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Final Report on the Safety Assessment of Sorbic Acid and Potassium Sorbate

Sorbic acid is a straight-chain monocarboxylic acid used in cosmetic formulations as a preservative at concentrations up to 1.0%.

Sorbic acid and potassium sorbate were practically nontoxic to rats and mice in acute oral toxicity studies. In subchronic studies no significant adverse effects were observed in rats, mice, or dogs when 10% sorbic acid was included in the diet.

Sorbic acid and potassium sorbate at concentrations up to 10% were practically nonirritating to the rabbit eye. Both ingredients at concentrations up to 10% were at most only slightly irritating.

Sorbic acid and potassium sorbate have been tested for mutagenic effects using the Ames test, genetic recombination tests, reversion assays, *rec* assays, tests for chromosomal aberrations, sister chromatid exchanges, and gene mutations. Results have been both positive and negative.

Potassium sorbate at 0.1% in the diet or 0.3% in drinking water of rats for up to 100 weeks produced no neoplasms. In other chronic studies, no carcinogenic effect was demonstrated by sorbic acid in rats or mice fed diets containing up to 10% sorbic acid.

No teratogenic effects have been observed in pregnant mice and rats administered potassium sorbate.

In three repeat insult patch tests, sorbic acid had overall sensitization rates of 0, 0.33, and 0.8%. All of the subjects sensitized were inducted with 20% sorbic acid and challenged with 5% sorbic acid. Formulations containing up to 0.5% sorbic acid and or potassium sorbate were not significant primary or cumulative irritants and not sensitizers at this test concentration. A formulation containing 0.01% sorbic acid was not a photosensitizer.

On the basis of the available data, it is concluded that sorbic acid and potassium sorbate are safe as cosmetic ingredients in the present practices of use and concentration.

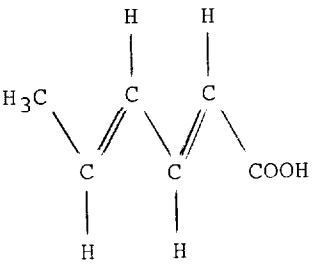
INTRODUCTION

The literature on sorbic acid and potassium sorbate dating from 1920 to 1975 has been previously reviewed in a generally recognized as safe

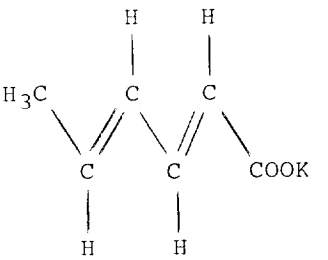
(GRAS) report and evaluation and is only briefly summarized here.[1,2] A survey of the most recent literature, pertinent articles not included in the GRAS report and evaluation, and the unpublished industry data have been incorporated here.

CHEMICAL AND PHYSICAL PROPERTIES

Sorbic acid is a straight-chain monocarboxylic acid also known as 2,4-hexadienoic acid. Potassium sorbate is the potassium salt of sorbic acid.[3,4] These ingredients conform to the following structures[4]:



SORBIC ACID



POTASSIUM SORBATE

Sorbic acid is a white, free-flowing, crystalline powder that is relatively soluble in alcohol and ether and only slightly soluble in water. It has a faint characteristic odor and a slightly acrid taste. Potassium sorbate is a white crystalline powder or white granules or pellets with no or slight odor. It is soluble in alcohol and freely soluble in water.[5-10] The physicochemical properties of sorbic acid and potassium sorbate are presented in Table 1.

Sorbic acid occurs naturally as the lactone, parasorbic acid, in berries of the mountain ash, *Sorbus aucuparia* L., *Rosaceae*. It can be synthesized by various processes, which include condensation of crotonaldehyde and acetic or malonic acid in pyridine solution,[6,10,11] condensation of crotonaldehyde and ketene in the presence of boron trifluoride,[4,10] preparation from 1,1,3,5-

TABLE 1. Physicochemical Properties

Property	*Values*	
	Sorbic acid	*Potassium sorbate*
Appearance	White, free-flowing powder[5,8]	White crystalline powder[5,8]
Odor, taste	Faint characteristic, odor,[3] slightly acrid taste[9]	No or slight odor[9]
Molecular weight	112.13[5,13]	150.22[5,13]
Boiling point (°C)	228 (decomposes)[10,14]	—
Melting point (°C)	134.5[10,14,15]	270 (decomposes)[4,5,10,14]
Flash point (°C)	127[10,14]	—
Ionization constant (at 25°C)	1.73×10^{-5}[1]	—
Density (19/4°C)	1.204 (19/4°C)[15]	1.36 (25/20°C)[14]
Maximum absorption (chloroform)	260 nm ($E = 2400$)[16]	—
pH	3.3 (0.20%)[17]	8.0 (0.3%)[18]
Solubility (%)		
Water	0.25 (at 30°C)[10] 58.2 (at 20°C)[10] 3.8 (at 100°C)[10]	
Propylene glycol	5.5 (at 20°C)[10]	—
Ethanol or methanol	12.90[10]	6.5[10]
Ethanol, 20%	0.29[10]	—
Glacial acetic acid	11.5[10]	—
Acetone	9.2[10]	—
Benzene	2.3[10]	—
Carbon tetrachloride	1.3[10]	—
Cyclohexane	0.28[10]	—
Dioxane	11.0[10]	—
Propanol	8.4[10]	—
Isopropyl ether	2.7[10]	—
Methyl acetate	6.1[10]	—
Toluene	1.9[10]	—
Chloroform	Relatively soluble[4]	Relatively insoluble[4]
Ether	Relatively soluble[4,8]	Relatively insoluble[4]
Loss on drying (% maximum)	0.5[5,13]	1.0[4,8,9,13]
Residue on ignition (% maximum)	0.2[5,8,9,13]	—
Arsenic as As (maximum)	3 ppm[5]	3 ppm[5]
Lead as Pb (maximum)	10 ppm,[5] 20 ppm[8]	10 ppm,[5] 20 ppm[8]

tetraalkoxyhexane,[10] and dealkanolation and hydrolysis of a 3,5-dialkoxyhexanal dialkyl acetal under oxidative conditions.[4] The trans,transisomer is usually obtained and is the commercial product.[10] Potassium sorbate is prepared by reacting sorbic acid with an equimolar portion of potassium hydroxide. The resulting potassium sorbate may be crystallized from aqueous ethanol.[4,6,10]

Numerous studies have been conducted on the stability of sorbic acid and its salts. From a study on the effects of acids, heavy metal ions, and sodium chloride on the autoxidation of sorbic acid in aqueous solution, it was determined that acetaldehyde and fumaraldehydic acid were formed as reaction products. Solutions of sorbic acid salts were stabilized against atmospheric oxidation by the inclusion of gluconic acid, δ-lactone, citric acid, EDTA, or erysorbic acid and its alkaline salts. Propyl gallate was also an effective antioxidant for sorbic acid solutions.[12]

McCarthy et al.[19] found that both temperature and type of container affected the breakdown of sorbic acid. Aqueous solutions of sorbic acid (0.1% w/v) stored for 12 weeks in polypropylene, polyvinyl chloride, polyethylene, and glass containers all had significant loss on storage, except when refrigerated or in the presence of an antioxidant (as occurs in polyethylene-92.2% sorbic acid remaining). The mechanism of decomposition was uncertain and in polyvinyl chloride and glass (at 50°C) was not linear. Although some solutions became increasingly acidic with time, leading to improved contact killing times, both dilution tests confirmed a loss in potency. These losses were not always proportional to the spectrophotometric results.

Gruntova et al.[20] also studied the stability of sorbic acid in aqueous and polysorbate solutions; sorbic acid was oxidized more readily in the polysorbate solutions, with the rate influenced by the packaging material. Kondrat'eva et al.[21] found that the amount of sorbic acid in petrolatum and emulsified bases stored at room temperature in metal containers started to decrease within 1 month and reached 60–80% of the initial content of the bases. They concluded that sorbic acid does not react with sodium lauryl sulfate or diethylene glycol stearate. Nielsen[22] found that sorbic acid incorporated in a cough syrup formulation did not decompose after 26 months of storage at room temperature.

Sorbic acid formed complexes with various starches by interacting with the amylose fraction of the starch. Sorbic acid complexed with acacia in aqueous solution and was also absorbed by nylon and cellulose acetate. The degree of sorbic acid uptake by nylon increased with both temperature and time and was dependent on the pH of the solution, indicating the undissociated molecule was the preferentially absorbed form.[12]

Shihab et al.[23] reported that urea, methylurea, ethylurea, 1,3-dimethylurea, an 1,3-diethylurea increased the solubility of sorbic acid in water. The ureas decreased the hydrophobic attraction between the acid molecules, thus allowing the formation of hydrogen bonds between the acid and water molecules. In another study on the solubility of sorbic acid in the presence of 12 macromolecules, it was found that the amount of solubilization was greatest with polysorbates.[12]

Sorbic acid and potassium sorbate are analyzed primarily by chromatographic techniques, including high-pressure liquid chromatography,[24–26] thin-layer chromatography (TLC),[27–29] gas-liquid chromatography,[30–32] gas chromatography,[33–35] and a combination of gas chromatography and mass spectrometry.[36] Other methods of analysis include ultraviolet spectrophotometry,[37–39] colorimetry,[37] and an isotachophoretic separation based on different electrophoretic mobilities.[40] Both sorbic acid and potassium sorbate can be identified by close matching to standard infrared spectra with no indication of foreign materials.[8]

There has been some concern in the past that sorbic acid may be contaminated with trace amounts of its isomer, parasorbic acid (5-hydroxy-2-hexanoic acid δ-lactone), which is a suspected carcinogen.[41] Stafford et al.,[42] using a new method combining column chromatography, thin-layer chromatography, and gas chromatography-mass spectral analysis, found no parasorbic acid in several food-grade samples of sorbic acid [method sensitive

down to concentrations of 20 ppm (20 mg/kg)]. Murphy and Wardleworth[43] described a more sensitive method in which parasorbic acid was extracted from aqueous potassium sorbate with dichloromethane and determined by gas chromatography using a flame ionization detector. They found no evidence of parasorbic acid down to a concentration of 0.5 mg/kg in the few samples of sorbic acid examined.

USE

Cosmetic

Sorbic acid and potassium sorbate are used in cosmetics and toiletries as preservatives and antimicrobials.[44-46] The 1986 U.S. Food and Drug Administration (FDA) data show that sorbic acid was used in a total of 445 products, including primarily makeup (44%), skin care (19%), eye makeup (16%), hair (7%), and bath (4%) preparations. Of these formulations, 62% incorporated sorbic acid at concentrations of $\leq 0.1\%$; 37% incorporated sorbic acid at concentrations of > 0.1–1%. Potassium sorbate was reported in 117 products, primarily skin care (including suntan preparations) (44%), hair (34%), and makeup (8%) preparations. Of the formulations, 56% incorporated potassium sorbate at concentrations of > 0.1–1%; 44% incorporated potassium sorbate at concentrations of $\leq 0.1\%$.[44]

The FDA cosmetic product formulation data presented in Table 2 are compiled through voluntary filing of such data in accordance with Title 21 Part 720.4 (d)(1) of the Code of Federal Regulations (1979). Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration is a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

The formulation data presented in Table 2 indicate that cosmetic products containing sorbic acid and potassium sorbate may contact all external body surfaces and hair, as well as ocular and vaginal mucosae. Sorbic acid additionally may contact the oral mucosae. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application can result in continuous exposure.

Noncosmetic

Sorbic acid and potassium sorbate are effective preservatives at low concentration for the control of mold and yeast in cheese products, based goods, fruit juices, fresh fruits and vegetables, wines, soft drinks, pickles, sauerkraut, and certain fish and meat products.[3] These ingredients are generally recog-

TABLE 2. Product Formulation Data

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)* > 1–5	> 0.1–1	≤ 0.1
	Sorbic acid				
Baby products	55	4		3	1
Bubble baths and other bath preparations	771	20		18	2
Eyeliner	235	12		1	11
Eye shadow	1406	26		2	24
Eye makeup remover	77	8		5	3
Mascara	325	10		4	6
Other eye makeup preparations	156	15		4	11
Fragrance preparations	1848	10		5	5
Powders (dusting, face, and talcum, excluding aftershave talc)	759	14		7	7
Hair conditioners, rinses, tonics and other hair-grooming aids	1204	25		15	10
Hair shampoos (noncoloring)	821	3		1	2
Hair shampoos (coloring)	27	3		3	
Blushers (all types)	472	19		14	5
Foundations	472	13		2	11
Lipstick	1552	32		32	
Makeup bases	462	106		1	105
Rouges	106	4		3	1
Other makeup preparations (not eye)	337	21		4	17
Manicuring preparations	77	3		1	2
Personal cleanliness products	506	6			6
Skin-cleansing preparations (cold creams, lotions, liquids, and pads)	1000	18	1	7	10
Face, body, and hand skin care preparations (excluding shaving) preparations)	1029	21		15	6
Moisturizing skin care preparations	802	23	1	13	9
Other skin care preparations	1042	22		7	15
Suntan preparations	202	7		1	6
1986 Totals		445	2	168	275
	Potassium sorbate				
Bubble baths and other bath preparations	478	4		3	1
Miscellaneous eye makeup	793	6		5	1
Hair conditioners	556	7		3	4
Hair shampoos (noncoloring)	838	18		4	14
Tonics, dressings, and other hair-grooming aids	350	3		2	1
Wave sets	160	12		2	10
Foundations	472	9		7	2
Skin and personal cleansing preparations	976	7		5	2
Face, body, and hand skin care preparations, including suntan preparations	3281	51		35	16
1986 Totals		117		66	51

Source: From Reference 44.

nized as safe direct food additives when used in accordance with good manufacturing practice.[47,48] Results of a survey of food manufacturers in 1970 indicated that the mean (weighted) level of the addition of sorbic acid to foods ranged from < 0.01 to 1.40% and that for potassium sorbate ranged from < 0.01 to 0.58%. The Grocery Manufactures of America has made an independent estimate of 0.5–0.3% for the range of sorbate addition to food.[2]

The Joint FAO–WHO (Food and Agriculture Organization–World Health Organization) Expert Committee on food additives has estimated the acceptable daily intake of sorbic acid and its salts (expressed as sorbic acid) as 25 mg/kg body weight.[49]

Potassium sorbate is also recognized as a GRAS indirect food additive as it migrates to food from paper and paperboard products used in food packaging.[50]

Sorbic acid and potassium sorbate are also used as preservatives in a variety of pharmaceuticals.[12,13,51–54] The Ophthalmic Advisory Review Panel of the FDA over-the-counter (OTC) drug review program has proposed that sorbic acid used alone in concentrations of 0.1–0.2% is not an effective antimicrobial agent because of its limited bactericidal effects. They also indicated that more data were required to establish the safety and effectiveness of sorbic acid used as a preservative in combination with other approved preservatives.[55,56] The OTC panel on contraceptives and other vaginal drug products has proposed that potassium sorbate at a concentration of 1–3% was safe and effective for OTC use as a vaginal douche for the relief of minor vaginal irritations. However, as potassium sorbate has not been marketed for this purpose to a material extent in the United States, it is considered by the FDA to be a new drug within the meaning of Section 201 (p) of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321 (p)].[57]

Sorbic acid and potassium sorbate also have various industrial uses. These ingredients are both used as preservatives in starch glue.[58,59] Sorbic acid is used to improve the characteristics of drying oils, the gloss in alkyd type coatings, and the milling characteristics of cold rubber.[10] Sorbic acid and potassium sorbate are used to prevent the premature sprouting of wheat seeds,[60,61] to dry out cut plants, such as alfalfa,[62] and in spray compositions applied to the foliage of crop plants to increase yield and growth.[63] Sorbic acid has also been used in insect disease control. Silkworm larvae consuming mulberry leaves that had been sprayed with 0.1–1.0% sorbic acid were protected from insect diseases caused by bacteria, fungi, and viruses.[64]

GENERAL BIOLOGY

Antimicrobial Effects

Sorbic acid and potassium sorbate have a broad spectrum of fungistatic activity but are less active against bacteria. Their antimicrobial activity depends upon the amount of undissociated acid, which is determined primarily by the dissociation constant (1.73×10^{-5} for sorbic acid) and the pH of the system. Optimum effectiveness is attained at pH values up to 6.5.[3,45] Table 3 gives the percentage of preservative undissociated related to pH value.

TABLE 3. Percentage of Sorbic Acid Undissociated Related to pH Value

pH	*% Undissociated*
3	98
4	86
5	37
6	6.0
7	0.6

Source: From References 45 and 65.

The anions of dissociated acids may be inactive owing to repulsion from the negatively charged microbial cell wall.[45,65] However, Eklund[66] has reported that the inhibition of bacteria by sorbic acid was due to both the undissociated and dissociated acid and has calculated the effect in accordance with a proposed mathematical model. Although the inhibitory action of the undissociated acid was 10–600 times greater than that of the dissociated acid, the latter was responsible for more than 50% of the growth inhibition of most of the organisms tested at pH levels above 6.

The antimicrobial activities of sorbic acid and potassium sorbate have been studied extensively. Bell et al.[67] tested sorbate against 66 species of filamentous fungi, 32 species of yeast, and 6 species of lactic acid bacteria. They reported that all organisms grew in media containing 0.1% sorbic acid at pH 7; however, this concentration of sorbic acid inhibited the yeasts and fungi at pH 4.5 and the bacteria at pH 3.5. Extensive tables on the antimicrobial spectrum of sorbic acid are found in the thesis by York.[68] The minimal inhibitory concentrations of sorbic acid for various common microbes are given in Table 4. The reader is referred to Woodford and Adams[12] and Sofos and Busta[69] for more in depth reviews of the antimicrobial effectiveness of sorbic acid and potassium sorbate.

Numerous mechanisms for microbial growth inhibition by sorbate are found in the literature; they indicate there was little or no agreement among

TABLE 4. Effective Concentrations of Sorbic Acid Against Common Microbials

Test organisms ($\sim 10^6$ colony-forming units per ml)	*Minimal inhibitory concentration (μg/ml) (serial dilution test; incubation times of 24 and 72 h; pH 6.0)*
Staphylococcus aureus	50–100
Clostridium sporogenes	100–500
Escherichia coli	50–100
Klebsiella pneumoniae	50–100
Pseudomonas aeruginosa	100–300
Pseudomonas fluorescens	100–300
Pseudomonas cepacia	50–100
Candida albicans	25–50
Saccharomyces cerevisiae	200–500
Aspergillus niger	200–500
Penicillium notatum	200–300

Source: *From Reference* 11.

scientists as to the manner in which sorbic acid inhibited microorganisms. Many investigators have suggested that sorbic acid works by inhibiting various enzyme systems and their reactions. Sorbic acid inhibition of sulfhydryl enzymes, including fumarase, aspartase, succinic dehydrogenase, and yeast alcohol dehydrogenase, has been noted.[69] Inhibition of the enzymes enolase,[11,69] proteinase,[70] catalase,[71] phosphopyruvic hydratase,[72,73] and cytochrome c oxidase[74] has also been reported. Reinhard and Radler[75] found that high concentrations of sorbic acid did not inhibit the enzymes aldolase, enolase, or pyruvate decarboxylase and assumed that sorbic acid inhibited the yeast cells mainly by influencing the cell membrane and its permeability. Cells of *Saccharomyces cerevisiae* rapidly adsorbed sorbic acid (primarily the undissociated form), and disturbances of cell growth may be caused by a reaction of sorbic acid with thiol groups of the surface of the yeast cell.[76] Harada et al.[77] suggested that sorbic acid inhibited the respiration of yeast through its competitive action with acetate at the site of acetyl-CoA formation. Deak and Novak[78] suggested that interference with active transport processes may play an important role in the inhibition of yeast by sorbic acid. Freese et al.[79] and Sheu et al.[80] generalized that lipophilic acid preservatives uncouple both substrate transport and oxidative phosphorylation from the electron transport system. Growth was inhibited by a reduction in cellular uptake of amino acids, organic acids, phosphate, and other compounds.

It has been variously reported that sorbic acid was both effective and ineffective as an antimicrobial in the presence of nonionic surfactants. Some of these discrepancies have been attributed to test conditions,[12] and it was generally accepted that sorbic acid was not strongly affected by the presence of nonionic surfactants.[81–84] In other interaction studies, pantothenic acid and biotin reduced the effectiveness of sorbate against *Verticillium dahliae* but thiamine did not.[85] The activity of sorbic acid may also be reduced by interaction with or loss through the containers.[45,86] Sorbic acid can also be degraded by microbes capable of using sorbic acid as a carbon source.[12,69,76] The mode of sorbic acid degradation has been postulated as being through decarboxylation.[87]

Potassium sorbate has synergistic antimicrobial activity with butylated hydroxyanisole and *tert*-butylhydroquinone against *Staphylococcus aureus* and *Salmonella typhimurium*.[88] It has acted synergistically with sodium nitrite and tripolyphosphate against *Clostridium botulinum*.[89] Potassium sorbate acted synergistically with heat to inactivate four types of molds; the addition of sucrose and sodium chloride further enhanced the inhibition of *Aspergillus flavus*.[90] The synergistic action of sorbate with sodium chloride[91–93] and sucrose[92] has been previously noted. A chlorine addition product of sorbic acid was more effective than sorbic acid alone and was less affected by pH.[45]

Biochemical and Cellular Effects

Sorbic acid did not affect the protein content or the biosynthesis of RNA and DNA in mouse embryo fibroblast cells in tissue culture.[94]

Sorbic acid (1.0 mmol/kg) and/or aminopyrine (0.4 mmol/kg) and sodium nitrite (1.0 mmol/kg) were orally administered to groups of five rats for 3

consecutive days. The rats were killed 24 h later and evaluated for alterations in biochemical parameters, namely, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase in serum, hepatic mincrosomal drug oxidation systems, and glucose-6-phosphate dehydrogenase and lysosomal enzymes in hepatic soluble fractions. The simultaneous administration of aminopyrine and sodium nitrite induced alterations in these parameters, believed to be due to the formation of *N*-nitrosodimethylamine. Sorbic acid did not inhibit the alterations produced by these chemicals, and when administered alone, did not significantly affect these parameters.[95]

Sorbic acid strongly inhibited both the peroxidase and oxidase activity of cabbage peroxidase and its isozymes. Sorbic acid produced a marked difference in action on the isozymes, being much more effective on isozyme III than I. Inhibition was noncompetitive, and the effectiveness depended on the concentration of sorbic acid, time of action, and pH of the medium. Inhibition increased with decreasing pH. Sorbic acid was the most effective organic acid assayed.[96]

Alimukhamedova and Mavlani[97] reported that sorbic acid affected the ultrastructural organization of yeast cells. *Saccharomyces vini* or *Rhodotorula glutinis* incubated with 250–500 mg sorbic acid had an accumulation of dense phospholipoprotein granules, numerous mitochondria of various sizes, and vacuoles within the cells, as well as the presence of irregular nuclei.

Sorbic acid was the second active compound of 35 food ingredients evaluated for a protective effect against cytogenetic radiation damages in the root tip of an onion (*Allium cepa*). Sorbic acid produced a 31% reduction in the rate of aberrant mitosis caused by 100 R irradiation.[98]

Sorbic acid combined in a 1:1 mixture with monolaurin effectively reduced (> 99.9%) the viability of the 14 human RNA and DNA enveloped viruses studied. The sorbic acid–monolaurin mixture was added to the cell culture at a concentration of 1% and incubated for 1 h at 23°C. The virucidal effect was attributed to the solubilization of the lipids and phospholipids in the envelope by the mixture, leading to a generalized disintegration of the viral envelope.[99] Similarly, sorbic acid enhanced the viral activity of the nucleopolyhedrosis virus in treated larvae of the gypsy moth *Lymantria dispar*.[100]

Sorbic acid had no inhibitory effect on the formation of plaque or the development of caries in rats; however, it did enhance the activity of dextranase on these factors.[101]

Metabolism and Excretion

The results of early metabolic studies indicated that sorbic acid was qualitatively metabolized in the same manner as the saturated or singly unsaturated fatty acids of the same C-atom number and was readily used as an energy source.[17]

The metabolism of [^{14}C] sorbic acid was studied after the administration by stomach tube of approximately 920 mg sorbic acid per kg body weight to female Sprague-Dawley rats. Within 4–10 h, 85% of the radioactivity was recovered in the expired carbon dioxide, 0.4% in the feces, 2% in the urine, 3%

in internal organs and blood, 3% in skeletal muscles, and 6.6% in other parts of the carcass. No radioactivity was in the liver or muscle glycogen, but some radioactivity was associated with the lipid fraction of the carcass, internal organs, and skin. The percentage of the radioactivity found in expired carbon dioxide was independent of dosage between 61 and 1213 mg sorbic acid per kg body weight. In similar tests, caproic acid was oxidized at the same rate and to the same extent.[2]

Sodium sorbate or sodium caproate was administered orally to fasted female rats at doses of 75 or 150 mg (calculated as acetone) per 100 m^2 of body surface. Administration was conducted daily in two divided doses of approximately 6 g sorbic acid per kg body weight; a similar proportion was excreted as ketone bodies. Sorbic acid and caproic acid were metabolized via acetone bodies, and under normal conditions sorbic acid was completely oxidized to carbon dioxide and water.[2]

In rabbits fed 3 g sorbic acid per kg body weight, the urine contained 0.1–0.2% *trans,trans*-muconic acid.[1,2] Small amounts of sorbic acid and muconic acid also have been found in the urine of mice orally administered aqueous solutions of sodium sorbate in doses of 40 and 3000 mg/kg body weight. Within 4 days, 81 ± 10% of the sorbic acid was oxidized to carbon dioxide and water; about 4% was found in urine, partially as muconic acid.[2] The metabolism of sorbic acid was identical in animals and humans.[1]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The oral LD_{50} for sorbic acid in rats has ranged from 7.36 to 12.5 g/kg body weight.[1,11,102] In rats fasted for 18 h prior to the administration of sodium sorbate, the LD_{50} (calculated as sorbic acid) was 3.6 g/kg for females and 4.3 g/kg for males. In rats that had not been fasted, the LD_{50} was 5.9 g/kg (also calculated as sorbic acid). The lower LD_{50} value for sodium sorbate compared with that for sorbic acid was attributed to its more rapid absorption from the gut.[2] The oral LD_{50} for sorbic acid in mice was greater than 8.0 g/kg body weight.[103]

Verrett et al.[104] also evaluated the toxicity of potassium sorbate using embryonating chicken eggs. They injected up to 10.00 mg potassium sorbate (in aqueous solution) into the air sac of the egg at 96 h incubation. The LD_{50} was 2.44 mg potassium sorbate per egg.

A formulation containing 5% sorbic acid was administered by intubation at a dose of 7.0 g/kg to five rats of unspecified strain and sex, and the rats were observed for 7 days. One of the rats died 1 day after treatment. The four surviving rats gained weight during the 7 day observation period.[105]

A 26 ml/kg dose of a cosmetic containing 0.15% potassium sorbate was administered orally to five male and five female fasted Harlan-Wistar rats. There were no signs of toxicity, and weight gains were normal during the 7 day observation period.[106] Groups of five male and five female fasted

Harlan-Fischer 344 rats were given 13 ml/kg of a bronzer[107] and a moisturizer[108] containing 0.15% potassium sorbate by gavage, and the animals were observed for 2 weeks. There were no deaths and no signs of toxicity.

Intraperitoneal

Sparfel et al.[103] reported an intraperitoneal (IP) LD_{50} for sorbic acid in mice of 2800 mg/kg body weight. Five mice were used for each dose. Aqueous solutions of sorbic acid were brought to a pH of 6 with sodium carbonate before injection of 0.5 ml/20 g. An IP LD_{50} value of 2820 mg/kg has also been reported for sorbic acid in mice.[109]

Potassium sorbate had an IP LD_{50} of 1300 mg/kg in mice.[110]

Subcutaneous

Sorbic acid had a subcutaneous LD_{50} of 2820 mg/kg in mice.[109]

Short-Term to Subchronic Toxicity

Oral

Numerous studies have been conducted on the short-term to subchronic oral toxicity of sorbic acid and potassium sorbate when administered to mice, rats, guinea pigs, and dogs. Results have varied with dose and length of administration.

Groups of 5–10 male and female albino rats were fed diets containing 10% sorbic acid for 30–120 days. The test animals had a higher liver-body weight ratio than the controls. Liver homogenates of the first-generation rats fed sorbic acid had lower oxygen consumption than controls; homogenates of liver from second-generation rats had a statistically significant decrease in oxygen uptake. Feed intake and reproduction were normal.[1]

Rats fed an 8% sorbic acid diet for 90 days had no adverse effects other than a slight enlargement of the liver. A 4% diet did not cause hepatic enlargement. Similarly, no adverse effects were found upon histopathologic examination of three dogs fed a diet containing 4% sorbic acid for 3 months.[2]

Rats and dogs fed diets containing up to 8% sorbic acid for 3 months were not adversely affected.[1]

A diet containing 2% sorbic acid (about 2 g/kg body weight) was fed to 8-week-old Wistar rats for 10 weeks. Growth was unaffected. Livers were slightly enlarged, although no microscopic abnormalities were noted.[2]

Groups of rats were fed diets containing 2 or 0.25% sorbic acid or potassium sorbate for 3 months. At the 2% dose, slight increases in the bilirubin and cholesterol content of the bile were noted as well as decreased pancreatic chymotrypsin and amylase. Potassium sorbate also reduced the lipase activity. At the 0.25% level, an increase was seen in the pancreatic juice secretion, its protein content, and the activity of all its enzymes.[1] It was concluded that the significance of these findings could not be assessed from the data.[2]

A 1% oily solution of sorbic acid administered orally to guinea pigs for 20 days produced a four- to sixfold increase in phagocytosis of staphylococcus.[1]

Groups of 50 male and 50 female mice were orally administered sorbic acid at a dose of 80 mg/kg per day for 3 months. Groups of 25 male and 25 female mice were similarly administered sorbic acid at a dose of 40 mg/kg per day or a polymeric impurity obtained from it at doses of 0, 8, or 800 mg/kg per day for 2 months. The mice were observed for general condition and behavior, survival, feed consumption, and weight gain. Tests were also conducted to determine the effects of hunger, physical stress, and carbon tetrachloride poisoning on the test animals compared to controls. Those mice administered sorbic acid for 2 months did not differ significantly from controls in survival, feed consumption, or weight gain. Weight gain was significantly increased in mice receiving the 800 mg/kg dose of the sorbic acid polymeric impurity. Mice administered sorbic acid for 3 months had slightly decreased weight gains compared to controls. Generally, the test mice reacted as well or better than controls to conditions of stress, hunger, and administration of carbon tetrachloride.[111]

In another study, sorbic acid was administered for a period of 3 months at a dose of 40–80 mg/kg per day to 400 albino rats and 1900 mice. No toxic effects on weight gain, feed consumption, or survival rate and no deleterious effects on reactions to stress were produced. The immunobiologic activity and detoxifying action of the liver were increased.[1]

Groups of 10 rats (5 males and 5 females) were fed a diet containing 0, 1, 2, 5, or 10% potassium sorbate for 3 months. The weight gain of the female rats fed 5 and 10% potassium sorbate was decreased initially. Relative hepatic weights were the same in all groups; renal weights were increased in the rats fed 10% potassium sorbate, to a lesser degree in those fed 5% potassium sorbate. No controls for high potassium intake were described.[2]

Potassium sorbate was administered in the diet for 3 months at concentrations of 1 and 2% to two groups of eight dogs each. Weight gains were comparable to those of the control group of four dogs. No adverse effects attributable to potassium sorbate were found upon gross examination at necropsy.[2]

Dermal

A dose of 2 ml/kg of a formulation containing 0.5% sorbic acid (pH not specified) was applied by inunction 5 days/week for 4 weeks to the clipped skin of the backs of three male and three female New Zealand albino rabbits. Plastic collars were worn to prevent ingestion of the test material. The skin of three of the rabbits was abraded. A control group consisted of three male and three female rabbits. Hematologic and biochemical measurements were made during the study. At the end of the study, the rabbits were killed, and the internal organs were examined microscopically. No adverse effects were produced on physical appearance, behavior, body weights, or survival, and no systemic effects were attributed to the formulation. No gross or microscopic lesions were produced. The intact and the abraded skin responses were the same. Slight to moderate erythema and edema were observed in all the rabbits during the first week, and this continued throughout the study. Slight atonia

was observed in all the animals during the second week and continued to be observed in four animals throughout the study. Slight desquamation was observed in two animals during week 2 and in two other animals during week 3, and this continued until the end of the study. The skin had a mild intradermal inflammatory response.[112]

A cream containing 0.15% potassium sorbate (pH not specified) was applied daily for 90 days to the clipped backs of five male and five female New Zealand rabbits. The cream was applied with a spatula to 10% of the total body surface of each animal in a dose of 6 mg/cm^2. Collars were worn to prevent ingestion of the test material. There were five male and five female control rabbits. The animals were observed for local and systemic effects. They were killed at the end of the study, and gross and microscopic examinations were performed. Two control rabbits and one treated rabbit died during the study from causes not considered treatment related. Mean feed consumption, body weights, and organ weights were normal, as were values obtained for hematology, clinical chemistry, urinalyses, and light microscopic examination. Incidental lesions in treated rabbits included granulomatous meningoencephalitis and acute colitis. All treated animals developed slight to moderate erythema and edema during the first week, and this continued throughout the study. Desquamation was slight to moderate in all the rabbits. Four animals developed fine fissures during week 3, and one animal had cutaneous fissures and bleeding on days 46–48. Papillae were observed on the backs of two animals during week 12. Histologically, compound-related dermatitis was observed in 8 of the 10 treated rabbits. The dermatitis was mild and was characterized by the presence of a few inflammatory cells in the upper dermis. No erosion or ulceration of the dermis was observed.[113]

Chronic Toxicity

Oral

Sorbic acid was evaluated for chronic oral toxicity in Wistar rats by administration of 0, 1.5, or 10% sorbic acid in the diet for 2 years. Experimental groups consisted of 48 males and 48 females each. For a similar caloric intake in all groups, a mixture of corn oil and starch (1:1) was added to the 0 and 1.5% sorbic acid diets at 10 and 8.5%, respectively. Body weight, feed and water consumption, mortality, and hematologic and urinalysis parameters were monitored. The organs of all rats were weighed and examined microscopically. No changes in appearance or behavior were noted. Mortality was similar in test and control groups. No significant effects attributable to sorbic acid treatment were found in the hematologic and serum evaluations, urinalyses, or microscopic examination. The total incidence of neoplasms (malignant and nonmalignant), as well as the distribution of affected tissues, was not influenced by sorbic acid treatment. The body weight gain in rats of the 1.5% sorbic acid group did not differ significantly from that in controls; however, rats of the 10% sorbic acid group had a statistically significant reduction in body weight gain from weeks 26 and 39 on in the females and males, respectively. This difference was only 5–10% of the control weight and was

not considered to represent a serious toxic effect because it did not affect mortality. No consistent differences were noted in feed consumption; the mean daily intake was calculated as 0.63 and 4.33 g/kg in males and 0.85 and 5.69 g/kg in females fed the diets with 1.5 and 10% sorbic acid, respectively. Male rats of the high-dose group had higher thyroid gland weights and higher thyroid gland-body weight ratios. All these animals had signs of advanced renal disease, and as prolonged renal damage in the rat can result in parathyroid gland hyperplasia,[114] the increased thyroid weights were considered due to increased parathyroid gland weights. The investigators claimed that their evaluation was further supported since this condition was found in males only, as glomerulonephrosis is usually less severe in females. Both males and females of the 10% sorbic acid group had higher relative liver weights, and the females additionally had higher relative kidney, small intestine, and ovary weights. The higher relative liver and kidney weights were not considered indicative of a serious effect as they were not associated with microscopic changes. (The livers of the high-dose females had only a marginal increase in fatty change and focal necrosis attributed to increased intakes of fatty acid.) In conclusion, the investigators stated that sorbic acid had a no-effect level of at least 1.5% (~ 750 mg/kg per day), although the lack of a carcinogenic effect and the "doubtful nature" of the other effects at 10% (~ 5 g/kg per day) indicate that the no-effect level may be closer to 5%.[115]

Sorbic acid was evaluated for chronic oral toxicity in a similar study in mice (strain ASH/CSI) by administration of 0, 1, 5, or 10% sorbic acid in the diet for 80 weeks. Experimental groups consisted of 48 male and 50 female mice each. To maintain the caloric intake in all groups, a mixture of corn oil and starch (1:1) was added to the 0, 1, and 5% sorbic acid diets at 10, 9, and 5%, respectively. Body weights, mortality, and hematologic parameters were monitored. At termination, the mice were killed and organs examined microscopically. Organ weights were also recorded. No adverse effects attributable to sorbic acid were noted on mortality, hematologic parameters, or the incidence of lesions, including neoplasms. A statistically significant reduction in body weight gain was noted in the males fed 5% sorbic acid and in both males and females fed the 10% sorbic acid diet; this reduction was more pronounced in the latter group. However, as mortality was unaffected and these mice had no other adverse effects, the lower weight was considered a "mildly unfavorable response." Statistically significant increases were noted in the relative organ weights of the brain, liver, kidney, stomach, and small intestine of males on both the 5 and 10% sorbic acid diets. All groups of females treated with sorbic acid had increased relative heart and liver weights, and females of the highest dietary group also had increased relative brain, small intestine, kidney, and spleen weights. The elevation in the relative weights of brain, spleen, stomach, and small intestine were not considered a toxic effect in that there were no significant differences in their absolute weights and no indication of microscopic change. The increased values for relative heart weights, occurring in females only, were not considered an effect of sorbic acid intake. The increased relative liver weights were considered to reflect an increase in metabolic demand resulting from increased fatty acid intake as there was a lower incidence of lesions in the livers of mice fed

sorbic acid than in controls. Similarly, the enlarged kidneys of these mice were not considered a serious toxic effect in that the incidence of lesions in the kidneys was significantly less in the treated mice than in controls. In conclusion, the investigators stated that the no-effect level of sorbic acid in mice may be considered 1% of the diet (~ 1400 g/kg per day), although because of the nature of the effects at concentrations up to 10%, the actual no-effect level may be substantially higher.[116]

Sorbic acid was administered in the diet at concentrations of 0, 0.1, 0.5, and 5.0% (0, 50, 250, and 2500 mg/kg per day) for a period of 1000 days to groups of 50 male and 50 female rats. No differences between test and control animals were noted in appearance, growth, mortality, or reproduction. Rats fed through the second generation a 0.1 or 0.5% sorbic acid diet had no signs of toxicity in respect to growth or reproduction. A group of 30 rats of the second generation maintained on a 5% sorbic acid diet for 252 days had no significant lesions. An unpublished report from the same laboratory described a study in which 50 male and 50 female rats were again fed 5% sorbic acid in the diet during their life span. Mortality was not significantly affected; the average life span of test males compared to control males was 811 and 709 days, and the test and control females lived an average of 789 and 804 days, respectively. No differences were reported in organ weights, and only two neoplasms were found in each of the control and test groups. No abnormalities were seen in the liver, kidneys, heart, or testes.[1,2]

Chronic oral administration of sorbic acid at concentrations of 1–500 times the amounts used in foods had no adverse effect on the blood or internal organs of rats, guinea pigs, rabbits, or dogs.[117]

Shtenberg and Ignatev[111] studied the toxicologic effects of some combinations of preservatives on both mice and rats. Groups of 25 male and 25 female mice were administered 40 mg/kg per day sorbic acid or 40 mg/kg per day sorbic acid plus 2 mg/kg per day nisin as a paste prior to the main feed. Administration continued for 17 months. A control group was fed the basal diet only. The mice were observed for their general appearance and behavior, feed consumption, weight gain, and survival. Organ weights were also recorded at the end of the study. Some of these mice were tested for the effects of physical stress (swimming with a 2 g weight on the tail) and feed restriction. After 8 months on test, some mice from the groups receiving the preservative combination or control diets were mated and reproduction was studied over five generations. The test mice were given the same combination (40 mg sorbic acid and 2 mg nisin per kg per day) from weaning to mating; litters were monitored for weight gain for 3.5 months after weaning. Mice receiving the sorbic acid-nisin combination had a lower survival rate than controls. Relative weights of the liver, kidneys, and testes of mice receiving only sorbic acid were lower than those in all other groups; however, these were not considered adverse effects. The litters from the five-generation study administered the sorbic acid-nisin combination gained more weight than those receiving a benzoic acid-sodium bisulfite mixture. Those mice administered sorbic acid or sorbic acid-nisin also had better scores on the stress tests than those receiving benzoic acid or the benzoic acid-sodium bisulfite mixture. No neoplasms were found in the control or sorbic acid-nisin groups.

The rats of this study similarly received 40 mg/kg per day sorbic acid (groups of 10 males and 10 females) or 40 mg sorbic acid and 2 mg nisin per kg per day (groups of 50 males and 50 females). Other groups received benzoic acid and/or sodium bisulfite. Feed and water consumption, weight gain, and hematologic parameters were monitored. The effects of stress factors were also recorded. These consisted of feed restriction, cold stress, centrifugation, a carbon tetrachloride detoxication test, and a renal function test. Rats fed the sorbic acid-nisin mixture gained more weight and fared better than those on the benzoic acid-sodium bisulfite diet under all stress conditions except feed restriction. The results of the latter study were inconclusive as rats in all test groups survived longer than the controls.[111] (The results of this study were not analyzed statistically.)

Several additive toxicity tests have been conducted with sorbic acid and other preservatives. Ohno et al.[118] studied the additive toxicity of sorbic acid and benzoic acid in groups of 20 male and 20 female Sprague-Dawley rats. The rats were administered diets for 1 year containing concentrations of 5% sorbic acid, 0.5% benzoic acid, 2% benzoic acid, 5% sorbic acid plus 0.5% benzoic acid, 5% sorbic acid plus 2% benzoic acid, or a basal diet with no supplementation. A slight growth inhibition was noted in the female rats receiving 5% sorbic acid after 6 months; no effects were noted on the males. No significant effects were noted in the hematologic values or in the serum and urine analyses of the test rats when compared to controls. No distinctive microscopic changes were noted in any experimental group. Sorbic acid and benzoic acid did not produce additive toxicity in the rat.

Because of concern about the possible contamination of sorbic acid with parasorbic acid, two chronic oral toxicity studies were conducted in rats and mice using sorbic acid deliberately adulterated with 1000 ppm parasorbic acid. Groups of 48 male and 48 female Wistar rats were fed diets containing sorbic acid or the adulterated sorbic acid at concentrations of 1.2% for 2 years.[119] Similarly, groups of 48 male and 48 female mice were fed diets containing the same concentrations of sorbic acid and adulterated sorbic acid for 80 weeks.[120]

The inclusion of parasorbic acid in the diet of rats had no significant effect on feed and water consumption, weight gain, hematologic values, renal function, serum analyses, or the incidence of lesions, including neoplasm incidence. Mortality was slightly greater in the females of the parasorbic acid group, but this was attributed to five rats that died or were killed between weeks 58 and 80. No comparable difference was observed in the males. The liver weights and relative liver weights of the females in the parasorbic acid group were increased compared to those of the sorbic acid group; however, this was not considered significant. The investigators concluded the sorbic acid diet was not made more toxic by the inclusion of 1000 ppm parasorbic acid.[119]

The inclusion of parasorbic acid in the diet of mice produced no statistically significant effects on weight gain, hematologic values, organ weights, or lesions, including the incidence of neoplasms. Mortality was slightly higher in the females of the parasorbic acid group (statistically significant for the last 3 weeks of the study) but was not attributable to the administration of parasorbic acid. Three moribund mice were killed because of severe middle ear

infection, generalized lymphoblastoma, and papillary adenoma of the lungs; both of the latter are common in mice. The other deaths were of differing etiology. The females receiving sorbic acid had only an unusually low mortality rate compared with historic controls from the same laboratory, and the mortality rate of the females receiving parasorbic acid was within the normal range for this strain of mice. The prolonged feeding to mice of a sorbic acid diet adulterated with 1000 ppm parasorbic acid did not lead to an increase in the toxic effects of sorbic acid and did not have a carcinogenic effect.[120]

Irritation

Ocular

A modified Draize ocular irritation test was conducted to evaluate the irritancy of sorbic acid and potassium sorbate to the rabbit eye. Sorbic acid (in petrolatum) and potassium sorbate (in aqueous solution) were evaluated at concentrations of 1, 5, and 10% (pH not specified). Three rabbits were used for each dose group. Eyes were scored at 1, 2, and 24 h and daily thereafter until all irritation had disappeared. Sorbic acid at concentrations of 1, 5, and 10% had ocular irritation indices (at 24 h) of 0.7, 0.7, and 2 (maximum = 110), respectively. Potassium sorbate had an ocular irritation index of 0 at all concentrations (at 24 h). Sorbic acid and potassium sorbate caused practically no ocular irritation and were well tolerated under these conditions.[121]

A 1% aqueous solution of potassium sorbate (pH not specified) was placed in the conjunctival sacs of one eye of each of three male and three female rabbits of unspecified strain. The Draize irritation score, 1 day after test material administration, was 0; the solution had no potential for eye irritation.[122] A 0.1 ml dose of a potassium sorbate solution of unspecified concentration was instilled into the conjunctival sac of one eye of each of six New Zealand white rabbits. The Draize irritation score at 24 h ranged from 2 to 11, and the average of the Draize scores for 24, 48, and 72 h was 4.7. No irritation was observed on day 7 after exposure. Some of the conjunctival tissue in two female rabbits was bleached white on day 1, and this was also observed in one of these rabbits on day 2. Conjunctival petechial hemorrhage was observed in the third female on days 1–3; on day 7, this was no longer observed.[123]

An eye makeup remover containing 0.10% sorbic acid was placed in one eye of each of six albino rabbits. The Draize irritation score was 0; the formulation was nonirritating.[124]

A 0.1 ml dose of a cosmetic containing 0.15% potassium sorbate was instilled into one eye of each of six albino rabbits of unspecified strain, and the animals were observed for 7 days. Slight conjunctival redness was observed 1 h after treatment, but this cleared within 24 h. Cornea and iris were not affected.[106] Groups of six New Zealand albino rabbits were used to evaluate the acute ocular irritation potential of a bronzer[107] and a moisturizer[108] containing 0.15% potassium sorbate. The undiluted formulations were instilled into one eye of each rabbit in a dose of 0.1 ml, and irritation was scored on days 1, 2, 3, and 7. Slight conjunctival hyperemia was

observed 1 h after treatment with both formulations, and this cleared within 24 h. No other signs of irritation were observed.

Dermal

A modified Draize irritation test was conducted to evaluate the dermal irritancy of sorbic acid and potassium sorbate in rabbits. Sorbic acid (in petrolatum) and potassium sorbate (in aqueous solution) were evaluated at concentrations of 1, 5, and 10% (pH not specified). Three rabbits were used for each dose group. Patches were applied under semiocclusive conditions. Sorbic acid at concentrations of 1, 5, and 10% had irritancy indices of 0, 0.2, and 0.5 (maximum = 8), respectively. Potassium sorbate had an irritancy index of 0 at all concentrations. Sorbic acid and potassium sorbate cause practically no dermal irritation and were well tolerated under these conditions.[121]

The primary skin irritation of a 1% aqueous potassium sorbate solution (pH not specified) was evaluated with nine rabbits of unspecified strain. A single occlusive patch was applied, and erythema and edema were scored 2 and 24 h after removal. The primary irritation index (PII) for the test material was 0.6 of a maximum possible of 4.0; the material was practically nonirritating.[125]

Sorbic acid, at a concentration of 5% in a lanoline-petrolatum paste, was applied daily, 6 days/week for 3 weeks, to the shaved skin of three rats. The paste was massaged lightly into the skin for 2 minutes, and the area was then washed with water and any excess paste wiped away. Three rats received similar applications of the lanoline-petrolatum paste without sorbic acid as controls. Weight gain was monitored over the 3 week period. All of the rats gained weight, those receiving petrolatum only at a rate of 5% and those receiving sorbic acid at a rate of 3%. No irritation or other adverse effects were reported.[103]

The primary skin irritation of a product containing 0.5% sorbic acid (pH not specified) was evaluated with nine rabbits of unspecified strain. Frythema and edema were scored 2 and 24 h after a single occlusive patch was removed. The PII of the test material was 0.72 of a maximum possible of 8.0; the skin irritation potential of the material was minimal.[126] An eye makeup remover that contained 0.10% sorbic acid (pH not specified) was evaluated for dermal irritation with 24 h occlusive patches on the intact and abraded skin of rabbits. The formulation did not irritate rabbit skin.[124]

Sensitization

Maurer et al.[127] compared the results of several methods used to assess the contact allergy of weak allergens in guinea pigs with the known epidemiologic data on the occurrence of hypersensitivity reactions in humans. Sorbic acid was evaluated for sensitization by an optimization method in 10 male and 10 female Pirbright white strain guinea pigs at a concentration of 0.1% in physiologic saline. The first week of induction consisted of intracutaneous injections of 0.1 ml of the test solution on Monday (flank and back), Wednesday (back), and Friday (back). The guinea pigs were chemically depilated 21 h after each injection, and the reactions were assessed 3 h later. The diameters of the two largest erythematous reactions (in vertical alignment) and the

skinfold thickness (as measured with a skinfold gauge) were used to determine the individual reaction volume for each animal for each reaction. For induction weeks 2 and 3, 0.1 ml of a 1:1 mixture of sorbic acid (in saline) and adjuvant was injected intracutaneously into the nuchal skin of each guinea pig on Monday, Wednesday, and Friday. The first challenge was administered 2 weeks after the last induction dose. A volume of 0.1 ml of 0.1% sorbic acid in physiologic saline was injected into a previously untreated site on the flank of each animal. The diameter and increase in skinfold thickness of each reaction were measured 24 h later to determine the individual reaction volumes. For each animal, the reaction volume at challenge was compared to the mean plus the standard deviation of the first four induction doses (considered the individual threshold value). If the reaction volume upon challenge exceeded the corresponding threshold value, the animal was considered sensitized. A second epidermal challenge was administered 2 weeks after the intradermal challenge. Occlusive patches containing 1% sorbic acid in soft white petrolatum were applied for 24 h to a shorn, previously untreated site. The reaction sites were chemically depilated 21 h after patch removal and the extent of erythema and skinfold thickness determined 3 h later. An allergic reaction was considered a clearly discernible reddening of the reaction site. The number of positive reactions to the first (intradermal) challenge was 4 of 20 ($P = 0.053$); the second (epidermal) challenge produced no positive reactions.

REACTIONS WITH NITRITE

The potential formation of mutagenic or DNA-damaging reaction products in the presence of sorbic acid or potassium sorbate and sodium nitrite has been studied extensively. Conflicting results have been reported. High concentrations of sorbic acid and sodium nitrite, reacting under acidic conditions, in most cases produced ethylnitrolic acid (ENA).[128–130] ENA has been reported by some as mutagenic and a potent inhibitor of *Escherichia coli*.[128,131,132] However, the results of an extensive study by Difate[133] established that nitrite, not ENA, was the mutagenic compound. He attributed the mutagenicity of ENA reported in earlier studies to possible free nitrite contamination of the test solutions.

Robach et al.,[134] evaluating the mutagenicity of sorbic acid-sodium nitrite reaction products produced in bacon-curing brines, reported that ENA was not formed at nitrite concentrations < 250 ppm and at a pH of 3.4. ENA was not formed at higher pH values (6) even with a nitrite concentration of 500 ppm. All sorbate-nitrite solutions and their ether extracts were negative in the Ames *Salmonella* assays.

Osawa and Namiki[131] analyzed the reactants of sodium nitrite with some sorbic acid analogs for mutagenicity using the *rec* assay and the Ames test. By a large-scale reaction of sodium nitrite with sorbic acid methyl ester, they isolated and identified 5-nitro-2,4-hexadienoic acid methyl ester and ENA as the main mutagens. The investigators concluded that a nitro group adjacent to the double bond is an important factor for the development of mutagenicity.

Khoudokormoff and Gist-Brocades[135] studied the mutagenicity of several food preservatives under conditions of pH and nitrite concentrations approximating those used in preserved foods. *Bacillus subtilis* mutant strain M45 *rec*$^-$, unable to repair DNA damage, was used as the test organism, with the wild-type strain H17$^+$ as control. Sorbic acid (0.1% as potassium sorbate) solutions containing 100, 200, or 400 ppm nitrite at a pH range of 3.0–6.5 consistently had a mutagenic activity that increased as the pH decreased and persited for 8 weeks. No mutagenicity was detected at pH $\geq$ 6. The results of similar experiments carried out with concentrations of SO_2^- approximating those used in wine (150 mg/liter) were a weak mutagenic effect after 2 weeks of exposure. Sorbic acid, nitrite, or bisulfite, tested alone at any pH, did not have mutagenic activity. The sorbic acid-nitrite complex was not mutagenic in two other microbiologic systems using Ames *Salmonella* strains and *E. coli* WP2 and WP2uvra$^-$. Both these systems had media buffered at pH 7, a pH at which sorbate and nitrite exert no mutagenic activity.

Namiki et al.[136,137] studied the effects of reaction conditions on the induced mutagenic (and antibacterial) activities of sorbic acid-sodium nitrite reactants. The pH of the medium, relative molar ratio of nitrite to sorbic acid, and time and temperature were all varied. Mutagenicity was assayed by the *B. subtilis rec* assay and by the Ames assay using *Salmonella typhimurium* TA-98 and TA-100 strains without metabolic activation. The reaction mixture (20 mM sorbic acid and 160 mM sodium nitrite reacted at 60°C for 1 h) obtained at a pH above 6.0 was inactive in the *rec* assay. DNA-damaging activity was produced at a pH between 2 and 5, with the maximum at 3.5–4.2. Mutagenic activity reached a maximum at a sorbic acid-$NaNO_2$ molar ratio of 1:8 (20 mM/160 mM), even though the formation of mutagenic compounds was detected at a ratio of 1:0.5.[137] Heating the reaction mixture to 60°C produced maximal DNA-damaging activity within 30 minutes, which then decreased gradually over time; the reaction carried out at 4°C had activity increasing slowly with time and reaching a maximum level between 48 and 96 h. By use of TLC and/or column chromatography, five C-nitro and C-nitroso compounds were isolated. These compounds included ENA, product Y, determined to be 1,4-dinitro-2-methylpyrrole, product B (total structure unknown), and products F and pre-F, considered primary products of the reaction that would lead to secondary and tertiary products. Tested individually for mutagenic activity, 1,4-dinitro-2-methylpyrrole was highly mutagenic by both the *rec* assay and the Ames assay. ENA was highly active in the *rec* assay but had no activity with *S. typhimurium* strain TA-98 and only weak activity with strain TA-100. Product B had no mutagenicity by the Ames assay and weak activity by the *rec* assay; products F and pre-F were inactive by all bioassays. Sorbic acid (100 mM) and sodium nitrite (160–800 mM) had no mutagenicity by the *rec* assay at the concentrations used in these experiments, although sodium nitrite has had mutagenic activity in other systems.[136] The addition of ascorbic acid or cysteine effectively inhibited the mutagen formation in this reaction system.[137]

Tanaka et al.[138] also tested for mutagenic activity in sorbic acid-nitrite reaction products. A compound designated compound I (the same as product

pre-F just discussed), as well as an unidentified product, were examined by the Ames assay with *S. typhimurium* strains TA-98, TA-100, and TA-1538. Neither compound gave positive results with or without metabolic activation. These compounds also gave negative results when evaluated for DNA-damaging activity using the *B. subtilis rec* assay. The reaction mixture itself (10 mM sorbic acid and 100 mM $NaNO_2$, pH 1, 37°C) was negative by the Ames assay when evaluated without metabolic activation.

Because sorbic acid reacts readily with nitrite, it was postulated that sorbic acid would inhibit the formation of carcinogenic nitrosamines from amines and nitrite. Numerous investigators have studied this under varying experimental conditions. Tanaka et al.[138] found that sorbic acid (20 mM) inhibited the in vitro formation of *N*-nitrosodimethylamine from dimethylamine and nitrite (40 mM $NaNO_2$, pH 2) by up to 74%. Sorbic acid (20 mM) also inhibited the formation of *N*-nitrosomorpholine from morpholine and nitrite (20 mM, pH 2) by up to 72%. Sorbic acid had no effect on the nitrosation of *N*-methylaniline. Ascorbic acid, tested under similar conditions, was equally inhibiting to the formation of *N*-nitrosodimethylamine but was a much stronger inhibitor of the formation of the other nitrosamines.

Lathia and Schellhob[139] also investigated the inhibition of nitrosamine formation in vitro by sorbic acid and/or ascorbic acid. Sorbic acid (0.05 mM) inhibited the formation of *N*-methyl-*N*-nitrosoaniline by 54% and *N*-nitrosomorpholine by 77% from *N*-methylaniline and morpholine, respectively. Reactions were carried out at pH 2 with equimolar amounts of potassium nitrite with either *N*-methylaniline (0.1 mM) or morpholine (10 mM). Increasing the concentration of sorbic acid to 0.1 mM decreased the amount of inhibition for both nitrosamines. Sorbic acid and ascorbic acid synergistically inhibited the formation of *N*-methyl-*N*-nitrosoaniline but not *N*-nitrosomorpholine.

Massey et al.[140] studied the effects of sorbic acid (and ascorbic acid) on *N*-nitrosamine formation in a heterogeneous, protein-based model system containing a 20% nonaqueous phase (glycerol tributyrate). The reactions were carried out at 37°C with an aqueous phase pH of 5.25. Sorbic acid (0.05 M) reduced the formation of nitrosopyrrolidine from pyrrolidine (0.05 M) and sodium nitrite (0.1 M) by 50% in both the aqueous and nonaqueous phases of the system.

Amundson et al.[141] compared the nitrosamine formation in fat and lean bacons cured with 0.26% potassium sorbate and either 40 or 120 ppm sodium nitrite. Nitrosamine formation was suppressed, although not eliminated, by the sorbate cure in both types of bacon.

Kawanishi et al.[142] reported that sorbic acid had no effect on nitrosamine formation from either aminopyrine or aminocycline reacting with sodium nitrite in guinea pig or rat stomachs.

Sorbic acid inhibited the formation of *N*-nitrosodimethylamine in human saliva from the interaction (in vitro) of salivary nitrite with aminopyrine or oxytetracycline. Inhibition ranged from 24 to 45% with 1 mM of sorbic acid and from 51 to 81% with 10 mM sorbic acid. Inhibition was greater at pH 3 than at pH 4.[143]

Potassium sorbate has been incorporated into cosmetic formulations to minimize *N*-nitrosamine contamination.[144]

MUTAGENICITY

The results of genetic recombination tests indicated that sorbic acid had a deleterious effect on the genetic material of *B. subtilis* 168. At concentrations of 20 and 30 μg/ml, sorbic acid (pH adjusted to 7) decreased the frequency of transformations to 77 and 75%, respectively. Concentrations of 1–10 μg/ml sorbic acid produced at 90–91% frequency of transformation. In further testing, sorbic acid (10 μg/ml) did not influence the reversion of characteristic genetics in cells of *B. subtilis* strains 3308, 112, 566, or 168. Sorbic acid (10 μg/ml) was also nonmutagenic by the Ames test with *S. typhimurium* strains 1535 and 1537.[145]

Morita et al.[146] evaluated sorbic acid for mutagenicity using a *rec* assay with wild and recombination-deficient strains of *B. subtilis* and a reversion assay using *S. typhimurium* strains TA-98 and TA-100, both with and without metabolic activation. Sorbic acid was negative by both the *rec* assay and reversion assay at concentrations up to 5.0 mg per disk and 10 μg per plate, respectively. Kada[129] also reported that sorbic acid was negative in the *rec* assay.

Potassium sorbate was evaluated for mutagenicity in a series of short-term assays using *S. typhimurium* strains TA-100 and TA-98 and silkworms for mutations, *B. subtilis* for *rec* assay (without metabolic activation, pH 5), and hamster lung fibroblast cells for chromosomal aberrations and sister chromatid exchanges (SCE; without metabolic activation), as well as rat bone marrow cells for chromosomal aberrations. Potassium sorbate was positive for chromosomal aberrations in hamster fibroblast cells and in the *rec* assay with *B. subtilis*; all other results were negative. No quantitative results were given.[147]

Potassium sorbate was evaluated for chromosomal aberrations and sister chromatid exchanges in a pseudodiploid Chinese hamster cell line at concentrations ranging from 5×10^{-3} to 4×10^{-2} M (maximum concentration of 2×10^{-2} M for the SCE test). Potassium sorbate produced a significant increase in SCE ($p = 0.05$) at concentrations of 1 and 2×10^{-2} M when compared with the mean value for the saline solvent, although this was not considered a dosage effect. However, a dose-related increase in chromosomal aberrations was noted. The investigators concluded that potassium sorbate induced aberrations but did not cause a pronounced increase in SCE.[148]

Ishidate et al.[149] studied the induction of chromosomal aberrations using a Chinese hamster fibroblast cell line in vitro. No metabolic activation was used. Potassium sorbate (in saline) at a maximum tolerated dose of 4.0 mg/ml produced chromosomal aberrations (chromatid gaps, breaks, and translocations) in 11% of the cells within 48 h. This was considered a positive response. Sorbic acid (in dimethylsulfoxide) was negative, producing aberrations in only 3% of the cells at a maximum tolerated dose of 1.0 mg/ml.

Ishidate et al.[149] conducted further studies using the Ames test with *S. typhimurium* strains TA-92, TA-1535, TA-100, TA-1537, TA-94, and TA-98 both with and without metabolic activation. Potassium sorbate (in distilled water) at a maximum dose of 3.0 mg per plate was negative. Sorbic acid (in dimethylsulfoxide) at a maximum dose of 10.0 mg per plate was also negative.

Potassium sorbate was evaluated for mutagenicity in a series of microbial assays. The results of plate tests using *S. typhimurium* strains TA-1535, TA-1537, and TA-1538 with a concentration of 2.5% (w/v) potassium sorbate (in phosphate buffer, pH 7.4) were negative for reversions. Suspension tests using the same strains of *S. typhimurium* and *S. cerevisiae* strain D4 with concentrations of 2.5 and 5.0% potassium sorbate were negative both with and without metabolic activation.[150]

Hasegawa et al.[151] studied the potential of sorbic acid, potassium sorbate, and sodium sorbate to induce chromosomal aberrations, SCE, and gene mutations in cultured Chinese hamster V79 cells. Sorbic acid was tested at concentrations of 350, 700, and 1050 μg/ml; potassium sorbate was evaluated at concentrations of 5000, 10,000, 15,000, and 20,000 μg/ml. Sorbic acid and potassium sorbate induced chromosomal aberrations in a significant number of cells (21 and 28%, respectively) only at the highest doses tested. The effect of sorbic acid and potassium sorbate on SCE was very limited, although concentration dependent, with the highest doses tested resulting in numbers of SCE 1.2 times the control level. The increase in the numbers of SCE was statistically significant at concentrations of 1050 μg/ml sorbic acid and at all concentrations $\geq$ 10,000 μg/ml potassium sorbate. The same test concentrations of sorbic acid and potassium sorbate produced no 6-thioguanine-resistant mutations. The effects of change in osmotic pressure caused by the addition of sorbic acid and potassium sorbate were also evaluated by substituting sodium chloride and potassium chloride. The induction of chromosomal aberrations could be partially attributed to the change in osmotic pressure, whereas the latter did not affect the number of SCE. Sodium sorbate was substantially more genotoxic than either sorbic acid or potassium sorbate.

Tsuchiya and Yamaha[152–154] conducted a series of mutagenicity tests on mice administered sorbic acid or potassium sorbate. Five groups of 77–79 male mice were fed diets containing 0 (control), 1.34, 6.7, and 20.1% potassium sorbate as well as 15% sorbic acid for periods of time up to 15 months. The first test evaluated the mutagenicity of the intestinal contents of these mice. Small and large intestinal contents were removed from 5–10 mice of all groups at week 1 and after 1, 3, and 6 months. The samples of contents taken from mice of the same dose group at the same time were combined, homogenized, and extracted with diethyl ether. The ether layer was evaporated, and one part was dissolved in dimethyl sulfoxide at a concentration of 0.70 mg/ml for the mutagenicity assay test and the other part subjected to fractionation (basic, neutral, and acidic). Using a modified Ames assay both with and without metabolic activation, the ether extracts of week 1 and 1 and 3 months were nonmutagenic in *S. typhimurium* TA-100 (extensive killing of bacteria occurred at 6 months); the extracts of contents sampled up through 6 months were nonmutagenic in *S. typhimurium* TA-98. These tests were repeated with strain TA-98 using the acidic, basic, and neutral components obtained by

fractionating the intestinal contents sampled at 3 and 6 months from the control and sorbic acid groups only. The results with basic and neutral components were negative. The acidic components from both samples of the sorbic acid group were slightly mutagenic but only with metabolic activation. The mutagenic ratios of acidic components at 3 months were slightly higher than those at 6 months; the distribution of acidic components at 6 months was 1.5 times greater than that at 3 months. The investigators suggested that mutagens were gradually produced in the intestine and moved into the liver, the site of metabolic activation.[152]

The urine of these mice was collected at 6 months and 100 μl samples assayed for mutagenicity using the Ames test with *S. typhimurium* strains TA-98 and TA-100. All results were negative. Samples (200 μl) from the control, 15% sorbic acid, and 20.1% potassium sorbate groups were also assayed using the Ames preincubation method; results were negative. Urinary samples from these mice treated with or without β-glucuronidase were fractionated by XAO-2 column chromatography and assayed for mutagenicity with strain TA-98. Only those samples from the 15% sorbic acid group were mutagenic when metabolically activated. Mutagenic ratios were unaffected by treatment with β-glucuronidase. Urine from mice on the 15% sorbic acid diet for 12 months was also collected and fractionated. These samples were nonmutagenic in strain TA-100 but gave positive results in TA-98 with metabolic activation. A comparison of the volume and pH of the urine between mice of the control and 15% sorbic acid groups at 6 months indicated a slight increase in volume and decrease in pH of urine of the sorbic acid group.[153]

Tsuchiya and Yamaha[154] further studied the mutagenicity of the intestinal contents, the glutathione content in the liver, and the relative body weight-liver weight ratios in these mice after administration of sorbic acid or potassium sorbate for 12 months. The acidic components of the intestinal contents of 10 mice from each group were assayed for mutagenicity using *S. typhimurium* strain TA-98 both with and without metabolic activation. Those samples taken from the 15% sorbic acid group were mutagenic with metabolic activation (mutagenicity increased with increasing amount in milligrams of acidic components per plate); slight mutagenicity was noted in samples from the 20.1% potassium sorbate group (with metabolic activation). The glutathione content in the liver of the 15% sorbic acid group at 3 months was decreased by 60% compared with that of the controls. This low concentration was maintained for up to 12 months. A close correlation was noted between the extent of depletion of hepatic glutathione content and the concentration of sorbic acid in the diet. The relative body-liver weight ratios of animals of the 15% sorbic acid group were clearly increased compared with those of the other groups.

CARCINOGENICITY

Potassium sorbate was administered orally to six rats at a concentration of 0.1% in the diet and to another six rats at 0.3% in the drinking water. No induced hepatic tumors were detected by laparotomy in these animals at 65

weeks, and the oral administration was therefore continued for 100 weeks (when all had died). The animals were examined postmortem, with microscopic examination when appropriate; no induced tumors were found in any of the rats.[18]

No carcinogenic effect was demonstrated by sorbic acid in Wistar rats[115] or ASH/CSI mice[116] fed diets containing up to 10% sorbic acid for periods of 2 years and 80 weeks, respectively. (See Chronic Toxicity: Oral section for more details). Ishizawa et al.[155] have reported a carcinogenic effect on the liver of mice fed diets containing up to 15% sorbic acid for 88 weeks.

TERATOGENICITY

Potassium sorbate was evaluated for teratogenicity in groups of approximately 20 pregnant mice (CD-1) and rats (Wistar-derived stock). The mice were administered potassium sorbate as a water suspension at doses of 4.6, 21.4, 99.1, and 460.0 mg/kg body weight; the rats received doses of 3.4, 15.8, 73.3, and 340.0 mg/kg body weight. Doses were administered daily by oral intubation on days 6–15 of gestation. Both vehicle and positive (aspirin) controls were used. No significant effects were noted on nidation or on maternal or fetal survival in either mice or rats. The number of abnormalities seen in soft and skeletal tissues of the potassium sorbate groups did not differ from the number occurring spontaneously in the vehicular controls.[156]

CLINICAL IRRITATION AND SENSITIZATION

Clemmensen and Hjorth[157] patch tested 91 dermatologic patients on the upper back with concentrations of 0.1, 1.0, 5.0, and 10% sorbic acid and benzoic acid in petrolatum. Occlusive patches were applied for 20 minutes and reactions scored upon removal. Sorbic acid produced erythema in 19.8, 61.5, 64.8, and 67.4% of the patients at each of the four increasing concentrations, respectively. Edema was produced by sorbic acid in 0, 1.1, 7.7, and 9.0% of the patients at successively increasing concentrations, respectively. The investigators noted that the positive reactions seemed to follow a dose-response curve with a plateau at 1%. A group of 10 patients with positive reactions to sorbic acid was selected to test the effects of local application of an antihistamine prior to patch testing. Mepyramine (2% in water or gel) was applied as a closed patch test 3 h prior to patch testing with sorbic acid. Mepyramine produced a mean reduction in erythemal responses of 31.4% (range 2.2–65.6%), although in no patient was the reaction totally abolished. Prick tests with histamine produced no reactions in these patients (See Table 5 for clinical irritation and sensitization results.)

Soschin and Leyden[158] studied the effects of sorbic acid on different body regions when used as an ingredient in steroid preparations and other vehicles. Patches containing a 0.1 ml sample of sorbic acid at concentrations of 0.1, 0.5, and 1.0% in 2-isopropanol and water 1:1 were applied to the deltoid muscle, the volar aspect of the forearm, and the upper portion of the back of 15–17

TABLE 5. Clinical Irritation and Sensitization

Ingredient	*Test method*	*No. of subjects*	*Results*		*Reference*
Sorbic acid					
0.1% in petrolatum	Single occlusive	Dermatologic patients	Erythema in:	Edema in:	157
1.0%	patch on upper back	91	18 (19.8%)	0(0%)	
5.0%	(20 minutes)	91	56 (61.5%)	1 (1.1%)	
10.0%		91	59 (64.8%)	7 (7.7%)	
		89	60 (67.4%)	8 (9.0%)	
			Investigators noted a dose-response curve with a plateau at 1%		
In isopropanol and water	Single occlusive patch on deltoid muscle, forearm, and upper back (20 minutes)	15–17	High prevalence of either erythema or edema at all body sites, with dose response evident by the intensity of the reaction; reactions most intense on the face		158
0.1%					
0.5%					
1.0%					
In ethanol and water	Single occlusive patch on cheeks and forehead (20 minutes)	15			
0.05%					
0.1%					
0.5%					
1.0%					
In petrolatum	Draize RIPT[a]				
Induction					
10		93	Induction with 10%: 0 of 93 sensitized		159
20		33	Induction with 20%: 1 of 33 sensitized		
Challenge, 5			Overall sensitization rate of 0.8%		
In petrolatum	Draize RIPT				
Induction					
10%		181	Induction with 10%: 0 of 181 sensitized		160
20%		121	Induction with 20%: 1 of 121 sensitized		
Challenge, 5%			Overall sensitization rate of 0.33%		
1.0% in petrolatum	Draize-Shelanski RIPT	50	49 totally negative responses; 1 subject with 2+ reaction at application 3, given 1 day rest, administration continued with 0.5% sorbic acid, no further reactions		161
0.10% in eye makeup remover	Schwartz and Peck prophetic patch test, with and without UV	102	Eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing		162
	Shelanski and Shelanski RIPT	Unspecified	Eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing		162
	Controlled use study (4 weeks)	54	No irritation was observed		163

TABLE 5. Continued

Ingredient	*Test method*	*No. of subjects*	*Results*	*Reference*
0.5% in formation, tested as a 0.5% aqueous solution	RIPT	86	Formulation did not induce contact sensitization; minimal to mild irritation	164
0.2% in bubble bath	RIPT	78	Bubble bath did not induce allergic sensitization; minimal irritation in three subjects	165
0.2% in formulation	RIPT	52	Formulation did not induce allergic sensitization; minimal irritation	166
0.2% in facial conditioner	RIPT	84	Facial conditioner did not induce allergic sensitization; minimal to mild irritation	167
0.2% in formulation	RIPT	98	Formulation did not induce allergic sensitization; minimal irritation	168
Potassium sorbate				
0.15% in cream	Cumulative irritation test	12	Very mild cumulative irritation was observed	169
0.15% in moisturizer	Cumulative irritation test	12	Very mild cumulative irritation was observed	170
	Cumulative irritation test	12	No cumulative irritation was observed	171
0.15% in formulation	Shelanski-Jordan RIPT	209–210	Formulation was not a strong irritant or a strong contact sensitizer	172
0.15% in bronzer	Modified Draize-Shelanski RIPT	199–204	Bronzer was not a primary irritant or an allergic contact sensitizer	173
0.15% in moisturizer	Modified Draize-Shelanski RIPT	202–205	Moisturizer was not a primary irritant or an allergic contact sensitizer	174
0.1% in facial scrub, tested diluted 1:100 in deionized water	RIPT	53	Facial scrub was a very mild cumulative irritant but was not a primary irritant and did not induce sensitization	175
	RIPT	53	Facial scrub was a very mild cumulative irritant but was not a primary irritant and did not induce sensitization	176
	RIPT	56	Facial scrub did not induce dermal irritation or sensitization	177
	RIPT	47	Facial scrub was not a sensitizer	178

[a]Repeat insult patch test.

subjects. The patches were occluded for 20 minutes, and sites were scored 10 minutes after patch removal using a maximum scale of 4. Of these subjects, 15 received an additional application of sorbic acid at concentrations of 0.05, 0.1, 0.5, and 1.0% on the cheeks and forehead. A high prevalence of either erythema or edema was observed at all sites, with the dose response evident by the intensity of the reaction. Reactions were most intense on the face; the number of scores of 3 or 4 on the face was significantly increased compared with the other body sites at 0.1% sorbic acid. No significant differences in the rate or intensity of the reaction rate were noted at higher concentrations of sorbic acid.

Soschin and Leyden[158] also investigated the effect of sorbic acid-induced reactions on the anti-inflammatory effects of corticosteroid creams. They compared the dermal effects of 0.1% sorbic acid in ethanol and water with the effects of hydrocortisone cream containing 0.1% sorbic acid or potassium sorbate in 17 subjects. Each was applied to the cheek and forehead without occlusion for 20 minutes. The intensity of the reaction rate was significantly less in the topical steroid preparations than in the ethanol-water vehicle. The anti-inflammatory effect was not affected by sorbic acid-induced erythema.

The results of further studies showed that pretreatment of skin with topical steroids to induce vasoconstriction diminished the response to sorbic acid. Oral administration of aspirin blocked the erythematous component, and the investigators suggested that prostaglandins were therefore important mediators. Systemic steroids, antihistamines, and hydroxyzine failed to influence the erythema and edema produced by sorbic acid. In electron microscope studies of tissue from the sites on the upper back, it was concluded that erythema, edema, and flare in response to sorbic acid were not associated with mast cell degranulation.[158]

Marzulli and Maibach have conducted two Draize repeat insult patch tests (RIPT) of sorbic acid. In each test, sorbic acid was applied in petrolatum at concentrations of 10 and 20% during the induction period and 5% for the challenge. The induction period consisted of ten 48 h occlusive patches (72 h on the weekends) applied over a period of 3–5 weeks. Each patch contained 0.5 g of the test material and was applied to the same site on the lateral arm above the elbow. Following a 2 week rest period, a challenge patch was applied for 72 h. All reactions were scored upon patch removal on a scale of 1–4. The results of the first test were 0 in 93 sensitized when treated with 10% sorbic acid and 1 in 33 sensitized when treated with 20% sorbic acid. This gave an overall sensitization rate of 1 in 126, or 0.8%.[159] The results of the second test were 0 in 181 sensitized when treated with 10% sorbic acid and 1 in 121 sensitized when treated with 20% sorbic acid. This gave an overall sensitization rate of 1 in 302, or 0.33%. The sensitization rate of 0.8% for the subjects treated with 20% sorbic acid was not statistically significant.[160]

Klauder[161] conducted a Draize-Shelanski RIPT with sorbic acid at a concentration of 1% in petrolatum. Closed patches containing sorbic acid were applied at the same site every other day for a total of 12 applications. It was not specified whether the patches were occlusive or nonocclusive. After a 2 week rest, a challenge patch was applied to the same site. Of the 50 subjects completing the test, 49 had negative responses. A single subject had a

2+ reaction to the third induction patch; he was given 1 day of rest and then patched with 0.5% sorbic acid. All subsequent reactions were negative. The investigator noted that this was probably an irritation response and was consistent with the results of the pilot study (2 and 4% sorbic acid producing irritation in 2 of 12 and 4 of 10 subjects, respectively).

An eye makeup remover containing 0.10% sorbic acid was tested for skin irritation in the Schwartz and Peck prophetic patch test using 102 panelists. Open and closed patches were scored on a 1+ to 3+ scale, and the effect of ultraviolet radiation was also determined. There were no reactions to open patches. There were five 1+ reactions to closed patches on day 1, two 1+ reactions on day 2, one 2+ reaction on days 1 and 2, respectively, and one reaction on day 2 following ultraviolet radiation. The same formulation was also tested in a Shelanski and Shelanski RIPT with an unspecified number of panelists. There were no reactions to open patches, and there were no reactions following ultraviolet radiation. There were up to three 1+ reactions to closed patches each day during the 11 days of the study. The eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing.[162]

An eye makeup containing 0.10% sorbic acid was tested for skin irritation in a controlled use study with 54 panelists. No irritation was observed in any panelist during the 4 weeks of the study. The eye makeup remover was nonirritating.[163]

A formulation containing 0.5% sorbic acid was tested for skin irritation and sensitization in an RIPT procedure using 86 panelists. The formulation was tested as a 0.5% aqueous solution. Occlusive 24 h induction patches were applied three times a week for 3 weeks to the upper backs of the subjects. An untreated site was challenged with a 24 h patch during week 6 of the study. Induction patches were each scored 24 h after removal, and the challenge patch was scored 24 and 48 h after removal. A total of 19 panelists reacted to induction patches: 7 had mild (pink uniform erythema covering most of the contact site) reactions, and 12 had at most barely perceptible (minimal faint uniform or spotty erythema) reactions. There were three barely perceptible reactions at the 24 h challenge reading and no reactions at the 48 h reading. The formulation under these test conditions did not induce contact sensitization.[164]

A bubble bath containing 0.2% sorbic acid was tested as a 0.25% aqueous solution for skin irritation and sensitization in an RIPT with 78 panelists. Three occlusive 24 h induction patches were applied to the upper back of each panelist each week for 3 weeks, and a 24 h challenge patch was applied to a previously untreated site after a 3 weeks rest. Reactions to induction patches were scored 24 h after patch removal, and reactions to challenge patches were scored 24 and 48 h after patch removal. Of the 78, 3 subjects had barely perceptible reactions to induction patches, and there were no reactions at challenge. The bubble bath formulation did not induce allergic sensitization.[165]

An RIPT was conducted with a formulation containing 0.2% sorbic acid. Occlusive 24 h induction patches were applied to the upper backs of 52 panelists three times a week for 3 weeks, and reactions were scored 24 h after the removal of each patch. A 24 h challenge patch was applied to an untreated site during week 6 of the study, and the reaction was scored 24 and

48 h after patch removal. Of 52 subjects, 9 had barely perceptible reactions to at least one induction patch. Another 2 subjects had barely perceptible reactions at one reading of the challenge site, 1 at 24 h and 1 at 48 h. Follow-up testing was performed with the subject with the reaction at the 48 h challenge reading; no reactions were observed. The original challenge reaction was of a nonspecific, irritant nature and was not due to allergy. Under these test conditions, this formulation did not induce allergic sensitization.[166]

The skin irritation and sensitization of a facial conditioner containing 0.2% sorbic acid was determined in an RIPT using 84 panelists. Three occlusive 24 h induction patches were applied to the upper back each week for 3 weeks, and a 24 h challenge patch was applied after a 3 week rest period. Induction reactions were scored 24 h after patch removal, and challenge reactions were scored 24 and 48 h after patch removal. Of 84 panelists, 23 had barely perceptible to mild reactions to at least one induction patch. A single panelist had a barely perceptible reaction, and another panelist had a mild reaction at the 24 h challenge reading. There were no reactions at the 48 h challenge reading. The facial conditioner did not induce allergic sensitization.[167]

A formulation that contained 0.2% sorbic acid was tested in an RIPT for skin irritation and allergic sensitization. A total of nine occlusive 24 h induction patches were applied to the upper backs of 98 panelists over a 3 week period. Reactions to these patches were scored 24 h after patch removal. Occlusive 24 h patches were applied to untreated sites in week 6 of the study, and reactions to these patches were scored 24 and 48 h later. Of 98 panelists, 10 had barely perceptible to mild reactions to at least one induction patch. There were two reactions to the challenge patch at the 24 h reading, and there were no reactions at the 48 h reading. Under the conditions of this RIPT, this formulation did not induce allergic sensitization.[168]

A white cream containing 0.15% potassium sorbate was tested for cumulative irritation in 12 subjects. Each day for 21 consecutive days, 23 h occlusive patches were applied to the backs of the subjects, and reactions to each patch were scored every 24 h. The total composite score for the cream was 83 of a maximum possible score of 756, and the total score with a base of 10 subjects was 69 of a maximum possible score of 630. There was a slight potential for very mild cumulative irritation under the conditions of this test; the cream is probably mildly irritating in normal use.[169]

The cumulative irritation potential of a moisturizer containing 0.15% potassium sorbate was evaluated with 23 or 47 h occlusive patches applied to the backs of 12 subjects. Patches were applied each day, with the exception of a holiday, for 20 consecutive days. Reactions were scored 1 h after patch removal. The composite total score for the 12 subjects was 169 of a possible maximum score of 720, and the total score with a base of 10 subjects was 140.83 of a possible maximum of 600. The moisturizer was probably a mild irritant in normal use; there was evidence of a slight potential for very mild cumulative irritation under the conditions of this test.[170]

A moisturizer containing 0.15% potassium sorbate was evaluated for cumulative irritation with 12 panelists. With the exception of a holiday, 23 or 48 h occlusive patches were applied to the backs of the subjects each day for 20 consecutive days. Reactions were scored 1 h after patch removal. The composite total irritation score for the 12 panelists was 52 of a possible maximum of

720, and the total score calculated for 10 panelists was 43.33 of a possible maximum of 600. There was essentially no evidence of cumulative irritation under the conditions of this test.[171]

A Shelanski-Jordan RIPT was conducted with a formulation containing 0.15% potassium sorbate. Occlusive induction patches were applied for 24 h to the backs of 209 to 210 subjects three times a week for a total of 10 applications. Reactions were scored at patch removal on a 0–4+ scale. A 48 h challenge patch was applied 10–14 days later, and this reaction was scored at patch removal. After another 7–10 days a second 48 h challenge patch was applied, and this reaction was scored 48 and 72 h after patch application. A single subject had a 2+ reaction to induction patches 9 and 10; these reactions appeared to be irritation due to occlusive patch testing. Another subject had a 2+ reaction at the 72 h reading of the second challenge; this reaction lacked signs of edema. No other reactions were observed. The formulation does not appear to be a strong irritant or a strong contact sensitizer.[172]

A bronzer containing 0.15% potassium srobate was evaluated for skin irritation and sensitization in a modified Draize-Shelanski RIPT. Occlusive induction patches were applied for 24 h to the upper backs or inner upper arms of 199–204 subjects three times a week for a total of 10 applications, and reactions were scored on a scale of 0–4+ at 24 or 48 h. To the same sites and to previously unpatched sites, 48 h occlusive challenge patches were applied 3 weeks later, and these reactions were scored 48 and 72 h after application. There were fourteen 1+ and three 2+ reactions to induction patches. There were six 1+ reactions to the challenge patch at the original site at the 48 h reading, two 1+ reactions to the challenge patch at the untreated site at the 48 h reading, five 1+ reactions to the challenge patch at the original site at the 72 h reading, and three 1+ reactions to the challenge patch at the previously untreated site at the 72 h reading. There were no other reactions. The 1+ and 2+ reactions were judged to be irritant in nature and were not considered clinically significant. The bronzer did not appear to be a primary irritant or an allergic contact sensitizer.[173]

A modified Draize-Shelanski RIPT was used to test the skin irritation and sensitization potential of a moisturizer containing 0.15% potassium sorbate. Occlusive induction patches were applied for 24 h three times a week for a total of 10 applications. These patches were applied to the upper backs or inner upper arms of 202–205 subjects, and reactions were scored on a scale of 0 to 4+ , 24 or 48 h later. After a 3 week rest period, 48 h occlusive challenge patches were applied to the original sites and to previously untreated sites. These reactions were scored 48 and 72 h after patch application. There were nine 1+ reactions and two 2+ reactions to induction patches; these reactions were judged irritant in nature and were not considered clinically significant. There was one 1+ reaction to the challenge patches applied to previously untreated sites at the 48 h reading, three 1+ reactions to the challenge patches applied to the original sites at the 72 h reading, and three 1+ reactions to the challenge patches applied to the previously untreated sites at the 72 h reading. The moisturizer appeared not to be a primary irritant or an allergic contact sensitizer.[174]

A facial scrub containing 0.1% potassium sorbate was diluted 1:100 in deionized water and was evaluated for skin irritation and sensitization in an

RIPT with 53 subjects. Eight semiocclusive induction patches, each 24 h in duration, were applied to the upper arm of each of the subjects over a 2 week period. Reactions were graded at patch removal. After a 2 week nontreatment period, a semiocclusive challenge patch was applied to a previously untreated site for 24 h. Reactions were graded at patch removal and 24, 48, and 72 h later. Seven minimal erythema reactions were observed during induction, and no reactions at any challenge reading. The facial scrub was a very mild cumulative irritant but was not a primary irritant. The formulation did not produce sensitization in any of the subjects tested.[175]

The skin irritation and sensitization of a facial scrub containing 0.1% potassium sorbate were evaluated in an RIPT using 53 panelists. The formulation was diluted 1:100 in deionized water. Four semiocclusive induction patches were applied to the upper arm of each subject for 24 h each week for 2 weeks. Reactions were scored at patch removal. A 24 h semiocclusive challenge patch was applied to a previously untreated site 3 weeks later. This reaction was evaluated at patch removal and 24, 48, and 72 h later. There were two minimal erythema reactions during induction and no other reactions. The facial scrub was a very mild cumulative irritant, but it was not a primary irritant. It did not produce sensitization in any of the subjects tested.[176]

An RIPT was conducted using 56 panelists and a facial scrub containing 0.1% potassium sorbate. The formulation was diluted 1:100 by weight with distilled water for the study. Eight 24 h semiocclusive induction patches were applied over a 2 week period to the lateral upper arm of each subject. Reactions were scored at patch removal. After an approximately 2 week rest period, a 24 h semiocclusive challenge patch was applied to a previously untreated site. Reactions to the challenge patch were graded at patch removal and 24 and 48 h later. Two slight, transient, questionable erythema reactions were observed during induction. No other reactions were observed during induction or challenge. The facial scrub did not induce dermal irritation or sensitization.[177]

The skin irritation and sensitization potential of a facial scrub containing 0.1% potassium sorbate was evaluated in an RIPT with 47 panelists. The formulation was diluted 1:100 in distilled water. Eight 24 h semiocclusive induction patches were applied to the lateral aspect of the upper arms of the subjects over a 2 week period, and reactions were scored on a scale of 0–5 at patch removal. After a 2 week rest period, a 24 h semiocclusive challenge patch was applied, and reactions were scored at patch removal and 24 and 48 h later. No reactions greater than 2 (moderate erythema) were observed during the induction period, and no reactions at challenge were indicative of sensitization.[178]

SUMMARY

Sorbic acid is a straight-chain monocarboxylic acid, also known as 2,4-hexadienoic acid. It is a white crystalline powder soluble in alcohol and ether but only slightly soluble in water. Potassium sorbate is the potassium salt of

sorbic acid and is a white crystalline powder or white granules or pellets freely soluble in alcohol and water.

Sorbic acid occurs naturally as the lactone, parasorbic acid, in berries of the mountain ash, *Sorbus aucuparia* L., Rosaceae. The sorbic acid used in cosmetics is synthesized by various commercial processes. Potassium sorbate is prepared by reacting sorbic acid with an equimolar portion of potassium hydroxide.

Solutions of sorbic acid are subject to autoxidation and atmospheric oxidation. Both the temperature and the type of container have also affected the breakdown of sorbic acid.

Sorbic acid and potassium sorbate are analyzed primarily by chromatographic techniques. Several analytic studies have been conducted to determine whether sorbic acid was contaminated with its isomer parasorbic acid, a suspected carcinogen. No traces of parasorbic acid were found (tests sensitive down to a concentration of 0.5 mg/kg).

Sorbic acid and potassium sorbate are used in cosmetics and toiletries as preservatives and antimicrobials generally at concentrations of $\leq 1\%$. According to the data voluntarily reported to the FDA through 1986, sorbic acid and potassium sorbate were used in 445 and 117 cosmetic formulations, respectively. These ingredients are primarily used in facial and eye makeup and skin care and hair preparations.

Sorbic acid and potassium sorbate are generally recognized as safe (GRAS) direct food additives. They are used as preservatives at low concentrations ($<0.01-1.40\%$) in many foods. Potassium sorbate is also a GRAS indirect food additive as it migrates to food from paper products used in packaging.

The Joint Food and Agricultural Organization–World Health Organization Expert Committee on food additives has estimated the acceptable daily intake of sorbic acid and its salts (expressed as sorbic acid) as 25 mg/kg body weight.

Sorbic acid and potassium sorbate are used as preservatives in a variety of pharmaceuticals. These chemicals also have various industrial uses.

Sorbic acid and potassium sorbate have a broad spectrum of fungistatic activity but are less active against bacteria. Their antimicrobial activity depends upon the amount of undissociated acid, which in turn is determined primarily by the dissociation constant and the pH of the system. Optimum effectiveness is attained at pH values up to 6.5. The mechanism by which sorbic acid inhibits microorganisms is not yet understood.

In biochemical studies, sorbic acid did not affect the protein content or the biosynthesis of RNA and DNA in mouse embryo fibroblast cells. Sorbic acid did not significantly affect biochemical parameters when administered orally to rats. Sorbic acid did inhibit both peroxidase and oxidase activity in cabbage and reduced the rate of aberrant mitosis caused by irradiation in onion root tips. Sorbic acid also affected the ultrastructural organization of yeast cells and effectively reduced the viability of the 14 human RNA and DNA enveloped viruses when combined with monolaurin.

The results of metabolic studies were that sorbic acid was qualitatively metabolized in the same manner as the saturated or singly unsaturated fatty acids of the same C-atom number. Under normal conditions, sorbic acid was almost completely oxidized to carbon dioxide and water.

Sorbic acid and potassium sorbate were practically nontoxic to rats and mice in acute oral toxicity studies. Intraperitoneal LD_{50} values in mice were 2800 and 2820 mg/kg for sorbic acid and 1300 mg/kg for potassium sorbate. Sorbic acid had a subcutaneous LD_{50} of 2820 mg/kg in mice. Formulations containing up to 5% sorbic acid administered orally at doses up to 7.0 g/kg were not toxic to rats.

In short-term to subchronic oral studies, sorbic acid did not produce significant adverse effects in rats, mice, or dogs at concentrations up to 10% (of the diet). Potassium sorbate was practically nontoxic in rats and dogs at concentrations up to 10 and 2%, respectively. Application to rabbit skin of formulations containing 0.5% sorbic acid or 0.15% potassium sorbate over short-term and subchronic periods, respectively, resulted in dermatitis.

Chronic oral studies in which sorbic acid was administered to mice and rats at concentrations up to 10% have established absolute no-effect levels of 1.5% in rats and 1.0% in mice. No significant toxic effects were noted in rats at a 5% concentration in the diet. Sorbic acid had no additive toxicity in rats when administered with benzoic acid. Adulteration of a 1.2% sorbic acid diet with 1000 ppm parasorbic acid produced not adverse effects in rats or mice administered these diets for 2 years and 80 weeks, respectively.

Sorbic acid (in petrolatum) and potassium sorbate (as aqueous solution) at concentrations of 1, 5, and 10% were practically nonirritating and nonirritating, respectively, to the rabbit eye. Formulations containing 0.1% sorbic acid or 0.15% potassium sorbate were nonirritating to the rabbit eye.

Sorbic acid (in petrolatum) and potassium sorbate (as aqueous solution) at concentrations of 1, 5, and 10% were slightly irritating and nonirritating, respectively, when evaluated using a modified Draize irritation test. In another Draize test, sorbic acid was classified a severe irritant after application of 1 mg under occlusive conditions. A 1% aqueous potassium sorbate solution was practically nonirritating to rabbit skin. No irritation or adverse effects were produced in rats by daily application, 6 days/week for 3 weeks, of 5% sorbic acid in a lanoline-petrolatum paste. A formulation containing 0.5% sorbic acid was not irritating to rabbit skin.

In a guinea pig sensitization test, sorbic acid produced four positive reactions to the first intradermal challenge although the reactions of all 20 guinea pigs were negative at the second epidermal challenge.

The results of studies of the potential formation of mutagenic or DNA-damaging reaction products in the presence of sorbic acid or potassium sorbate and sodium nitrite have varied. Sorbic acid and sodium nitrite, when reacted under acidic conditions, produced ethylnitrolic acid, considered by some to be mutagenic. Other reaction products, only partially identified, were both mutagenic and nonmutagenic. On the other hand, sorbic acid, in that it reacts readily with nitrite, has inhibited the formation of some carcinogenic nitrosamines from amines and nitrites.

Sorbic acid and potassium sorbate have been extensively tested for mutagenic effects using the Ames test, genetic recombination tests, reversion assays, *rec* assays, and tests for chromosomal aberrations, sister chromatid exchanges, and gene mutations. These tests have been conducted in various systems: *B. subtilis* strains 3308, 112, 566, and 168; *S. typhimurium* strains

TA-98, TA-100, TA-1535, TA-1537, and TA-1538; *S. cerevisiae* strain D4; silkworms; Chinese hamster cells; and rat bone marrow cells. The results have been both positive and negative.

A series of mutagenicity tests has also been used to evaluate the intestinal contents and urine of mice fed sorbic acid and potassium sorbate for periods of up to 15 months. The concentration of glutathione in the liver and the relative body weight–liver weight ratios were evaluated as well. Acidic components of the intestinal contents and the urine of those mice administered a diet containing 15% sorbic acid were mutagenic in *S. typhimurium* strain TA-98, but only with metabolic activation. The concentration of lipid peroxide in the livers increased almost linearly with the concentration of sorbic acid in the diet. Sorbic acid decreased hepatic glutathione concentrations and increased the relative body weight–liver weight ratios in these mice.

The oral administration of potassium sorbate as 0.1% of the diet or 0.3% of the drinking water for up to 100 weeks produced no neoplasms in rats. No carcinogenic effect was demonstrated by sorbic acid in rats or mice fed diets containing up to 10% sorbic acid for periods of 2 years and 80 weeks, respectively. A diet containing up to 15% sorbic acid has been reported to have a carcinogenic effect in the liver of mice after 88 weeks' administration.

No teratogenic effects have been observed in pregnant mice and rats administered potassium sorbate at doses of up to 460 and 340 mg/kg body weight, respectively.

In three repeat insult patch tests using a total of 478 subjects, sorbic acid had overall sensitization rates of 0, 0.33, and 0.8%. All the subjects sensitized were inducted with 20% sorbic acid and challenged with 5% sorbic acid. Formulations containing up to 0.5% sorbic acid or 0.15% potassium sorbate were not cumulative irritants or were very mild cumulative irritants. They were not primary irritants and were not sensitizers. A formulation containing 0.01% sorbic acid was not a photosensitizer.

CONCLUSION

On the basis of the data included in this report, the CIR Expert Panel concludes that sorbic acid and potassium sorbate are safe as cosmetic ingredients in the present practices of use and concentration.

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