmitate is L-Ascorbic Acid, Dihexadecanoate (Wenninger and McEwen 1997).

Ascorbyl Stearate

Ascorbyl Stearate (CAS No. 25395-66-8) is the ester of ascorbic acid and stearic acid. It conforms generally to the formula:

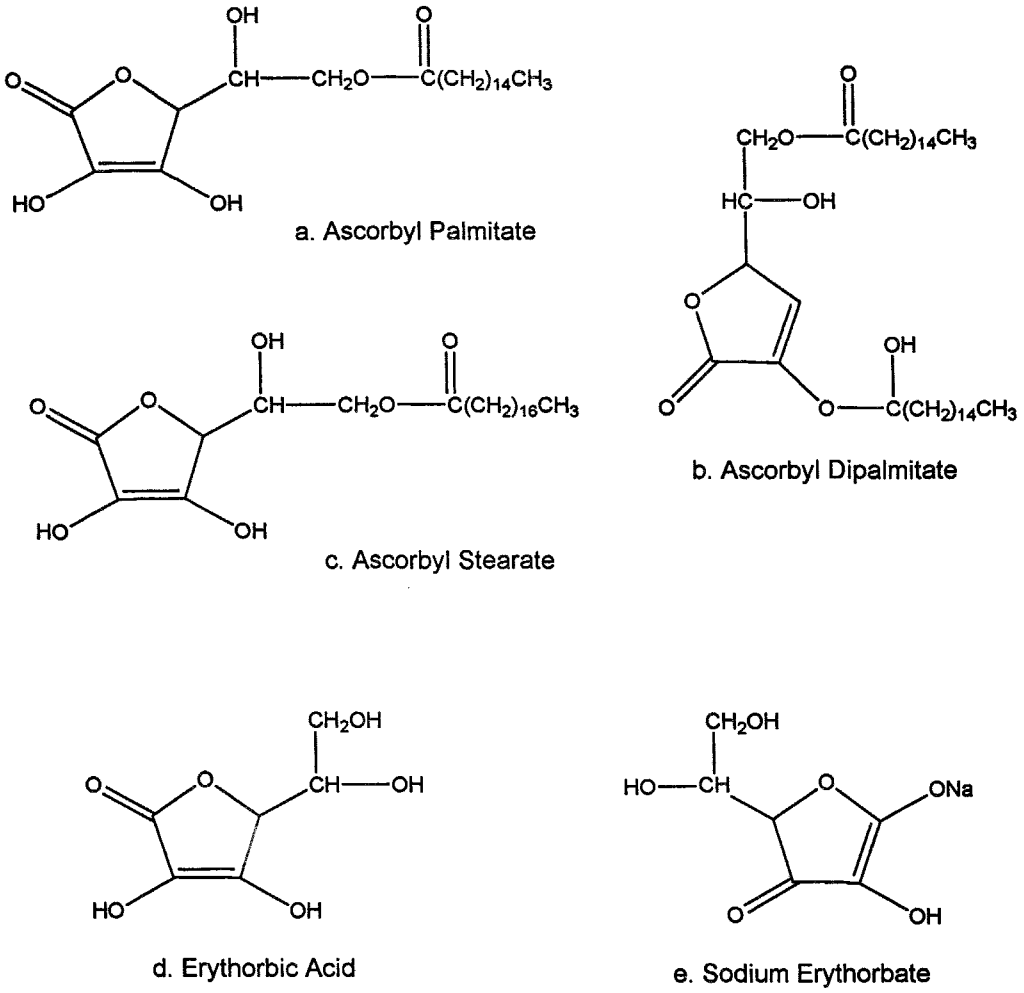

FIGURE 1
Chemical formulae of (a) Ascorbyl Palmitate, (b) Ascorbyl Dipalmitate, (c) Ascorbyl Stearate, (d) Erythorbic Acid, and (e) Sodium Erythorbate.

shown in Figure 1c (Wenninger and McEwen 1997). Ascorbyl Stearate may contain Ascorbyl Palmitate (SCC 1993; FAO/WHO 1996). Synonyms for Ascorbyl Stearate are L-Ascorbic Acid, Monooctadecanoate (Wenninger and McEwen 1997); L-Ascorbyl Stearate; 6-Stearoyl-3-Keto-L-Gulofuranolactone; 2,3-Dehydro-L-Threo-Hexono-1,4-Lactone-6-Stearate; and Vitamin C Stearate (FAO/WHO 1996).

Erythorbic Acid

Erythorbic Acid (CAS No. 89-65-5) conforms to the formula shown in Figure 1d (Wenninger and McEwen 1997). Other names for this compound include Isoascorbic Acid, D-Isoascorbic Acid, Araboascorbic Acid, D-Araboascorbic Acid, Erycorbin, D-Erythorbic Acid, Glucosaccharonic Acid, Isovitamin C, Mercate 5, Neo-Cebicure, Saccharosonic Acid (Registry of Toxic Effects of Chemical Substances [RTECS] 1994; HSDB 1994), D-Erythro-Hex-2-Enonic Acid Gamma Lactone (HSDB 1994; Wenninger and McEwen 1997; FAO/WHO 1996), D-Erythro-Ascorbic Acid, D-Erythro-3-Ketohexonic Acid Lactone, and D-Erythro-3-Oxohexonic Acid Lactone (HSDB 1994).

Sodium Erythorbate

Sodium Erythorbate (CAS No. 6381-77-7) is the sodium salt of Erythorbic Acid which conforms generally to the formula shown in Figure 1e (Wenninger and McEwen 1997). Synonyms for Sodium Erythorbate include D-Erythro-Hex-2-Enonic Acid, γ-Lactone, Monosodium Salt; Sodium Isoascorbate (Wenninger and McEwen 1997); Isoascorbic Acid, Sodium Salt; Erythorbic Acid Sodium Salt; Sodium D-Isoascorbate; Mercate 20; Isona; Neo-Cebitate (Radian Corporation 1991); 3-Keto-D-Gulofuranolactose; Sodium Enolate Monohydrate (FAO/WHO 1996);

TABLE 1
Physical and chemical properties of Ascorbyl Palmitate

Property	Description	Reference
Molecular formula	$C_{22}H_{38}O_7$	Wenninger and McEwen 1997
Molecular weight	414.54	Gennaro 1990
Appearance	White or yellowish-white powder at room temperature	Lewis 1993; FASEB 1979
Odor	Citrus-like	Lewis 1993
Melting point	107–117°C	Osol 1980; Yakuji Nippo, Ltd. 1979
	116–117°C	Lewis 1993; Sax 1979
Solubility	Soluble in alcohol and vegetable oils, slightly soluble in water	Lewis 1993
	1 g in >1000 ml of water, 125 ml of alcohol, >1000 ml of chloroform, or >1000 ml of ether	Gennaro 1990
Assay	Not less than 95.0% of $C_{22}H_{38}O_7$ calculated on dried basis. Dissolve about 300 mg in 50 ml alcohol, and 30 ml water, and titrate with 0.1 N iodine to a yellow color. 1 ml 0.1 N iodine = 20.73 mg $C_{22}H_{38}O_7$	NAS 1996
Specific rotation	$[\alpha_D]^{25}$: +21 to +24 (1 g in 10 ml methanol)	NAS 1996
Loss on drying	2.0% maximum in vacuum at 60°C for 1 hour	Nikitakis and McEwen 1990; NAS 1996
Residue on ignition	Not more than 0.1%	NAS 1996
Sulfated ash	0.1% minimum	Nikitakis and McEwen 1990
Arsenic (as As)	3 ppm maximum	Nikitakis and McEwen 1990
Lead (as Pb)	20 ppm maximum	Nikitakis and McEwen 1990
	10 ppm maximum	NAS 1996

and Sodium D-Erythro-3-Oxohexonate Lactone (Ema, Itami, and Kanoh 1985).

Physical and Chemical Properties

The physical and chemical properties of Ascorbyl Palmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate are summarized in Tables 1 to 4, respectively. No information was available describing the physical and chemical properties of Ascorbyl Dipalmitate.

Ascorbyl Palmitate has greater fat solubility than ascorbic acid (Food and Drug Administration [FDA] 1983). Erythorbic Acid gradually darkens in color when exposed to light. It is

TABLE 2
Physical and chemical properties of Ascorbyl Stearate

Property	Description	Reference
Molecular formula	$C_{24}H_{42}O_7$	Wenninger and McEwen 1997
Appearance	White crystalline powder	Yakuji Nippo, Ltd. 1979
Odor	"Slight characteristic odor"	Yakuji Nippo, Ltd. 1979
Melting point	115–118°C	Yakuji Nippo, Ltd. 1979
Assay	Dry for 4 h in desiccator (sulfuric acid), dissolve 0.3 g in 50 ml ethanol, add 25 ml 0.1 N iodine, add 3 drops methylene blue solution (1/1000), titrate with 0.1 N sodium thiosulfate immediately until solution turns blue and continues for 30 min. 1 ml 0.1 N sodium thiosulfate = 22.130 mg $C_{24}H_{42}O_7$	Yakuji Nippo, Ltd. 1979
Loss on drying	Not more than 2% (1 g, reduced pressure, sulfuric acid for 4 h)	Yakuji Nippo, Ltd. 1979
Residue on ignition	Not more than 0.1%	Yakuji Nippo, Ltd. 1979
Arsenic (as As)	2 ppm maximum	Yakuji Nippo, Ltd. 1979

TABLE 3
Physical and chemical properties of Erythorbic Acid

Property	Description	Reference
Molecular formula	$C_6H_8O_6$	Wenninger and McEwen 1997
Molecular weight	176.12	Budavari 1989; Aldrich 1992
	176.03	Grant 1972
	176.12	RTECS 1994
Appearance	Shiny granular crystals from water or dioxane	Budavari 1989
	White to yellowish-white crystals	Yakuji Nippo, Ltd. 1979
Odor	Odorless	Yakuji Nippo, Ltd. 1979
Melting point with decomposition	174°C	Budavari 1989
	164–169°C	Lewis 1993
	169–172°C	Aldrich 1992
Solubility	Soluble in water, alcohol, pyridine	
	Moderately soluble in acetone	
	Slightly soluble in glycerol	
	Free acid more soluble in water than sodium salt	Budavari 1989; Lewis 1993 Budavari 1989
Assay	Not less than 99.0% of $C_6H_8O_6$. Dissolve about 400 mg in 100 ml water and 25 ml diluted sulfuric acid, titrate with 0.1 N iodine, add starch near endpoint. 1 ml 0.1 N iodine = 8.806 mg $C_6H_8O_6$	NAS 1996
Specific rotation	$[\alpha_D]^{16.5}$: −17 $[\alpha_D]^{20}$: −16.6	Budavari 1989; FASEB 1979
	$[\alpha_D]^{20}$: −16.2 ~ −18.2 (1.0 g, water, 10 ml, 100 mm)	Yakuji Nippo, Ltd. 1979
pH (aqueous)	Between 5–6 (16 g in 100 ml water)	HSDB 1994
	Between 7.2–7.9 (10% aqueous solution from commercial grade)	Budavari 1989
Loss on drying	Not more than 2%	Yakuji Nippo, Ltd. 1979
Residue on ignition	Not more than 0.3%	NAS 1996
Arsenic (as As)	Not more than 3 ppm	NAS 1996
Lead (as Pb)	Not more than 10 ppm	NAS 1996

reasonably stable in air unless in solution, when it deteriorates rapidly (National Academy of Sciences [NAS] 1996). Sodium Erythorbate is sensitive to prolonged exposure to light (Radian Corporation 1991), and deteriorates in the presence of air, trace metals, and heat when in solution. In the dry state, it is fairly stable in air (NAS 1996). Sodium Erythorbate is combustible, and is incompatible with strong oxidizing agents (Radian Corporation 1991).

Method of Manufacture

Ascorbyl Palmitate is prepared by condensing palmitoyl chloride and ascorbic acid in the presence of a dehydrochlorinating agent such as pyridine (Gennaro 1990). It is also formed in the reaction of L-ascorbic acid and palmitic acid (HSDB 1994).

Ascorbyl Stearate is produced by the reaction of L-ascorbic acid and stearic acid (SCC 1993).

Erythorbic Acid is produced by the fermentation of D-glucose to 2-keto-D-gluconic acid by *Pseudomonas fluorescens* bacteria. The fermentation product is esterified and heated in basic solution to yield Sodium Erythorbate. Upon acidification of the salt in a water-methanol solution, Erythorbic Acid is formed (FDA 1983). Erythorbic Acid can also be prepared by reacting 2-keto-D-gluconate with sodium methoxide, synthesized from sucrose, or naturally produced by *Penicillium* species (Budavari 1989). Sodium Erythorbate is prepared from D-glucose by a combination of biosynthesis and chemical synthesis via the intermediate 2-keto-D-gluconic acid (FDA 1983; Rothschild 1990).

Analytical Methods

Ascorbyl Palmitate can be identified through infrared spectroscopy and high performance liquid chromatography (Suzuki et al. 1987). A 1:10 dilution of Ascorbyl Palmitate in alcohol decolorizes 2,6-dichlorophenol-indophenol TS (NAS 1996).

A 1:50 dilution of Erythorbic Acid reduces alkaline cupric tartrate TS at both room temperature and when heated. A 1:50 solution turns blue when sodium nitroferricyanide TS and sodium

TABLE 4
Physical and chemical properties of Sodium Erythorbate

Property	Description	Reference
Molecular formula	$C_6H_8O_6Na$	Radian Corporation 1991
Molecular weight	199.12	Radian Corporation 1991
Appearance	White, crystalline powder or granules Fluffy, off-white powder	Radian Corporation 1991; NAS 1996
Odor	Odorless	Radian Corporation 1991; NAS 1996
Melting point	154–164°C (decomposes)	Radian Corporation 1991
Solubility (18°C)	≥100 mg/ml water; ~1 g/7 ml water 1–10 mg/ml DMSO <1 mg/ml ethanol (95%) or acetone	Radian Corporation 1991
Assay	Not less than 98.0% and not more than 100.5% $C_6H_8O_6Na$. Dissolve about 400 mg in 100 ml alcohol, and 25 ml 2 N sulfuric acid, and titrate with 0.1 N iodine. Add strarch TS near endpoint. 1 ml 0.1 N iodine = 10.81 mg $C_6H_8O_6Na$	NAS 1996
pH (1:20 solution)	5.5–8.0	NAS 1996
Specific gravity	1.2	NAS 1996
Specific rotation	$[\alpha_D]^{25}$: between +95.5° and +98.0°	NAS 1996
Heavy metals (as Pb)	Not more than 10 mg/kg	NAS 1996
Lead (as Pb)	Not more than 5 mg/kg	NAS 1996

hydroxide are added. Erythorbic Acid can also be identified by infrared spectroscopy and nuclear magnetic resonance (Tsao, Dunham, and Leung 1992).

A 1:50 dilution of Sodium Erythorbate slowly reduces alkaline cupric tartrate TS at 25°C, but more readily upon heating. A transient blue color is produced when a few drops of sodium nitroferricyanide TS and 1 ml of 0.1 N sodium hydroxide are added to 2 ml of a 1:50 dilution of Sodium Erythorbate that has been acidified with 0.5 ml of 0.1 N hydrochloric acid (NAS 1996).

Impurities

The National Formulary (NF) states that Ascorbyl Palmitate must contain between 95.0% and 100.5% of $C_{22}H_{38}O_7$, based on the dried weight (NF 1995). Depending on the method of manufacture, Ascorbyl Palmitate could contain stearic acid, because palmitic acid samples contain large quantities of stearic acid. Likewise, Ascorbyl Stearate could contain palmitic acid (SCC 1993; FAO/WHO 1996). Ascorbyl Stearate, when dried, contains not less than 93% of ascorbyl-L-stearate (Yakuji Nippo, Ltd. 1979). In data submitted by Clairol (1996a), the minimum purity of Erythorbic Acid is 99%. Due to its method of synthesis (starting materials, sucrose and 2-keto-D-gluconate), Erythorbic Acid is not expected to contain significant impurities.

Ultraviolet Absorbance

Isoascorbic acid (D- or L-form not specified), ascorbic acid, sodium ascorbate, and potassium isoascorbate had strong absorption bands at approximately 265 nm due to the π-π^* excitation of the C=C double bond (Lohmann, Pagel, and Penka 1984). The maximum absorbance of Erythorbic Acid (0.5% in water) occurred at 260 nm and there was no significant absorption above 290 nm (Clairol 1996a).

USE

Cosmetic

Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate function as antioxidants in cosmetic formulations (Wenninger and McEwen 1997). Product formulation data submitted to the Food and Drug Administration (FDA) in January 1996 state that Ascorbyl Palmitate, Erythorbic Acid, and Sodium Erythorbate are used in 561, 728, and 19 cosmetic product formulations, respectively, as detailed in Tables 5 to 7. Ascorbyl Dipalmitate and Ascorbyl Stearate are each used in one lipstick (FDA 1996).

Concentration of use values are no longer submitted to the FDA by the cosmetic industry (FDA 1992), but product formulation data submitted in 1984 stated that Ascorbyl Palmitate and Erythorbic Acid were both used at concentrations up to 1%. Sodium Erythorbate was used in hair coloring preparations at concentrations >50% (FDA 1984).

Clairol (1996a) reported that Erythorbic Acid was used at up to 0.6% in both oxidative and semipermanent hair dyes as an antioxidant to protect the dye ingredients from air oxidation during manufacture. Additionally, 0.5% Erythorbic Acid serves as an additive in semipermanent toners to neutralize any residual persulfate in the freshly bleached hair to which the toner is

TABLE 5
Product formulation data for Ascorbyl Palmitate (FDA 1996)

Product category	Total no. of formulations in category	Total no. containing ingredient
Eyebrow pencil	99	3
Eyeliner	533	1
Eye shadow	588	51
Eye lotion	22	2
Other eye makeup preparations	136	17
Powders	307	2
Other fragrance preparations	195	3
Hair conditioners	715	1
Hair sprays (aerosol fixatives)	334	4
Rinses (noncoloring)	60	2
Shampoos (noncoloring)	972	1
Tonics, dressings, and other hair grooming aids	604	3
Blushers (all types)	277	50
Face powders	313	31
Foundations	355	57
Lipstick	997	215
Makeup bases	154	16
Rouges	30	2
Other makeup preparations	157	8
Cuticle softeners	26	1
Other personal cleanliness products	339	1
Shaving cream	158	5
Shaving soap	3	1
Cleansing	820	2
Face and neck (excluding shaving)	300	5
Body and hand (excluding shaving)	1012	13
Moisturizing preparations	942	24
Night	226	4
Paste masks (mud packs)	300	12
Other skin care preparations	810	15
Suntan gels, creams, and liquids	196	6
Indoor tanning preparations	67	1
Other suntan preparations	68	2
1996 total		**561**

applied. In hair dyes, use concentrations would not be greater than 1%. In oxidative hair dyes, the Erythorbic Acid was rapidly oxidized when the color lotion was mixed with hydrogen peroxide immediately prior to application (Clairol 1996a).

Ascorbyl Palmitate was used at concentrations of 0.02% in body cleanser, 0.02% in cologne, 0.01% in body oil, and 0.2% in eye cream (Cosmetic, Toiletry, and Fragrance Association 1996).

TABLE 6
Product formulation data for Erythorbic Acid (FDA 1996)

Product category	Total no. of formulations in category	Total no. containing ingredient
Permanent waves	434	4
Hair dyes and colors	1612	702
Hair tints	57	19
Basecoats and undercoats	54	1
Mouthwashes and breath fresheners	45	1
1996 total		**728**

International

Ascorbyl Palmitate, Erythorbic Acid, and Sodium Erythorbate are included in the *Japanese Standards of Cosmetic Ingredients*, 2nd edition. The inclusion of any ingredient does not guarantee whether that ingredient is safe for use as a cosmetic ingredient, or that the use of the substance as a cosmetic ingredient complies with the laws and regulations governing such use in Japan (Rempe and Santucci 1992). In Japan, Ascorbyl Palmitate was used at concentrations of 0.1–4% in cosmetic preparations to inhibit the formation of melanine (SCC 1993).

Ascorbyl Palmitate, Erythorbic Acid, and Sodium Erythorbate are not included among the ingredients listed as prohibited for use in cosmetic products marketed in the European Union (European Economic Community [EEC] 1994). In Europe, Ascorbyl Palmitate and Erythorbic Acid were reportedly used in cosmetic products at concentrations $\leq$2%. Ascorbyl Palmitate is used at a concentration of 0.5% in cosmetic water/oil emulsions as an antioxidant for vegetable oils with large concentrations of unsaturated fatty acids (SCC 1993). Erythorbic Acid is prohibited for use in food in most European countries (Tsao and Salimi 1983; SCC 1993).

Noncosmetic

The Acceptable Daily Intake (ADI) of Ascorbyl Palmitate or Ascorbyl Stearate is 0–1.25 mg/kg. The ADIs of both Erythorbic Acid and Sodium Erythorbate are not specified, meaning that the

TABLE 7
Product formulation data for Sodium Erythorbate (FDA 1996)

Product category	Total no. of formulations in category	Total no. containing ingredient
Hair dyes and colors	1612	18
Other hair coloring preparations	71	1
1996 total		**19**

food additives were of such low toxicity at use concentrations that "the establishment of an ADI expressed in numerical form [was] not deemed necessary" (FAO/WHO 1996).

Ascorbyl Palmitate

Ascorbyl Palmitate is an antioxidant. It is used as a chemical preservative food additive (Sax 1979) and as an antioxidant in pharmaceuticals (Gennaro 1990). This ingredient is a generally recognized as safe (GRAS) chemical preservative when used according to good manufacturing practice (Code of Federal Regulations [CFR] §182.3149). Its use as a preservative in margarine is limited to 0.02% by weight of the finished food (CFR §166.110). Ascorbyl Palmitate in foods prevents rancidity and the browning of cut apples. It is also used in meat curing to preserve canned and frozen foods (Gennaro 1990), as a source of vitamin C, and as a stabilizer and emulsifier (Lewis 1993).

Erythorbic Acid

Erythorbic Acid, an antioxidant, is a GRAS chemical preservative when used according to good manufacturing practice (CFR §182.3041). It serves as a preservative in food (NAS 1996), a reducing agent in photography (Lewis 1993), and an antimicrobial agent (FDA 1995). Erythorbic Acid prevents beer haze when used as an antioxidant in the brewing industry (Grant 1972; Lewis 1993). It is limited to a maximum concentration of 150 ppm in canned applesauce (CFR §145.110).

Sodium Erythorbate

Sodium Erythorbate is an antioxidant and preservative in foods (Rothschild 1990; Radian Corporation 1991; NAS 1996), and serves to stabilize color, reduce fat oxidation, and inhibit nitrite reactions in meat curing (Radian Corporation 1991). Sodium Erythorbate is a antioxidant, curing agent, and pickling agent in alcoholic beverages, nonalcoholic beverages and beverage bases, breakfast cereals, and meat, milk, and poultry products. When used to accelerate color fixation in cured beef and pork cuts, or in cured comminuted meat food products, 0.88 oz. of Erythorbic Acid per 100 lb. of meat or meat by-product is allowed. Sodium Erythorbate is an adjunct used to prevent oxidation in brewing at a level of 70 ppm. A 10% solution may be added to the surfaces of cured cuts prior to packaging. When employed as a pickling agent, 87.5 oz of Sodium Erythorbate per 100 gallons pickle (at 10% pump level) is used (Rothschild 1990). Sodium Erythorbate is not listed as a GRAS ingredient under FDA regulations, but FDA has acknowledged that its use as a chemical preservative is GRAS in numerous opinion letters that have been issued since 1960. A proposal was made by the FDA to affirm the ingredient's GRAS status (FDA 1983), but this was withdrawn in 1991 (Rothschild 1991).

Foliar application of Sodium Erythorbate sprays and dusts are used to control young tree decline in citrus trees (Leonard 1977) and to reduce ozone damage to Thompson seedless grapes (Brewer, Carroll, and Cookston 1987).

GENERAL BIOLOGY

Ascorbyl Palmitate has a vitamin C activity approximately equal to that of L-ascorbic acid (FDA 1983). Erythorbic Acid, a stereoisomer of ascorbic acid, has 1/20 of its vitamin C activity (HSDB 1994; Budavari 1989), as does its sodium salt, Sodium Erythorbate (FDA 1983).

Vitamin C is an essential cofactor for prolyl and lysyl hydroxylases, the enzymes involved in the intracellular biosynthesis of collagen (Shimizu, McCann, and Keech 1965a; Anderson, McLean, and Elliott 1991; Oikarinen 1992). Erythorbic Acid at concentrations of 100–200 μg/ml was as effective as ascorbic acid in promoting collagen synthesis in cultured human dermal fibroblasts (Shimizu, McCann, and Keech 1965b).

Kawanishi et al. (1981a) injected sodium nitrite and other chemicals (e.g., aminopyrine) directly into the lumen of Wistar rat (male and female) and Hartley guinea pig (male) stomachs. After 10–30 minutes, the stomach was excised and the gastric content washed out with 10 ml of deionized water. Three minutes after washing out, 5 ml of 20% sulfamic acid was added to consume nitrite. Nitrosamine formation was determined using gas chromatography. The formation of nitrosamines in the rat stomach by the reaction of aminopyrine (40 μmol/kg) and sodium nitrite (200 μmol/kg) was inhibited by the simultaneous injection of Sodium Erythorbate or ascorbic acid at a concentration of 25 μmol/kg. The observed inhibition was dose-dependent; 100 μmol/kg prevented nitrosamine formation by more than 50% (Kawanishi et al. 1981a). Sodium Erythorbate and ascorbic acid also repressed hepatic injury induced by *N*-nitrosodimethylamine formed by the reaction of aminopyrine and sodium nitrite, as compared to controls (Kawanishi et al. 1981b).

Erythorbic Acid inactivated various phages, including J1, T5, T4, MS2, ϕ6, and $\phi \times 174$, at concentrations ranging from 1×10^{-4} to 1×10^{-3}. Inactivation occurred due to free radicals formed during the autoxidation of Erythorbic Acid (Murata et al. 1986).

Absorption, Distribution, Metabolism, and Excretion

Ascorbyl Palmitate

When applied topically to guinea pigs, Ascorbyl Palmitate penetrated the skin barrier so that ascorbic acid content in the skin, liver, and blood increased eight-, seven-, and fourfold, respectively, when compared to control animals that did not receive Ascorbyl Palmitate (Koroleva et al. 1981).

^{14}C-Ascorbyl Palmitate was applied to the skin of scorbutic (affected by scurvy) guinea pigs. Following the topical application, ascorbic acid concentrations in the skin, liver, kidneys, and blood were four to eight times greater than in the control (SCC 1993).

Ascorbyl Palmitate dissolved in a sodium taurocholate solution was hydrolyzed by homogenates of the liver, pancreas, and intestines of guinea pigs. Approximately 80% of Ascorbyl Palmitate was hydrolyzed to free ascorbic acid by homogenates of the small intestine and pancreas. In the same study, Ascorbyl

Palmitate (the equivalent of 20 mg of ascorbic acid) was orally administered to guinea pigs, and the amount of free ascorbic acid excreted in the urine was measured. Greater amounts of acid were excreted at 0–24 hours than at 24–48 hours. A similar trend was found in these organs of free ascorbic acid content when L-ascorbic acid was administered instead, but a reverse tendency was observed with Ascorbyl Palmitate (Inagaki and Kawaguchi 1966).

Ascorbyl Dipalmitate

Ascorbyl Dipalmitate dissolved in a sodium taurocholate solution was hydrolyzed by homogenates of the liver, pancreas, and intestines of guinea pigs. Ascorbyl Dipalmitate (~20%) was hydrolyzed to free ascorbic acid by homogenates of the small intestine and pancreas. In the same study, Ascorbyl Dipalmitate (the equivalent of 20 mg of ascorbic acid) was orally administered to guinea pigs, and the amount of free ascorbic acid excreted in the urine was measured. Greater amounts of acid were excreted at 0–24 hours than at 24–48 hours. A similar trend was found in these organs of free ascorbic acid content when L-ascorbic acid was administered instead, but a reverse tendency was observed with Ascorbyl Dipalmitate (Inagaki and Kawaguchi 1966). In this and a subsequent study (Inagaki, Arakawa, and Atsumi 1968), a difference was found in the body retention or availability of Ascorbyl Palmitate and Ascorbyl Dipalmitate due to the differences in the extent and rate of hydrolysis of the two esters.

Erythorbic Acid

The absorption of Erythorbic Acid through the human buccal mucosa was studied in healthy adult subjects. Absorption of a solution of 10 mM Erythorbic Acid, buffered to pH 6, was 13.0 ± 0.74 μmol/5 minutes. No statistical difference was found between the absorptions of Erythorbic Acid and L-ascorbic acid (Sadoogh-Abasian and Evered 1979).

Several different studies have explored the absorption of Erythorbic Acid in relation to L-ascorbic acid absorption in male Hartley guinea pigs. In one study three groups of guinea pigs (numbers not stated) were fed a diet that included 0.3 mg/100 g L-ascorbic acid (Hughes and Hurley 1969). Guinea pigs of Group A received no supplement; animals of Group B were fed 1.5 mg/100 g Erythorbic Acid; and Group C guinea pigs were fed an additional 1.5 mg/100 g L-ascorbic acid. The animals were killed and the adrenal glands and spleen were extracted to determine the combined concentration of Erythorbic Acid and L-ascorbic acid. No significant difference was observed in *total* ascorbic acid concentration between the control (Group A) group values and those of the supplemented Erythorbic Acid group (Group B). The ascorbic acid concentration was greatest in Group C guinea pigs that were supplemented with additional ascorbic acid. The data indicated that Erythorbic Acid was not deposited in these tissues at the administered concentration. In the same study, the supplements were given intramuscularly instead of orally with the same experimental setup. Parenteral administration of Erythorbic Acid increased the concentration of total ascorbic acid by 144% in the adrenal glands and 61% in the spleen when compared to controls. Group C, receiving the L-ascorbic acid supplement, had the greatest amount of ascorbic acid of the extracted tissues. These results indicated that the gastrointestinal permeability differed between the two chemicals (Hughes and Hurley 1969).

Another Hartley guinea pig study explored the effects of graded doses of Erythorbic Acid. The doses ranged from 1 to 100 mg/day of Erythorbic Acid for 16 days. Body weights increased during the experiment. On the 16th day, the guinea pigs were killed and the liver, adrenal glands, spleen, and kidneys of each were analyzed by high-performance liquid chromatography (HPLC). A small amount of Erythorbic Acid was detected in tissues of animals of the higher dose groups (Suzuki et al. 1987).

In studies in which L-ascorbic acid intake was low, the greatest concentrations of Erythorbic Acid were in the liver, adrenal glands, spleen, and kidneys (Suzuki et al. 1986). When different groups of guinea pigs were given a diet supplemented with ascorbic acid (5 mg/day), concentrations of ascorbic acid in the above tissues were always greater than when Erythorbic Acid (100 mg/day) and ascorbic acid (5 mg/day) were administered simultaneously (Arakawa et al. 1986).

Hornig (1977) reported that absorption was approximately four-to-one in favor of ascorbic acid, but the availability of ascorbic acid decreased by 40–60% when administered with Erythorbic Acid. Further data indicated that Erythorbic Acid promoted the acceleration of oxidative destruction of ascorbic acid in the liver.

Another study by Suzuki, Kurata, and Arakawa (1991) determined that Erythorbic Acid was absorbed less efficiently than ascorbic acid in the small intestine of male Hartley guinea pigs. The data also suggested that Erythorbic Acid was absorbed from the small intestine by the same active transport mechanism as used for ascorbic acid. The absorption rates of both in the small intestine could be dependent on the concentration of ascorbic acid already present in the tissues of the guinea pig.

Ascorbic acid transport in the brush border vesicles of the guinea pig small intestine was Na^+-dependent and electroneutral in the presence of Na^+. The ascorbic acid transporter had the kinetic characteristics of a mobile carrier ($K_m = 0.3$ mM). Ascorbic acid transport was subject to heterologous inhibition by D-glucose due to the increased intravesicular Na^+ concentration. Erythorbic Acid ($K_i = 20$ mM) was a poorer substrate of the same transporter and inhibited the transport of ascorbic acid (Siliprandi et al. 1979). Similar results occurred in the human small intestine (Toggenburger, Landoldt, and Semenza 1979).

Tsao and Salimi (1983) gave 21 Swiss Webster female mice (4-week-old) feed containing 5% ascorbic acid or Erythorbic Acid crystals for 2 months. The mice (five per group) were then fed diet containing 10% ascorbic acid or Erythorbic Acid for 5 additional months. Eleven mice received ascorbic acid-free diet throughout the experiment. Urine was collected and analyzed 2 weeks before termination of the study. The mice were killed

and their brains and livers were removed and stored for analysis. The amount of urinary Erythorbic Acid excreted from mice given Erythorbic Acid was approximately twice that of mice given ascorbic acid. Urinary ascorbic acid differed significantly (increased 60-fold) in mice given ascorbic acid when compared to controls. Ascorbic acid and Erythorbic Acid plasma concentrations were greater in the treated mice than the controls. The investigators reported that Erythorbic Acid replaced 45% of ascorbic acid in the liver and 28–39% of ascorbic acid in the brain.

Erythorbic Acid was not transported in vivo into the brain, cerebrospinal fluid (Hornig 1977), white blood cells, adrenal glands, and the globes as effectively as L-ascorbic acid (Pelletier 1969).

The reduced form of Erythorbic Acid was incorporated into human erythrocytes at the rate of 20% per 2 hours and the rate of uptake of this form was proportional to the extracellular concentration. The oxidized form of Erythorbic Acid, D-dehydroisoascorbic acid, became incorporated more rapidly than the reduced form, at a rate of 50% per 5 minutes, and 80% of the acid absorbed was subsequently reduced within the cells. The reduced form of Erythorbic Acid was more stable in plasma than the oxidized form, of which 61% was degraded in 60 minutes. In erythrocytes, the reduced form was stable, as in plasma, and the oxidized form slightly less so (Teruuchi and Okamura 1972).

The results of both short- and long-term studies indicated that Erythorbic Acid was readily metabolized and did not affect the urinary excretion of free ascorbic acid (Joint Expert Committee on Food Additives [JECFA] 1974). Erythorbic Acid administered orally to male 6-week-old F344 rats for 16 weeks caused a lowering of urinary pH (to pH 6.04), but did not produce significant changes in the concentrations of sodium, potassium, calcium, chloride, phosphorus, and magnesium in the urine. Additionally, no increase of crystal production was reported (Fukushima et al. 1986).

Erythorbic Acid apparently was not reabsorbed after glomerular filtration, and, therefore, was excreted from the kidneys more rapidly than L-ascorbic acid. In dogs, this resulted in a half-life of approximately 30 minutes for Erythorbic Acid in the plasma (Silber 1956). Wang, Fisher, and Dodds (1962) reported that Erythorbic Acid was excreted faster than L-ascorbic acid in humans.

Sodium Erythorbate

Male F344 rats (five per group, 6-week-old) were given 5% Sodium Erythorbate in feed for 22 weeks. The rats eliminated totals of 203.3 ± 33.2 mg/100 ml erythorbic acid and 9.0 ± 5.1 mg/100 ml dehydroerythorbic acid during the study. Ascorbic acid and dehydroascorbic acid were not detected. Urine pH was 6.98 ± 0.31, which was significantly different from that of rats given basal diet alone (6.31 ± 0.18; $p < 0.05$). Urine osmolarity also differed significantly from controls; osmolarity was 1378 ± 277 mOsmol/kg H_2O in rats given Sodium Erythorbate and 1756 ± 200 mOsmol/kg H_2O in rats of the control group. Crystals were detected in urine of rats given basal diet and Sodium Erythorbate or basal diet alone (Fukushima et al. 1984).

Pharmacologic Effects

Ascorbyl Palmitate

Male MF1 mice in which hepatotoxicity had been induced by the feeding of 600 mg/kg acetaminophen had covalent binding of acetaminophen metabolites to hepatic proteins, a depletion of hepatic nonprotein sulphydryl groups after 2 hours, and a dramatic increase in plasma alanine aminotransferase activity after 24 hours. The coadministration of acetaminophen and Ascorbyl Palmitate reduced this binding within 2 and 4 hours (to 31% and 22%, respectively), reduced the depletion in nonprotein sulphydryl groups and aminotransferase activity, and completely prevented the 35% mortality observed at 24 hours after acetaminophen treatment alone. Ascorbyl Palmitate appeared to prevent hepatic damage by removing the reactive acetaminophen metabolites and by having a sparing action on reduced hepatic glutathione (Jonker et al. 1988).

In a similar study, Mitra et al. (1988) gavaged male Swiss-Webster mice with 600 mg/kg acetaminophen to cause increases in liver weight/body weight ratio and hepatic glutathione depletion. Coadministration with Ascorbyl Palmitate (600 mg/kg) prevented these effects by reducing the reactive intermediate back to the parent compound. Additionally, Ascorbyl Palmitate produced an antipyretic effect at 15 and 30 minutes post dosing.

Primary cultures of heart endothelial and muscle cells prepared from 2–5-day-old Wistar rats were exposed to heated corn oil (Bird and Alexander 1981). Thermally oxidized fats when ingested can result in toxicity signs such as altered fatty acid composition of tissue lipids, depressed growth, formation of pyknotic nuclei, and necrosis of other tissues. Those oxidized lipids also increased the severity of vitamin E deficiency. When 10 mg Ascorbyl Palmitate in 100 ml ethanol was emulsified and used to treat the monolayer heart cell cultures at 96 hours, the mitotic index increased, but the mean cell count values were not different. Treatment with Ascorbyl Palmitate also tended to prevent necrosis, but did not reduce intracellular lipidosis (Bird and Alexander 1981).

Rapidly growing tumor cells in rodents and humans have high glutathione *S*-transferase (GST) activity, and GST can be involved in tumor cell drug resistance. Ascorbyl Palmitate significantly inhibited human term placental and fetal liver GST activity towards its second substrate, 1-chloro-2,4-dinitrobenzene ($K_i = 10.0\ \mu M$). The I_{50} (μM) for Ascorbyl Palmitate was 45 ± 0.3 when tested using cultures of human fetal liver, and 6 ± 1.5 using cultures of rat liver (Mitra et al. 1992).

Yamazaki et al. (1993) studied the effects of various antioxidants, including Ascorbyl Palmitate, on rabbit platelet functions by means of thromboxane B2 synthesis and EIA. Ascorbyl Palmitate inhibited A-23187–induced thromboxane B2 synthesis at 10^{-5} M and above, and thrombin-induced synthesis at 10^{-7} M when added simultaneously. The pretreatment of platelets with Ascorbyl Palmitate also inhibited both agonist-induced

syntheses unless the platelets had been stimulated with thrombin. When the rabbits were fed ADI concentrations of Ascorbyl Palmitate for 5 days, agonist-induced activation of platelets also was reduced considerably.

Ascorbyl Stearate

Ascorbyl Stearate reduced acetaminophen-induced hepatotoxicity in MF1 mice by reducing the binding of acetaminophen metabolites to hepatic proteins and the depletion of nonprotein sulfhydryl groups and aminotransferase activity. Treatment with Ascorbyl Stearate prevented the 35% mortality caused by administration of acetaminophen alone and prevented hepatic damage by reducing the reactive acetaminophen metabolites to the parent compound and by having a sparing action on reduced hepatic glutathione (Mitra et al. 1988).

Ascorbyl Stearate significantly inhibited human term placental and fetal hepatic GST activity towards its second substrate, 1-chloro-2,4-dinitrobenzene (K_i = 3.1 μM). The I_{50} (μM) for Ascorbyl Stearate was 15 $\pm$ 1.9 when tested using cultures of human fetal liver, and 21 $\pm$ 2.1 using cultures of rat liver. The inhibitory action of Ascorbyl Stearate on human term placental GST was reversible (Mitra et al. 1992).

Erythorbic Acid

Intravenous injection of a solution of pure tetracycline·HCl induced nephrotoxicity, manifested as marked azotemia, a rise in plasma creatinine, diuresis development, and lesions of tubular necrosis. Lightly etherized female Charles River CD rats were given 25 mg/ml tetracycline·HCl in normal saline or water via tail vein (50 mg/kg), and 6 adult mongrel dogs (12–17 kg) were injected via leg vein with 4.3 mg/ml for a dose of 50 mg/kg. Feed was withheld for 24 hours after injection; water was supplied ad libitum. The rats were anesthetized with ether at 24 hours, blood was drawn by cardiac or aortic puncture, and the rats were killed. The dogs were given a stock diet following the 1-day fast. A dose of 150 mg/kg Erythorbic Acid was given before administration of tetracycline or 125 mg/kg administered in solution with the antibiotic prevented azotemia in rats, and the tetracycline was excreted. Erythorbic Acid did not protect dogs. Results of studies using radioactive tetracycline suggested that Erythorbic Acid increased the rate of disappearance of tetracycline from plasma (rat or human), but not from saline. In dialysis and ultrafiltration studies, Erythorbic Acid decreased the binding of plasma protein to tetracycline (Polec, Yeh, and Shils 1971).

Erythorbic Acid decreased the viscosity of calf thymus DNA solutions when Cu^{2+} (as $CuSO_4$) was added. Erythorbic Acid, with or without the cupric ion, caused single- and double-strand scissions of DNA that were determined by sucrose density gradient centrifugation. The enediol group has an essential role in nucleic acid degradation (Omura et al. 1974, 1975).

Both ascorbic acid and Erythorbic Acid (at pH 7.5) inhibited dimeric dihydrodiol dehydrogenases of rabbit and pig lenses. Dihydrodiol dehydrogenases convert naphthalenedihydrodiol into the catechol metabolite, dihydroxynaphthalene, which mediates cataract formation in rabbits. The IC_{50}s of Erythorbic Acid were 0.43 mM for the pig enzyme and 0.13 mM for the rabbit enzyme. Erythorbic Acid was a more potent inhibitor than ascorbic acid (IC_{50}s equal 0.45 mM and 0.90 mM, respectively). Enzyme inhibition was instantaneous and reversible, and the inhibitory potency was decreased by addition of ascorbate oxidase. The inhibition of dihydrodiol dehydrogenases by ascorbic acid and Erythorbic Acid was pH-dependent; maximal inhibition occurred at pH 7–8 and inhibition decreased at lower and higher pH ranges. The investigators suggested that inhibition required the anionic form of ascorbic acid or Erythorbic Acid, such that one hydroxy group of the enediol structure was ionized. Because ascorbic acid accumulated in the lens (concentrations in lenses of the rabbit and pig were 0.7 mM and 1.2 mM, respectively), the concentration of ascorbic acid seemed to prevent the contribution of dihydrodiol dehydrogenases in the formation of naphthalene-induced cataracts. Ascorbic acid and the dihydrodiol dehydrogenases can be regulators of lens growth in the rabbit and pig (Hara et al. 1991).

Sodium Erythorbate

Sodium Erythorbate and ascorbic acid at low concentrations caused NADPH oxidase–induced lipid peroxidation and lysosome labilization. Their actions were additive and occurred via a "cofactor" action. At concentrations greater than 1 mM, the acids inhibited lipid peroxidation and lysosome labilization (Abe et al. 1979).

ANIMAL TOXICOLOGY

Acute Toxicity

In rats, the lowest effect level (LEL) of Ascorbyl Palmitate was 2500 mg/kg/day, and caused a decrease in body weight (FDA 1995). The acute oral LD_{50}s of Ascorbyl Palmitate (33.3% suspension) and Ascorbyl Dipalmitate (15% suspension) were 2 g/kg and >5 g/kg in mice and rats, respectively. The acute dermal LD_{50} of Ascorbyl Palmitate in guinea pigs was >3 g/kg (SCC 1993).

No adverse effects were observed when rats were fed 100–3000 mg/kg Ascorbyl Stearate; however, no details were available (SCC 1993).

In male rats, the LEL of Erythorbic Acid was >2500 mg/kg/day. Additionally, the LEL observed in dogs was greater than 7500 mg/kg/day (FDA 1995). The LD_{50} for orally administered Erythorbic Acid was 8.3 g/kg in mice and 18.0 g/kg in rats (Orahovats 1957).

The acute oral LD_{50} of Sodium Erythorbate in 10 fasted albino rats was >5.0 g/kg of Sodium Erythorbate in a 50% aqueous suspension. The treated rats had soft, pasty stools within 3 hours of dosing, followed in 2 hours by marked diarrhea that persisted for 24 hours (Clairol 1996b). Sodium Erythorbate powder (2 g/kg) was applied to the intact and abraded skin of six

rabbits. Each test site was moistened with physiological saline just prior to dosing. After application of the test material, the exposure area was covered with a double layer of surgical gauze and a piece of rubber dam. The trunk of each rabbit was wrapped in a stockinette, which was secured to the body with tape. The dressings were removed after 24 hour, and the amount of residual sample and signs of localized irritation were noted (see Animal Toxicology—Dermal Irritation in this report). The exposure area was cleaned by thorough wiping, and the rabbits were observed for signs of toxicity for 14 days. At 72 hours, a significant amount of residual compound was found at the exposure site of each rabbit. Behavior, body weight gain, and consumption of feed and water were normal, and no signs of toxicity were observed (European Cosmetic, Toiletry, and Perfumery Association (COLIPA) 1982; SCC 1993).

Short-Term Toxicity

Female mice fed Ascorbyl Palmitate for 63 days had no signs of toxicity during a tumor inhibition study at doses up to 3000 mg/kg/day (Huang et al. 1992).

Male and female B6C3F$_1$ mice (10 per sex per group) were given drinking water containing 0.625%, 1.25%, 2.5%, 5.0%, or 10% Sodium Erythrobate for 10 weeks. Water and feed were available ad libitum. The untreated control group consisted of 20 male and 20 female mice. By the end of the 1st week of treatment, six male mice and one female mouse of the 10% dosing group had died. Of the male mice given 5.0% Sodium Erythrobate, the average weekly body weight gain was slightly less than 90% that of the control female mice. Body weight gain was increased in female mice given Sodium Erythrobate at a concentration of 5.0%, compared to that of control mice. The maximum tolerated dose (MTD) of Sodium Erythrobate in drinking water was 2.5% for male mice and 5.0% for female mice. Mice given doses greater than the MTD had marked atrophy of both hepatocytes and splenic lymphoid follicles, as well as hydropic degeneration of the renal tubular epithelium. No significant changes were observed in the visceral organs of untreated mice or mice given the dose less than or equal to the MTD of Sodium Erythrobate (Inai et al. 1989).

Chronic Toxicity

Ascorbyl Palmitate

Rats fed 125 mg Ascorbyl Palmitate per kg per day for 728 days (0.25% of diet) had no harmful effects. This intake was the equivalent of 53 mg/kg of ascorbic acid per day. In the same study, however, a different group of rats had body weight decreases at dietary doses of 2500 mg/kg/day and above. The highest dose that did not cause a toxicologic effect was 1000 mg/kg/day. Additionally, oxalate stones were observed in the urinary bladders of two of the eight rats in the second group when a dose of 2500 mg/kg/day was administered (Fitzhugh and Nelson 1946).

Rats (10 per group) were given 2% and 5% Ascorbyl Palmitate in feed for 9 months. Significant growth retardation occurred in rats given 5% Ascorbyl Palmitate, and 2 of 10 had bladder stones and hyperplasia of the bladder epithelium. One rat of the high dosing group had lesions of nephritis. Slight growth retardation occurred in rats fed the 2% diet, but no other signs of toxicity were observed (SCC 1993).

Rats (eight per group) were fed a diet of heat-treated lard containing 2% or 5% Ascorbyl Palmitate (equivalent to 424 mg/kg and 1060 mg/kg, or 0.05% and 0.25% of the total diet) during a 2-year study. Growth rate decreased at the higher dose, and two of eight rats had oxalate stones after 9 months of treatment (SCC 1993).

Erythorbic Acid

No adverse clinical effects or microscopic lesions were observed when male rats (10 per group) were fed 1% Erythorbic Acid in the diet for 36 weeks up to 2 years. No differences were observed in rate of growth, mortality, or incidences of gross or microscopic abnormalities between treated rats and untreated control rats (World Health Organization [WHO] 1974; SCC 1993).

Lehman et al. (1951) noted no difference in weight gain between controls and rats fed Erythorbic Acid at 1% of the diet for six months. After two years, no lesions were observed that could be attributed to inclusion of Erythorbic Acid in the diet (Lehman et al. 1951). In a different study, one group of four beagles was fed 1 g Erythorbic Acid daily for 240 days, and a second group (4 beagles) 5 g for 50 days and then 7.5 g for an additional 190 days. Neither group had signs of toxicity at any time during the experimental period, and body weight changes and hematograms remained normal (Orahovats 1957).

Dermal Irritation and Sensitization

A 10% aqueous solution of Ascorbyl Palmitate and undiluted Ascorbyl Dipalmitate were nonirritating to the intact, shaved skin of albino rabbits in a modified Draize dermal irritancy test when the test materials were applied with occlusive patches for 24 hours (SCC 1993).

Dermal irritation data on Erythorbic Acid were not found, but its stereoisomer, ascorbic acid, produced barely perceptible erythema and no edema during a dermal irritation test using rabbits. Ascorbic acid was applied as a 0.5 ml volume of a 30% aqueous solution to the shaved, intact, and abraded skin of the back. In a Maurer optimization test using female guinea pigs, ascorbic acid did not produce dermal sensitization with or without intradermal injection of Freund's Complete Adjuvant. For this study, ascorbic acid was injected intradermally (0.1 ml) as a 0.1% solution in physiological saline, or applied epicutaneously under occlusive patches (0.05 ml/cm^2) as a 10% dispersion in petroleum ether (SCC 1993).

Sodium Erythorbate powder was applied to the intact and abraded skin of six rabbits as a single 2 g/kg dose (see Animal Toxicology—Acute Toxicity in this report). A substantial amount of residual compound was observed 24 hours after dosing. No erythema, edema, or other signs of dermal irritation

were observed at five of six test sites. One rabbit (abraded skin) had slight (1+) erythema at 24 hours that cleared by 48 hours (COLIPA 1982).

Ocular Irritation

An aqueous (10%) solution of Ascorbyl Palmitate and undiluted Ascorbyl Dipalmitate were instilled into the conjunctival sac of albino rabbits in a modified Draize ocular irritancy test. The test volume was 0.1 ml. Ascorbyl Palmitate was not irritating to the eyes of rabbits, and Ascorbyl Dipalmitate was minimally irritating (SCC 1993).

Ocular irritation data on Erythorbic Acid were not found. Ascorbic acid (0.1 ml) at a concentration of 30% in water caused slight to well-defined redness when instilled without rinse-off into the conjunctival sac of rabbits. The cornea and iris were unaffected, and all redness was gone by 72 hours (SCC 1993).

Sodium Erythorbate powder (100 mg) was instilled into the conjunctival sac of albino rabbits (10 male and 2 female). The eyes of half of the treated rabbits were rinsed after 5 seconds. Reactions were comparable in rinsed and unrinsed eyes, and were slight and transient in nature. One hour after dosing, two of six unrinsed eyes had congestion of the iris, but the iris reacted normally to light. Varying degrees of redness were observed in the lids of all unrinsed eyes. Slight redness of the nictitating membrane or palpebral conjunctiva at the medial canthus was observed in two unrinsed eyes. At one hour, 1+ iritis was observed in one rinsed eye. Five of six rinsed eyes had slight redness that was limited to only the nictitating membrane in three cases. At 24 hours, all eyes were normal, with the exception of one that had slight reddening of the conjunctiva at the medial canthus. All eyes, rinsed and unrinsed, were normal at 48 hours. The mean ocular irritation scores were 0.33/110 (unrinsed eyes) and 0.17/110 (rinsed eyes) (COLIPA 1982; SCC 1993).

Phototoxicity

Topical application of 10% vitamin C elevated cutaneous concentrations of vitamin C in pigs such that the treated skin was protected from ultraviolet B (UVB) damage (as measured by erythema and sunburn cell formation). After treatment with vitamin C, the number of sunburn cells/4 mm was reduced by 42% compared to controls. Vitamin C protected porcine skin from UVA-mediated phototoxic reactions as well; vitamin C–induced protection was due to the reducing properties of the molecule. Topical application of 10% Erythorbic Acid reduced the number of sunburn cells by 41%, compared to the vehicle control. The vehicle (control) was 20% propylene glycol (v/v) with 0.5% hydroxypropylcellulose as a thickener (Darr et al. 1992).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Various dosages of Sodium Erythorbate were administered via gastric intubation to mated female albino CD-1 outbred mice on days 6–15 of gestation. The test volume was 10 ml/kg in water. The positive control was 150.0 mg/kg of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. The mice were observed for appearance and behavior, as well as feed consumption. All control and test mice survived to term. Of the negative control mice, 21 of 30 became pregnant. Twenty-one of 23 mice of the positive control group were pregnant. Of the mice given Sodium Erythorbate, the number of pregnant females per group was 22 of 25 (10.3 mg/kg), 20 of 25 (47.8 mg/kg and 1030.0 mg/kg), and 21 of 28 (221.9 mg/kg), respectively.

All dams were subjected to caesarean section on day 17, and the numbers of implantation sites, resorption sites, and the number of live and dead fetuses were recorded (Table 8). The body weights of the live pups were determined. The fetuses were examined for the presence of external (gross) congenital abnormalities, and one-third of the fetuses underwent detailed visceral examination. The remaining fetuses were examined for skeletal defects. One pup of a dam of the positive control group had exophthalmos, encephalomeningocele, and gastroschisis. A cleft palate was observed in a pup of the 1030.0 mg/kg treatment group. The investigators concluded that the "administration of up to 1030 mg/kg of [Sodium Erythorbate] to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls" (Food and Drug Research Laboratories, Inc. 1974).

The same researchers gave Sodium Erythorbate via oral intubation to mated female albino Wistar rats on days 6 to 15 of gestation in a similar study. The positive control was 250 mg/kg of aspirin. The dams were dosed with 9.0 mg/kg, 41.8 mg/kg, 194.0 mg/kg, or 900.0 mg/kg. Of the mated rats, 20 of 24 (negative control), 20 of 22 (positive control), 20 of 20 (9.0 mg/kg and 41.8 mg/kg), 20 of 21 (194.0 mg/kg), and 20 of 24 (900.0 mg/kg) became pregnant; all survived to term. All dams were subjected to caesarean section on day 20, and the dams and fetuses were examined as above (Table 9). No soft tissue abnormalities were observed in rats of the negative control group or in rats given Sodium Erythorbate. Abnormalities were observed in rats given aspirin. The investigators concluded that "the administration of up to 900 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival" (Food and Drug Research Laboratories, Inc. 1974).

Ema, Itami, and Kanoh (1985) fed 0.05, 0.5, or 5.0% Sodium Erythorbate to mated female Wistar rats on days 7–14 of gestation. The rats were weighed daily and feed consumption was determined. Five to seven rats per group were killed on day 20. The peritoneal cavity was opened, and the numbers and positions of live and dead fetuses and resorptions were noted. The fetuses were removed by caesarean section, weighed, sexed, and examined for external anomalies. Half of the fetuses were examined for skeletal defects; the remaining fetuses were examined for visceral anomalies. Five pregnant rats of the 5% and 0.05% treatment groups were allowed to deliver spontaneously. The numbers of live and dead pups were recorded. Live pups were

TABLE 8
Reproductive and developmental toxicity of Sodium Erythorbate in CD-1 mice (Food and Drug Research Laboratories, Inc. 1974)

Endpoint	Treatment group					
	Negative control	Positive control	10.3 mg/kg	47.8 mg/kg	221.9 mg/kg	1030.0 mg/kg
Pregnancies (total no./no. to term)	21/21	21/21	22/22	20/20	21/21	20/20
No. corpora lutea (average/dam)	268 (8.93)	256 (11.1)	291 (11.6)	274 (11.0)	283 (10.1)	265 (10.6)
No. live litters	21	21	22	20	21	20
No. implant sites (average/dam)	243 (11.6)	232 (11.1)	267 (12.1)	246 (12.3)	264 (12.6)	250 (12.5)
No. resorptions	6	18	9	15	8	13
Dams with ≥1 site resorbed	6	12	8	9	7	7
Dams with all sites resorbed	—	—	—	—	—	—
% Partial resorptions	28.6	57.1	36.4	45.0	33.3	35.0
% Complete resorptions	—	—	—	—	—	—
No. live fetuses (average/dam)	235 (11.1)	214 (10.2)	254 (11.6)	230 (11.5)	256 (12.2)	235 (11.8)
M/F ratio	0.73	1.12	0.92	0.93	0.87	0.76
No. dead fetuses	2	—	4	1	—	2
Dams with ≥1 dead	2	—	4	1	—	2
Dams with all dead	—	—	—	—	—	—
% Partial dead	9.52	—	18.2	5.00	—	10.0
% All dead	—	—	—	—	—	—
Average fetus weight (g)	0.83	0.83	0.85	0.84	0.87	0.87
Live fetuses examined at term*	163/21	152/21	177/22	159/20	176/21	162/20
Sternebrae—incomplete ossification	34/15	29/14	57/17	66/18	37/13	28/12
Sternebrae—bipartite	—	4/4	1/1	1/1	3/3	1/1
Sternebrae—missing	27/11	22/11	29/14	37/13	12/9	18/7
Ribs—incomplete ossification	1/1	—	—	—	—	—
Ribs—>13	22/10	33/15	24/13	19/9	25/14	25/11
Vertebrae—incomplete ossification	12/5	8/4	6/6	10/3	1/1	11/3
Tail defects	1/1	1/1	—	—	—	—
Skull—incomplete closure	—	—	—	—	—	1/1
Extremities—incomplete ossification	7/4	6/3	2/2	11/3	1/1	8/3
Miscellaneous—hyoid missing	46/16	40/15	53/16	51/13	39/15	43/12
Miscellaneous—hyoid reduced	26/14	22/10	17/11	11/7	23/15	16/10

*Numerator = number of fetuses affected; denominator = number of litters affected.

sexed, weighed, and examined for external anomalies. The litter size was culled to eight offspring (four males, four females). The offspring were weighed weekly and weaned on day 21 after birth, when the dams were killed. The number of implantation remnants was determined. Offspring were weighed weekly until 10 weeks after birth.

Body weight gain of pregnant rats given 0.5% and 5% Sodium Erythorbate was comparable to that of the control group. Body weight gain of dams given 0.05% Sodium Erythorbate was greater than that of the control group. Feed consumption did not differ between groups. No clinical signs of toxicity were observed in dams of any group. The incidence of intrauterine fetal death, number of live fetuses/dam, sex ratio of the fetuses, fetal body weight of both sexes, and the placental weight did not differ from controls. External skeletal and internal visceral examinations of the fetuses produced no evidence of teratogenesis in any group. Offspring delivered normally had a high survival rate and normal growth rate (Ema, Itami, and Kanoh 1985).

TABLE 9

Reproductive and developmental toxicity of Sodium Erythorbate in Wistar rats (Food and Drug Research Laboratories, Inc. 1974)

Endpoint	Treatment group					
	Negative control	Positive control	9.0 mg/kg	41.8 mg/kg	194.0 mg/kg	900.0 mg/kg
Pregnancies (total no./no. to term)	20/20	20/20	20/20	20/20	20/20	20/20
No. corpora lutea (average/dam)	268 (11.2)	252 (11.5)	266 (13.3)	257 (12.9)	270 (12.9)	254 (10.6)
No. live litters	19	20	20	20	20	20
No. implant sites (average/dam)	244 (12.2)	227 (11.4)	237 (11.9)	233 (11.7)	238 (11.9)	234 (11.7)
No. resorptions	13	46	2	2	3	4
Dams with ≥1 site resorbed	5	11	2	2	3	4
Dams with all sites resorbed	1	—	—	—	—	—
% Partial resorptions	25.0	55.0	10.0	10.0	15.0	20.0
% Complete resorptions	0.96	1.10	0.82	0.94	0.84	1.09
No. live fetuses (average/dam)	231 (11.6)	181 (9.05)	235 (11.8)	231 (11.6)	230 (11.5)	230 (11.5)
M/F ratio	0.96	1.10	0.82	0.94	0.84	1.09
No. dead fetuses	—	—	—	—	5	—
Dams with ≥1 dead	—	—	—	—	1	—
Dams with all dead	—	—	—	—	—	—
% Partial dead	—	—	—	—	5.00	—
% All dead	—	—	—	—	—	—
Average fetus weight (g)	3.95	2.62	3.96	3.95	3.87	3.92
Live fetuses examined at term*	161/19	129/20	166/20	161/20	160/20	161/20
Sternebrae—incomplete ossification	28/10	52/16	55/15	21/11	56/16	27/14
Sternebrae—bipartite	—	11/6	—	—	—	—
Sternebrae—fused	—	1/1	—	—	—	—
Sternebrae—extra	—	2/2	—	—	—	—
Sternebrae—missing	8/2	106/20	13/5	8/5	21/10	4/4
Ribs—incomplete ossification	—	19/8	—	—	—	1/1
Ribs—fused/split	—	5/3	—	—	—	—
Ribs—wavy	18/8	52/14	8/5	14/7	8/5	12/4
Ribs—>13	4/3	39/14	2/1	—	2/2	2/1
Vertebrae—incomplete ossification	7/2	60/16	—	—	7/4	1/1
Vertebrae—extra centers of ossification	—	2/1	—	—	—	—
Skull—incomplete closure	30/12	54/15	17/10	16/8	15/5	26/13
Skull—missing	—	1/1	—	—	—	—
Extremities—incomplete ossification	—	7/2	—	—	—	—
Miscellaneous—hyoid missing	22/9	52/16	16/8	19/9	16/8	23/11
Miscellaneous—hyoid reduced	21/11	5/2	15/10	15/7	14/7	14/10

*Numerator = number of fetuses affected; denominator = number of litters affected.

MUTAGENICITY

Ascorbyl Palmitate

Prival, Simmon, and Mortelmans (1991) performed both the Ames test, using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, and the tryptophan reversion assay using *Escherichia coli* strain WP2. Doses of Ascorbyl Palmitate (dissolved in 0.067 M potassium or sodium sulfate buffer at pH 7.0) from 0.01 to 3.3 mg per plate were tested, and

doses greater than 3.3 mg per plate were toxic to bacteria. In both assays, Ascorbyl Palmitate was nonmutagenic.

Erythorbic Acid

Litton Bionetics, Inc. (1974) performed activation and nonactivation suspension tests using homogenates of liver, lungs, and testes and suspensions from adult male ICR mice, Sprague-Dawley rats, and *Macaca mulatta* primates. The indicator organisms were *S. typhimurium* strains TA-1535, TA-1537, and TA-1538, and *Saccharomyces cerevisiae* strain D4. In all tests, Erythorbic Acid was nonmutagenic (Litton Bionetics, Inc. 1974). Ishidate et al. (1984) performed primary mutagenicity screening on 242 food additives, including Erythorbic Acid. Erythorbic Acid (99.6% pure; 50.0 mg/plate) in phosphate buffer was mutagenic in *S. typhimurium* strain TA100. With S9 activation, Erythorbic Acid caused 222 revertants/plate. Without metabolic activation, 144 revertants/plate were observed. Erythorbic Acid (0.25 mg/plate) was nonmutagenic in the chromosomal aberration assay using Chinese hamster fibroblasts (Ishidate et al. 1984). When the Ames test was performed using Erythorbic Acid and the TA100 strain of *S. typhimurium* (without S9 activation), only 4 revertants/mg were induced (Ishidate, Sufuni, and Nohmi 1990). Zeiger (1993) affirmed that Erythorbic Acid was weakly mutagenic using the Ames test. Erythorbic Acid had DNA-damaging potential in the more sensitive liquid *Bacillus subtilis* rec assay using strains H17 and M45 (Nonaka 1989). Erythorbic Acid was weakly mutagenic in the Ames test and nonmutagenic in both the chromosomal aberration and dominant lethal assays (Clairol 1996b).

Sodium Erythorbate

Sodium Erythorbate is listed as a mutagen in the RTECS and National Toxicology Program (NTP) Chemical Repository databases (Radian Corporation 1991; RTECS 1994). In mice, Sodium Erythorbate was not mutagenic in the host-mediated assay using *S. typhimurium*, and it did not increase the mitotic recombination frequency in the host-mediated *S. cerevisiae* D3 assay. Sodium Erythorbate was not mutagenic to five strains of *S. typhimurium* in the Ames test, with or without metabolic activation. At a concentration of 5%, Sodium Erythorbate did not increase the mitotic recombination frequency of *S. cerevisiae* D3 in vitro. Dominant lethal tests using rats produced no consistent responses. Sodium Erythorbate did not induce heritable translocation heterozygosity in male mice when Sodium Erythorbate was added to the diet for 7 weeks (Newell, Jorgenson, and Simmon 1974; Jorgenson et al. 1978a). Sodium Erythorbate (99.8% pure; 5.0 mg/plate) was nonmutagenic in *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537 with and without S9 activation. Sodium Erythorbate (0.25 mg/ml/plate) was also negative in the chromosomal aberration assay using Chinese hamster fibroblasts; Sodium Erythorbate did not induce the formation of polyploid cells after 48 hours, and caused 1% chromosomal breaks after 24 hours (Ishidate et al. 1971; 1984). Sodium Erythorbate was not mutagenic in a number of assays, including the Ames test using *S. typhimurium* strains TA98 and TA199 (with or without activation), rec assay using *B. subtilis* (with or without activation), and the chromosomal aberration test using Chinese hamster fibroblasts. Sodium Erythorbate did not cause chromosomal aberrations or sister chromatid exchanges in cultured human embryo fibroblasts. The compound caused chromosome aberrations in vivo using rat bone marrow cells, but did not cause mutations in silk worms (Kawachi et al. 1980). Sodium Erythorbate was nonmutagenic in the dominant lethal test system using rats (Jorgenson et al. 1978b) and the chromosomal aberration test using Chinese hamster cells (Matsuoka, Hayashi, and Ishidate 1979).

CARCINOGENICITY

Ascorbyl Palmitate

Female 5-week-old CF-1 mice were either fed a control diet or a diet containing 2% Ascorbyl Palmitate for 2 weeks. One half of each group (12 mice per group), was injected subcutaneously with 10 mg/kg azoxymethanol in saline, which induces formation of focal areas of dysplasia (FADs), and the other half with normal saline once weekly for 6 weeks, while fed their respective diets. One week after the last injection, the mice were given intraperitoneal injections of 25 μCi [^{3}H]thymidine in water. An hour later, the animals were sacrificed and the colons removed and processed for microscopic examination. Multiple 500-μm sections of distal colon from each mouse were examined for the number of FADs, observed as cells with variable shape and size nuclei, lack of nuclear polarity, and a loss of mucin.

Oral administration of Ascorbyl Palmitate did not produce signs of toxicity or affect body weight. Subcutaneously administered azoxymethanol induced proliferation of colonic epithelial cells and the expansion of the proliferative compartment as well as the formation of FADs. No FADs were observed in either the control mice or those fed Ascorbyl Palmitate. Also, Ascorbyl Palmitate did not inhibit proliferation of or reduce the number of induced FADs (Huang et al. 1992).

Erythorbic Acid

Male 6-week-old F344 rats were given doses of 5% Erythorbic Acid for 168 days in a study in which various chemicals and their sodium salts (with promoting potential) or basal diet (control) were orally administered. Parameters of urinary excretion were investigated and the urinary bladder epithelium was examined using light and scanning electron microscopy at weeks 8, 16, and 24. The urine of rats fed Erythorbic Acid had increased pH, elevated content of crystals and sodium, and decreased osmolality; however, no morphological alterations such as hyperplasias were detected in the mucosa. The urine values and urinary bladder mucosa were similar to controls at doses below 5 g/kg/day (Shibata et al. 1985).

In a chronic toxicity study by Fitzhugh and Nelson (1946), no neoplasms related to treatment were observed when Erythorbic Acid was administered to weanling rats at 1.0 and 2.5 g/kg/day doses for 9 months.

Sodium Erythorbate

F344/DuCrj rats of both sexes (6-week-old) were given 1.25% or 2.5% Sodium Erythorbate in drinking water for 104 weeks and untreated water for 8 additional weeks. Rats of the control group were given untreated water only. Each group consisted of 52 male and 50 female rats. Cumulative consumption of Sodium Erythorbate by male rats was 217 g/rat (1.25%) and 430 g/rat (2.5%). Consumption by females was 206 g/rat (1.25%) and 583 g/rat (2.5%). Body weight of rats given 2.5% Sodium Erythorbate was reduced by 8.5% for males and 15.5% for females at weeks 88 and 85, respectively, compared to controls. Body weight gain was normal in rats of the low dose group. All male treated and control rats (except two of the high-dose group) had testicular interstitial cell tumors. Various tumors occurred in 80% of control males, 69% of males given the low dose, and 78% of males given the high dose. A 6–18% incidence of leukemia, pheochromocytoma, mammary fibroadenoma, and mesothelioma was observed. Of the females of the control, 1.25%, and 2.5% dose groups, 94%, 88%, and 78% had tumors, respectively. Twenty to 43% of females (all groups) had leukemia, mammary fibroadenoma, endometrial stromal polyp and/or pituitary adenoma. Females given 2.5% Sodium Erythorbate had significantly fewer tumors than control females. The pattern of occurrence of the various types of tumors was similar among the groups. Sodium Erythorbate did not enhance the development of rare spontaneous tumors or transform benign tumors (e.g., solid adenoma of the thyroid) to carcinomas. The investigators concluded that Sodium Erythorbate was not carcinogenic in F344 rats (Abe et al. 1984).

Male 6-week-old F344 rats were given doses of 5% Sodium Erythorbate in feed for 168 days. Parameters of urinary excretion were investigated and the urinary bladder epithelium was examined using light and scanning electron microscopy at weeks 8, 16, and 24. The urine of rats fed Sodium Erythorbate had increased pH, elevated content of crystals and sodium, and decreased osmolality; however, no morphological alterations such as hyperplasia were detected in the mucosa. The urine values and urinary bladder mucosa were similar to controls at doses below 5 g/kg/day (Shibata et al. 1985).

F344 male rats (15 per group; 5-week-old) were given powdered basal diet containing various chemicals, including 5% Sodium Erythorbate for 24 weeks. Urine samples were obtained and analyzed during weeks 8, 16, and 24 of treatment. The dietary addition of Sodium Erythorbate increased urinary pH and sodium ion concentration of the urine. Sodium Erythorbate also reduced significantly the osmolality of the urine. The urinary sediments, including the casts, epithelial cells, white and red blood cells, and bacterial contaminants, were similar for treated and control rats. Other parameters (e.g., bilirubin, protein, and glucose) were normal. Rats given Sodium Erythorbate had simple hyperplasia of the urinary bladder epithelium, consisting of diffuse thickening of the epithelium with four to eight layers of transitional epithelial cells. The observed hyperplasia was slight at week 8 and had regressed by week 16. The mucosa appeared normal at week 24. Papillary or nodular hyperplasia did not occur in the urinary bladders of Sodium Erythorbate–treated rats. At week 8, irregularly shaped foci with slight cellular elevation were observed on the luminal surface of the urinary bladder using the scanning electron microscope. Most of the cells in these foci were covered with short, uniform microvilli and with "ropy or leafy" microridges. Pleomorphic microvilli were observed on the luminal surface of several cells (Fukushima et al. 1986).

Sodium Erythorbate was administered in drinking water to male B6C3F$_1$ mice at concentrations of 1.25% and 2.5% (the MTD; see Animal Toxicology—Short-Term Toxicity in this report). Female mice received 2.5% and 5% (MTD). Each group contained 50 mice. Treatment continued for 96 weeks; the study was terminated at week 110. Feed and water were available ad libitum. No significant difference was observed between groups in the amount of water intake (Table 10). As a consequence, the males in the high dose group received a dose of Sodium Erythorbate that was approximately 1.5 times greater than that of the low-dose group. For the female groups, Sodium Erythorbate intake by the high-dose group was approximately 1.8 times greater than that of the low-dose group. The average body weights of the treated mice were similar to controls. Nine male and seven female mice died accidentally within week 21 and were excluded when the percent survivals were determined. Of the male mice (without tumors) that survived beyond week 43, dose-dependent reductions in the heart and brain weights were observed. The weights of the heart, lungs, kidneys, and brain of female mice (without tumors) were significantly different between the high-dose group and the control group. The tumors observed in the male mice were hepatocellular tumors, subcutaneous sarcoma, adenoma and carcinoma of the lungs, and lymphoma/leukemia. The time-adjusted analysis of tumor incidence was performed on the hepatocellular tumors and subcutaneous sarcomas, the incidence of which was significant. The lymphoma/leukemia had the highest incidence in female mice treated with Sodium Erythorbate, but this was not significant. Overall, tumor incidence, time to death with tumors, and the distribution of tumors in treated mice did not differ significantly from mice of the control group (Inai et al. 1989).

Sodium Erythorbate was administered to rats at doses of 1.25% and 2.5% in drinking water for 4 weeks. No evidence of carcinogenic potential was observed, and female rats treated at 2.5% had a slight reduction in certain spontaneous tumors (SCC 1993).

COCARCINOGENICITY

In a series of studies, F344 male rats were given Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, or Sodium

TABLE 10
Carcinogenicity of Sodium Erythorbate (Inai et al. 1989)

Dose group	Water intake (ml/day)	Sodium Erythorbate intake (g/kg/day)	No. effective mice[a]	Mean time to death in effective mice (weeks)[b]	Mice with tumor (%)	Mean time to death in mice with tumor (weeks)[b]
Male						
Control	8.6	—	38	69 ± 21	9 (24)	84 ± 21
1.25%	9.1	3.3	38	75 ± 19	14 (37)	79 ± 21
2.5%	6.9	4.9	43	84 ± 20[c]	19 (44)[d]	92 ± 14
Female						
Control	4.6	—	45	91 ± 18	10 (22)	91 ± 18
2.5%	4.8	4.0	44	95 ± 11	16 (36)	106 ± 6[c]
5.0%	4.2	7.0	46	101 ± 12[c]	15 (33)	101 ± 13

[a]Effective mice survived beyond week 43.
[b]Mean ± standard deviation.
[c]Significantly different from control group at $p < 0.05$ by chi-square test.
[d]Significantly different from control group at $p < 0.01$ by t test.

Erythorbate at a concentration of 5% in feed after initiation with the carcinogens *N*-butyl-(4-hydroxybutyl)nitrosamine (BBN), *N*,*N*-dibutylnitrosamine (DBN), or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). These studies are discussed below and presented in Table 11.

Ascorbyl Dipalmitate and Ascorbyl Stearate

Fukushima et al. (1987a) initiated F344 male rats with BBN and administered 5% Ascorbyl Dipalmitate or Ascorbyl Stearate. Body weight gain was decreased significantly in initiated rats given the ascorbyl esters compared to rats given BBN alone (control). The average body weights of rats given BBN and either Ascorbyl Dipalmitate or Ascorbyl Stearate were significantly lower than the weights of rats of the control group. No lesions of the liver or kidneys were observed in rats of any treatment group. Rats were given powdered basal diet containing 5% Ascorbyl Dipalmitate or 5% Ascorbyl Stearate. Rats of the control group received basal diet alone. Treatment with Ascorbyl Stearate caused significant increases in urine osmolality, but urinary pH and electrolyte parameters did not differ from controls. Administration of either ascorbyl ester did not increase crystals in urinary sediment (Fukushima et al. 1987a).

Erythorbic Acid

In a similar study by these authors, F344 rats were initiated with BBN and fed 5% Erythorbic Acid. Initiated rats fed Erythorbic Acid (group 1) had reduced weight gain compared to rats given BBN alone (group 2). Rats of group 1 had fewer neoplastic lesions in the urinary bladder as compared to rats of group 2, but the difference was not significant. Rats of group 3 (Erythorbic Acid alone) had no neoplasms. The microvilli in areas of induced mild epithelial hyperplasia became altered in the urinary bladders of rats of group 3. Other changes occurred in urinary pH, Na^+ ion concentration, membrane potential, and induction of ornithine decarboxylase activity. In this study, Erythorbic Acid had a total lack of promotion of urinary bladder neoplasms with or without prior initiation by the carcinogen BBN (Fukushima et al. 1987a).

In the same laboratory, male 6-week-old F344 rats were given 0.05% BBN in their drinking water for 4 weeks, and then were administered various antioxidants in their diet, including 5% Erythorbic Acid, for 32 weeks. The development of preneoplastic papillary or nodular hyperplasia, papilloma, or carcinoma in the urinary bladder was measured to determine the promoting potential of the chemicals. Administration of Erythorbic Acid after initiation with the carcinogen BBN did not significantly increase the incidence and average number (per 10 cm basement membrane) of lesions in the urinary bladder (Kurata et al. 1986).

Sodium Erythorbate

Fukushima et al. (1984) fed 5% Sodium Erythorbate to 15 rats for 22 weeks. Urinary pH and sodium content were increased in treated rats, and crystals of $MgNH_4PO_4$ were detected in the urinary sediment. Urine osmolality was significantly reduced.

Shirai et al. (1985) fed 5% Sodium Erythorbate to 21 F344 male rats 1 week after initiation with 150 mg/kg MNNG (in dimethyl sulfoxide). Body weights of rats given Sodium Erythorbate were slightly reduced. Treatment with MNNG alone caused multiple gastric tumors in the nonglandular portion and a small number of neoplasms in the glandular stomach. Rats given both MNNG and Sodium Erythorbate had a lower incidence of papilloma than the rats given MNNG alone. Rats given Sodium Erythorbate alone had no lesions of the glandular or nonglandular stomach (Shirai et al. 1985).

Abe et al. (1983) administered MNNG, at a total dose of 300 mg/kg (50 mg/kg, twice a week), to 16 F344 male rats by gastric intubation. By itself, the carcinogen caused 15 squamous

TABLE 11
Lesions in F344 male rats after initiation with various carcinogens

Study length (weeks)	No. rats	Initiator used[a]	Dietary treatment[b]	Lesions—type, incidence (%), and/or number (per 10 cm basement membrane)	Reference
			Nonglandular stomach		
36	21	MNNG	5% SE	Hyperplasia, 21 (100) Papilloma, 5 (23.8) Squamous cell carcinoma, 1 (5.0)	Shirai et al. 1985
	21	MNNG	—	Hyperplasia, 21 (100) Papilloma, 10 (47.6) Squamous cell carcinoma, 1 (5.0) Leiomyosarcoma, 3 (14.3)	
	14	—	5% SE	No lesions observed	
36	21	DBN	5% SE	Papilloma, 1 (5)	Fukushima et al. 1987b
	21	DBN	—	No lesions observed	
	20	—	5% SE	No lesions observed	
			Glandular stomach		
36	21	MNNG	5% SE	Dysplasia, 5 (23.8) Adenoma, 2 (9.5)	Shirai et al. 1985
	21	MNNG	—	Dysplasia, 13 (61.9) Adenocarcinoma, 1 (4.9)	
	14	—	5% SE	No lesions observed	
			Urinary bladder		
36	20	BBN	5% AD	PN hyperplasia, 8 (40), 0.3 ± 0.6[c] Papilloma, 2 (10), 0.1 ± 0.3 Carcinoma, 1 (5), 0.1 ± 0.2	Fukushima et al. 1987a
	20	BBN	5% AS	PN hyperplasia, 4 (20), 0.2 ± 0.4	
	20	BBN	—	PN hyperplasia, 5 (25), 0.3 ± 0.6	
	15	—	5% AD	No lesions observed	
	15	—	5% AS	No lesions observed	
36	25	BBN	5% EA	PN hyperplasia, 8 (40), 0.3 ± 0.4 Papilloma, 1 (5), 0.0 ± 0.1 Carcinoma, 1 (5), 0.0 ± 0.2	Fukushima et al. 1987a
	25	BBN	—	PN hyperplasia, 11 (44), 0.5 ± 0.8 Papilloma, 5 (20), 0.1 ± 0.3 Carcinoma, 2 (8), 0.1 ± 0.2	
	20	—	5% EA	No lesions observed	
24	20	BBN	5% SE	PN hyperplasia, 9 (45), 0.6 Papilloma, 3 (15), 0.1	Miyata et al. 1985
	44	BBN	—	PN hyperplasia, 3 (7), 0.1	
	16	—	5% SE	PN hyperplasia, 2 (13), 0.2	
36	23	BBN	5% SE	Simple hyperplasia, 23 (100) PN hyperplasia, 23 (100), 5.32 ± 4.18 Papilloma, 19 (83), 1.44 ± 1.19 Cancer, 15 (65), 0.77 ± 0.72	Fukushima et al. 1984
	24	BBN	—	Simple hyperplasia, 14 (58) PN hyperplasia, 8 (33), 0.48 ± 0.96 Papilloma, 5 (20), 0.19 ± 0.38 Cancer, 1 (4), 0.04 ± 0.20	
	26	—	5% SE	No lesions observed	

TABLE 11
Lesions in F344 male rats after initiation with various carcinogens *(Continued)*

Study length (weeks)	No. rats	Initiator used[a]	Dietary treatment[b]	Lesions—type, incidence (%), and/or number (per 10 cm basement membrane)	Reference
				Urinary bladder (cont.)	
36	23	BBN	5% SE	PN hyperplasia, 23 (100), 5.32 ± 4.18 Papilloma, 19 (83), 1.44 ± 1.19 Cancer, 15 (65), 0.77 ± 0.72	Ito et al. 1986
	24	BBN	—	PN hyperplasia, 8 (33), 0.48 ± 0.96 Papilloma, 5 (20), 0.19 ± 0.38 Cancer, 1 (4), 0.04 ± 0.20	
36	21	DBN	5% SE	PN hyperplasia, 6 (29), 0.4 ± 0.8 Papilloma, 9 (43), 0.7 ± 1.1 Carcinoma, 1 (5), 0.1 ± 0.2	Fukushima et al. 1987b
	21	DBN	—	PN hyperplasia, 9 (43), 0.6 ± 0.9 Papilloma, 8 (38), 0.4 ± 0.7	
				Liver	
36	21	DBN	5% SE	Hyperplastic foci, 21 (100) Hyperplastic nodules, 5 (24) Carcinoma, 2 (10)	Fukushima et al. 1987b
	21	DBN	—	Hyperplastic foci, 21 (100) Hyperplastic nodules, 8 (38) Carcinoma, 5 (24)	
	20	—	5% SE	No lesions observed	
				Esophagus	
36	21	DBN	5% SE	PN hyperplasia, 18 (86) Papilloma, 3 (14) Carcinoma, 1 (5)	Fukushima et al. 1987b
	21	DBN	—	PN hyperplasia, 16 (76)	
	20	—	5% SE	No lesions observed	

[a]MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 150 mg/kg by gastric intubation; BBN = *N*-butyl-(4-hydroxybutyl)nitrosamine, 0.05% in drinking water; DBN = *N*,*N*-dibutylnitrosamine, 0.05% in drinking water.
[b]AD = ascorbyl dipalmitate; AS = ascorbyl stearate; EA = erythorbic acid; SE = sodium erythorbate.
[c] PN = papillary or nodular.

cell carcinomas of the nonglandular stomach, 8 adenocarcinomas of the glandular stomach, and 1 sarcoma within the wall of the stomach. Of those lesions, 11 of the squamous cell carcinomas, and 6 of the adenocarcinomas were "larger than a soybean size." The number of large adenocarcinomas was a slight, but not significant difference ($p = 0.069$). When Sodium Erythorbate was given to 17 rats at a concentration of 2.5% in their drinking water from the 8th week of age, 2 weeks before initiation, until the rats were killed, 16 squamous cell carcinomas of the nonglandular stomach and 3 adenocarcinomas of the glandular stomach were detected. Of those 16 and 3 tumors, seven and one, respectively, were larger than "soybean size". Tumor incidence at sites other than the stomach was not significantly different among the experimental groups and groups from earlier studies. Interstitial cell tumors of the testis were observed in almost all rats that survived 100 weeks of age (control, 9/10; MNNG, 13/16; MNNG and Sodium Erythorbate, 14/17; Sodium Erythorbate, 10/10), and leukemia was the next frequent in occurrence (control, 3/10; MNNG, 1/16; MNNG and Sodium Erythorbate, 4/17; Sodium Erythorbate, 2/10). Pheochromocytomas, thyroid solid adenomas, subcutaneous fibromas, peritoneal mesotheliomas, and adenoacanthomas were observed in two or more of the rats tested, but their incidences were not significant. The investigators concluded that Sodium Erythorbate did not enhance or convincingly reduce MNNG-induced carcinogenesis in the stomach.

In another study, rats were initiated with DBN and fed 5% Sodium Erythorbate. The initial and final mean body weights of rats given Sodium Erythorbate and DBN were 128 ± 7 g and 422 ± 18 g, respectively. The final mean body weight was significantly different ($p < 0.001$) from the control group given DBN alone. Mean feed consumption was 14.4 g/day for rats given Sodium Erythorbate and DBN. Rats not initiated with DBN had initial and final mean body weights of 131 ± 6 g and 430 ± 17 g,

respectively. The mean feed consumption per rat was 14.8 g/day (Fukushima et al. 1987b).

This laboratory also conducted a study in which rats were fed 5% Sodium Erythorbate after initiation with BBN. The development of preneoplastic papillary or nodular hyperplasia, papilloma, or carcinoma in the urinary bladder was measured to determine the promoting potential of the chemicals. Administration of Sodium Erythorbate after initiation with the carcinogen BBN significantly increased the incidence and average number (per 10 cm basement membrane) of lesions in the urinary bladder. Other details were unavailable (Kurata et al. 1986).

Sodium Erythorbate did not have any modifying effects on second-stage carcinogenesis of the nonglandular and glandular stomach, colon, liver, kidneys, mammary gland, ear duct, or thyroid gland in F344 rats. Sodium Erythorbate did, however, enhance second-stage carcinogenesis of the urinary bladder (Ito et al. 1986).

Sixteen F344 male rats were treated with 200 mg/kg diethylnitrosamine (DEN), given a basal diet for 2 weeks, then were given diet containing 5.0% Sodium Erythorbate for 6 weeks. Three weeks after administration of DEN, the rats were subjected to partial hepatectomy. Preneoplastic lesions were determined using a placental form of glutathione *S*-transferase (GST-P) as a marker of altered enzyme foci. The number of GST-P–positive foci was $8.09 \pm 3.14/cm^2$. The area was 0.70 ± 0.26 mm^2/cm^2. These values did not differ significantly from the DEN-treated control group. The number of foci was $9.09 \pm 2.05/cm^2$ and the area was 0.77 ± 0.19 mm^2/cm^2 (Ito et al. 1986, 1988).

Ito and Fukushima (1989) reported that sodium and potassium salts, including Sodium Erythorbate, increased the tumor incidence in initiated rats by increasing the sodium concentration and pH of the urine. These latter increases caused an increase in bladder epithelium intracellular pH that stimulated cell replication, thereby enhancing tumor formation (Ito and Fukushima 1989). Concurrent ingestion of NH_4Cl in the drinking water prevented the increases in urine pH and sodium concentration and inhibited the promotion of bladder tumors after ingestion of ascorbate salts and saccharin salts (Fukushima et al. 1987a; Anderson 1991; Cohen et al. 1995).

Tumor Inhibition

Ascorbyl Palmitate when topically applied at small doses inhibited 12-O-tetradecanoylphorbol-13-acetate-induced (TPA-induced) ornithine decarboxylase activity, tumor production, and DNA synthesis in mouse epithelial cells (Smart et al. 1987). A dose of 4 μmol of Ascorbyl Palmitate inhibited by 60–70% after one topical application of 2 nmol TPA. When 5 nmol TPA was administered with 5 μmol Ascorbyl Palmitate twice weekly to previously initiated mice, 91% of tumors were inhibited per mouse.

Both Ascorbyl Palmitate and Erythorbic Acid at 0.017 mmol injected subcutaneously inhibited the growth of sarcoma 180 in mice (Omura et al. 1974). L-ascorbate and its oxidative product dehydroascorbate were cytotoxic or lethal to fast-growing malignant cells (at concentrations of 0.2 to 0.5 mM in the medium), but were less toxic to nonmalignant cells. Erythorbic Acid also had similar activities despite its lower antiscorbutic potency and high turnover rate (Tsao, Dunham, and Leung 1992; Leung et al. 1993).

The antitumor activities of ascorbic acid and Erythorbic Acid increased in the presence of cupric ion, a catalyst for the oxidation of ascorbic acid and its stereoisomers (Leung et al. 1993). Omura et al. (1974, 1975) reported that Erythorbic Acid lowered the viscosity of DNA solutions, and that this action was facilitated by the presence of Cu^{2+}. Erythorbic Acid, with or without the cupric ion catalyst, caused both single- and double-strand scissions of DNA.

A group containing a minimum of six female young adult BDF_1 mice received subrenal implantations of human mammary carcinoma MX-1 and, beginning 1 day later, were injected intraperitoneally with Erythorbic Acid and other derivatives of ascorbic acid daily for 5 days. Also, the experimental mice were administered the test chemicals in their drinking water at 1 g/L so that the total daily dose was 200 mg/kg. Six control mice received the xenografts, but were given doses of saline. After the experimental period of 6 days, the mice were killed, weighed, and the tumor-bearing kidneys removed so that final tumor sizes could be measured. No toxic effects were seen during the course of the experiment and tumor growth decreased in the treated mice as compared to the control mice. The data indicated that Erythorbic Acid reduced the mitotic activity of the tumor cells, thereby inhibiting their metabolism. Additionally, the degradation products of Erythorbic Acid could have been the responsible agents (Tsao, Dunham, and Leung 1992).

Erythorbic Acid induced chromosome aberrations in cultured cells that served to either suppress cell growth or kill the cells. When HT-1080, CCRF-CEM, P388, and L1210 cells were incubated in the presence of ascorbic acid or Erythorbic Acid at the ED_{50} concentration, many dead cells and much debris were observed. When the treated cells were washed and recultured in medium not containing ascorbic acid or its stereoisomers, no growth was observed, indicating a direct cell-killing, not cytostatic, action. The cytocidal activity of Erythorbic Acid was similar to that of ascorbic acid, despite the differences in their biological properties (Leung et al. 1993).

Tsao, Dunham, and Leung (1992) used young adult female BDF_1 mice as xenograft recipients. Tumors from human breast tumor cell line MX-1 were implanted beneath the renal capsule, and Erythorbic Acid in water was then administered (at 75 and 150 mg/kg/day doses) by intraperitoneal injection starting 24 hours later, and continued daily for 6 days. No weight loss was recorded for the mice, including the controls, and no signs of toxicity were observed. Tumor size decreased during Erythorbic Acid treatment, and the change was even greater when cupric sulfate was co-administered.

Leung et al. (1993) suggested that the cytotoxicity observed was not direct in nature or due to the vitamin metabolism of

L-ascorbic acid. Instead, the properties of the chemicals and their ability to produce active cytotoxic agents during oxidation resulted in cell death. When 12 other derivatives of ascorbic acid were tested, 6 inhibited tumor growth (to varying degrees, up to 65%) and 1 accelerated growth (Tsao, Dunham, and Leung 1992).

L-ascorbic acid, its isomers, and derivatives all include an enediol lactone ring, which could be at least partly responsible for its toxic effect (Leung et al. 1993). If the oxidized enediol group was blocked, antitumor and DNA-degrading activities were both lost, which suggested that the initial step in the inhibition of tumor growth could be the polymerization of DNA (Omura et al. 1974).

CLINICAL ASSESSMENT OF SAFETY

A maximization test using 15 female and 13 male subjects was performed to determine the contact sensitization potential of an eye cream containing 0.2% Ascorbyl Palmitate. Skin sites on the upper outer arm, volar forearm, or back of each subject were pretreated with 0.1 ml of sodium lauryl sulfate (SLS) at a concentration of 0.25% and covered with occlusive patches for 24 hours. At patch removal, 0.1 ml of the test eye cream was applied to the same site and covered with occlusive tape. This induction patch remained in place for 48 hours, after which it was removed and the test site was examined for signs of irritation. If no irritation was observed, the pretreatment-treatment procedure was repeated at the same skin site for a total of five induction exposures. If irritation was observed, the treatment patch only was applied for the duration of the study. Ten days after the last induction patch application, 0.1 ml of SLS (5.0%) was applied to an untreated skin site under an occlusive patch for 1 hour. The SLS patch was removed and replaced with a challenge patch containing the eye cream. At 48 and 72 hours, the skin sites were examined for sensitization. Twenty-six subjects completed the study; the remaining 2 subjects withdrew for reasons unrelated to the study. No adverse reactions or signs of dermal sensitization were observed during this study (Ivy Laboratories 1995).

One hundred nineteen subjects were enrolled in a modified Draize assay for skin sensitization potential (International Research Services, Inc. 1996). Of those, 106 completed the study. Ascorbyl Palmitate at concentrations of 1%, 3%, and 5% in petrolatum (0.025 g) was applied under occlusive conditions to the scapular back using a Finn Chamber. The test materials were applied three times per week for 3 consecutive weeks, and once in the 4th week (10 applications). The patch sites were evaluated 48 or 72 hours after application. Twelve days after the last patch was removed, a challenge patch was applied to an untreated skin site on the scapular back. The patch was removed at 48 hours, and the site was scored at patch removal and at 96 hours. One subject had a rash on his torso during the 3rd week of the study, but this was attributed to exclusionary medication that was not reported on the subject's personal and medical history, and was not related to the test substances. During induction, the 1% preparation caused seven 1+ reactions in a single subject. No reactions were noted for the 3% preparation, and one subject had five 1+ reactions after being treated with the 5% preparation. Under the conditions of this study, the investigators concluded that 1–5% Ascorbyl Palmitate was not sensitizing.

SUMMARY

Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate function as antioxidants in cosmetic formulations. Product formulation data submitted to the FDA in 1996 stated that Ascorbyl Palmitate, Erythorbic Acid, and Sodium Erythorbate are used in 561, 728, and 19 cosmetic formulations, respectively. Ascorbyl Dipalmitate and Ascorbyl Stearate are each used in one lipstick. Ascorbyl Palmitate is used in cosmetics at concentrations of 0.01–0.2% in eye cream, body cleanser, cologne, and body oil. Erythorbic Acid is used at concentrations of 0.5–1% in hair dyes and toners.

Ascorbyl Palmitate and Ascorbyl Dipalmitate are the ester and diester of ascorbic acid and palmitic acid, respectively. Ascorbyl Stearate is the ester of ascorbic acid and stearic acid. Ascorbyl Palmitate could contain Ascorbyl Stearate, and vice versa. Erythorbic Acid and Sodium Erythorbate are stereoisomers of ascorbic acid and its sodium salt, respectively.

Ascorbyl Palmitate is produced by the condensation of palmitoyl chloride and ascorbic acid in the presence of a dehydrochlorinating agent such as pyridine; Ascorbyl Palmitate is also formed by the reaction of L-ascorbic acid and palmitic acid. Ascorbyl Stearate is produced by the reaction of L-ascorbic acid and stearic acid. Erythorbic Acid is produced when the fermentation product of D-glucose by *Pseudomonas fluorescens*, 2-keto-D-gluconic acid, is esterified and heated in basic solution to Sodium Erythorbate. Acidification of this compound in a water-methanol solution results in the formation of Erythorbic Acid. Alternatively, Erythorbic Acid can be synthesized from sucrose, naturally produced by *Penicillium* species, or prepared by the reaction of 2-keto-D-gluconate sodium methoxide.

Ascorbic acid derivatives typically have strong absorption bands at 256 nm. The maximum absorbance of Erythorbic Acid was 260 nm; Erythorbic Acid does not significantly absorb UV light at wavelengths above 290 nm.

Ascorbyl Palmitate has vitamin C activity approximately equal to that of L-ascorbic acid. Erythorbic Acid has 1/20 of the vitamin C activity of its stereoisomer, as does its salt, Sodium Erythorbate. Vitamin C is a cofactor for enzymes involved in collagen biosynthesis; Erythorbic Acid is as effective as L-ascorbic acid in promoting collagen synthesis in vitro.

Ascorbyl Palmitate applied topically to guinea pigs penetrated the skin such that ascorbic acid content in the skin, liver, and blood increased eight-, seven-, and four-fold, respectively. Homogenates of the liver, pancreas, and intestines hydrolyzed Ascorbyl Palmitate and Ascorbyl Dipalmitate to free ascorbic acid. No statistical difference was observed between the absorption of Erythorbic Acid and ascorbic acid by the skin of the human cheek. When L-ascorbic acid intake by guinea pigs was low, Erythorbic Acid was retained at greater concentrations in

the liver, adrenal glands, spleen, and kidneys. This suggested that Erythorbic Acid was absorbed into the small intestine by the same active transport mechanism used by ascorbic acid.

One researcher reported that absorption was approximately four-to-one in favor of ascorbic acid over Erythorbic Acid, but ascorbic acid availability decreased by 40–60% when Erythorbic Acid was coadministered. Additional animal studies produced data that Erythorbic Acid promoted the acceleration of oxidative destruction of ascorbic acid in the liver. Rats given Sodium Erythorbate in feed eliminated Erythorbic Acid and dehydroerythorbic acid, but not ascorbic acid or dehydroascorbic acid.

Ascorbyl Palmitate and Ascorbyl Stearate reduced acetaminophen-induced hepatotoxicity in MF1 mice by reducing the binding of acetaminophen metabolites to hepatic proteins and the depletion of nonprotein sulphydryl groups and aminotransferase activity. Treatment with Ascorbyl Palmitate and Ascorbyl Stearate prevented the 35% mortality caused by administration of acetaminophen alone and prevented hepatic damage by reducing the reactive acetaminophen metabolites to the parent compound and by having a sparing action on reduced hepatic glutathione.

Ascorbyl Palmitate counteracted the formation of pyknotic nuclei and increased the mitotic index in primary cultures of Wistar rat heart endothelial and muscle cells by exposure to thermally oxidized fats.

Ascorbyl Palmitate significantly inhibited human term placental and fetal hepatic GST activity towards the second substrate 1-chloro-2,4-dinitrobenzene. Ascorbyl Palmitate also inhibited thromboxane B2 synthesis induced by both A-23187 and thrombin and the agonist-induced activation of platelets in rabbits.

Erythorbic Acid increased the rate of disappearance of tetracycline from rat and human plasma and decreased plasma protein binding of tetracycline. In other animal studies, Erythorbic Acid decreased the viscosity of calf thymus DNA solutions when copper was added. Erythorbic Acid caused single- and double-strand scissions of DNA with or without the addition of copper. Erythorbic Acid inactivated various phages in vitro.

The acute oral LD_{50}s of 33.3% Ascorbyl Palmitate and 15% Ascorbyl Dipalmitate were 2 g/kg and >5 g/kg, respectively, in both rats and mice. The acute dermal LD_{50} of Ascorbyl Palmitate in guinea pigs was >3 g/kg. The lowest effect level (LEL) of Ascorbyl Palmitate in rats was 2500 mg/kg/day, which caused body weight reductions. Rats given up to 3 g/kg of Ascorbyl Stearate in feed had no adverse effects. The LEL of Erythorbic Acid in rats was >2500 mg/kg/day and was >7500 mg/kg/day in dogs. The LD_{50} of Erythorbic Acid was 8.3 g/kg and 18.0 g/kg in mice and rats, respectively. The acute oral LD_{50} of 50% Sodium Erythorbate in rats was >5 g/kg. No adverse effects were observed when 2 g/kg of Sodium Erythorbate powder was applied to the intact and abraded skin of rabbits for 24 hours.

Mice fed Ascorbyl Palmitate for 63 days and rats fed Erythorbic Acid for 168 days had no signs of toxicity at the doses administered. In a 728-day chronic toxicity study using rats, the no-observed-adverse-effect level was 1000 mg/kg/day Ascorbyl Palmitate. At 2500 mg/kg/day and above, the rats had decreased body weight gain and the formation of oxalate stones in the urinary bladder. Rats given 2–5% Ascorbyl Palmitate in feed for 9 months had significant growth retardation, bladder stones, and hyperplasia of the urinary bladder epithelium. In 2-year feeding studies using rats, no lesions were seen after administration of Erythorbic Acid. Dogs fed Erythorbic Acid for 240 or 330 days did not have signs of toxicity during the testing periods. The maximum tolerated dose (MTD) of Sodium Erythorbate in drinking water was 2.5% in male mice and 5% in female mice after 10 weeks of treatment.

Ascorbyl Palmitate (10%) and Ascorbyl Dipalmitate (100%) were not irritating to the intact skin of albino rabbits during a 24-hour modified Draize dermal irritancy test. Dermal irritation data were not available on Erythorbic Acid, but ascorbic acid (30%) caused barely perceptible erythema to the intact and abraded skin of rabbits. Sodium Erythorbate powder did not cause signs of dermal irritation when applied to the intact and abraded skin of rabbits. Ascorbic Acid was not a sensitizer. Erythorbic Acid (10%) applied topically to porcine skin reduced UVB-induced phototoxicity.

Ascorbyl Palmitate at a concentration of 10% did not irritate the eyes of rabbits. Ascorbyl Dipalmitate was minimally irritating. Ocular irritation data on Erythorbic Acid were not available, but its stereoisomer, ascorbic acid caused slight to well-defined reddening of the eyes of rabbits when instilled at a concentration of 30%. Instillation of Sodium Erythorbate powder to the conjunctival sac of rabbits caused slight and transient reddening of the conjunctiva that cleared within 24 hours.

Sodium Erythorbate did not cause maternal or fetal toxicity when administered to female rats and mice by oral intubation at dosages up to 1030 mg/kg/day during gestation. Developmental toxicity did not occur after pregnant rats were given up to 5% Sodium Erythorbate in feed during a 13-week teratogenesis study.

Ascorbyl Palmitate was nonmutagenic in both the Ames test and the tryptophan reversion assay. Erythorbic Acid had little mutagenic potential in the Ames test, but demonstrated DNA-damaging potential in the liquid *Bacillus subtilis* rec assay. In activation and nonactivation tests using mouse, rat, and primate homogenates of liver, lungs, and testes, Erythorbic Acid was nonmutagenic. Erythorbic Acid was not genotoxic in chromosomal aberration and dominant lethal assays. Erythorbic Acid reduced the mitotic activity of human mammary carcinoma MX-1 cells that were implanted subrenally in BDF_1 mice. Sodium Erythorbate produced negative results in the Ames test, the host-mediated assay using *S. typhimurium*, chromosomal aberration tests using Chinese hamster ovary fibroblasts, the dominant lethal test using rats, and the *B. subtilis* rec assay. Sodium Erythorbate did not cause sister chromatid exchanges or chromosomal aberrations in cultured human embryo fibroblasts, but did cause chromosomal aberrations in rat bone marrow cells in vivo. Sodium Erythorbate did not increase the mitotic recombination

frequency of *S. cerevisiae* D3 in vitro, and the compound did not induce heritable translocation heterozygosity in male mice.

Formation of focal areas of dysplasia, body weight changes, and signs of toxicity were not observed in CF-1 mice fed 2% Ascorbyl Palmitate during a 2-week feeding study. Rats given 5% Erythorbic Acid or Sodium Erythorbate in feed for 168 days had no morphological alterations such as hyperplasias of the urinary bladder mucosa. Sodium Erythorbate did not enhance the development of rare spontaneous tumors or transform benign tumors to carcinomas after administration to F344 rats in feed at concentrations up to 2.5%. During a 24-week study, F344 rats given 5% Sodium Erythorbate in feed had simple hyperplasia of the urinary bladder epithelium. The addition of 1.25–2.5% Sodium Erythorbate to drinking water did not significantly increase tumor incidence, time to death with tumors, or the distribution of tumors in $B6C3F_1$ mice after 96 weeks of treatment.

F344 rats were given 5% Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, or Sodium Erythorbate after initiation with various carcinogens. Rats given Ascorbyl Dipalmitate or Ascorbyl Stearate after initiation with *N*-butyl-(4-hydroxybutyl)nitrosamine (BBN) had reduced body weights, but no changes of the liver or kidneys were observed. Erythorbic Acid did not promote urinary bladder neoplasms with or without prior initiation with BBN. Sodium and potassium salts are known to enhance tumor formation and increase tumor incidence in initiated rats. Sodium Erythorbate did not have modifying effects on second-stage carcinogenesis of the nonglandular and glandular stomach, colon, liver, kidneys, mammary gland, ear duct, or thyroid gland, but it did increase the incidence and average number of lesions of the urinary bladder after initiation with BBN.

Small topical doses of Ascorbyl Palmitate inhibited ~91% of tumors in previously initiated mice. Ascorbyl Palmitate also inhibited TPA-induced ornithine decarboxylase activity, tumor production, and DNA synthesis in mouse epithelial cells. Ascorbyl Palmitate and Erythorbic Acid inhibited the growth of sarcoma 180 in mice when the antioxidants were injected subcutaneously. The antitumor activities of both compounds increased in the presence of cupric ion, a catalyst for their activation.

Ascorbyl Palmitate at concentrations of 0.2% in formulation and at 1–5% in petrolatum produced no signs of dermal irritation or sensitization in clinical studies.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel noted that antioxidants, particularly those derived from vitamin C, are used in cosmetic formulations with increasing frequency. In its review of the antioxidants Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate, the Expert Panel acknowledged the potential beneficial effects, for example, the promotion of collagen biosynthesis, but focused on the assessment of cosmetic safety.

The CIR Expert Panel noted that the ester forms Ascorbyl Palmitate, Ascorbyl Dipalmitate, and Ascorbyl Stearate penetrate the skin readily, whereas Erythorbic Acid and Sodium Erythorbate are not likely to penetrate.

The Expert Panel recognized that the highest use concentrations of Erythorbic Acid and Sodium Erythorbate are in hair dyes, whereas the ester forms are used at lower concentrations in leave-on formulations. Erythorbic Acid and its sodium salt are used as neutralizers in oxidative hair dyes; the ingredients function as antioxidants in the color section of the mix to prevent premature oxidation of the color. As the antioxidants are completely consumed in the reaction, the Expert Panel concluded that Erythorbic Acid and Sodium Erythorbate are safe as used in hair dyes. The available animal and clinical toxicity data also supported the safety of Ascorbyl Palmitate, Ascorbyl Dipalmitate, and Ascorbyl Stearate as used in other cosmetic formulations.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate are safe for use as cosmetic ingredients in the present practices of use.

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