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Final Report of the Safety Assessment of Kojic Acid as Used in Cosmetics

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Abstract

Kojic acid functions as an antioxidant in cosmetic products. Kojic acid was not a toxicant in acute, chronic, reproductive, and genotoxicity studies. While some animal data suggested tumor promotion and weak carcinogenicity, kojic acid is slowly absorbed into the circulation from human skin and likely would not reach the threshold at which these effects were seen. The available human sensitization data supported the safety of kojic acid at a use concentration of 2% in leave-on cosmetics. Kojic acid depigmented black guinea pig skin at a concentration of 4%, but this effect was not seen at 1%. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the 2 end points of concern, dermal sensitization and skin lightening, would not be seen at use concentrations below 1%; therefore, this ingredient is safe for use in cosmetic products up to that level.

Keywords

cosmetics, kojic acid, safety

Introduction

Kojic acid is an antioxidant used by the cosmetics industry and has been described as an alternative to hydroquinone in skin lightening.[1] Kojic acid was discovered in 1907 through isolation from the mycelia of *Aspergillus oryzae* grown on steamed rice (the term koji means steamed rice in Japanese).[2]

While kojic acid is purported to have skin-whitening properties, it is currently not approved by the US Food and Drug Administration (FDA) for such use in over-the-counter pharmaceutical products.

Chemistry

Kojic acid (CAS No 501-30-4) is the heterocyclic compound that conforms to the structure depicted in Figure 1. Technical names, traced names, and trade mixture names for this ingredient are listed in Table 1.[3]

Physical and chemical properties of kojic acid are described in Table 2. UV absorption appears to vary as a function of the pH.

According to a review article by Beelik, the enolic hydroxyl group at C5 gives kojic acid its weakly acidic property and allows it to form salts with a number of metals.[2]

Kojic acid is naturally produced as a secondary metabolite in the following *Aspergillus* strains: *Aalbus*, *A alliaceus*, *A awamori*, *A arachidicola*, *A bombycis*, *A caelatus*, *A candidus*, *A clavatus*, *A effusus*, *A flavus*, *A fumigatus*, *A giganteus*, *A glaucus*, *A gymnosardae*, *A leporis*, *A luteovirescens*, *A lutescens*, *A minisclerotigenes*, *A nidulans*, *A nomius*, *A parasiticus*, *A parvisclerotigenus*, *A pseudotamarii*, *A tamarii*, and *A wentii*.[2,9] It is also the secondary metabolite of several strains of *Penicillium* and *Acetobacter* fungi and several species of acetic acid bacilli.[2,10,11]

Kojic acid can be detected with chromatographic or electrophoretic techniques.[9,10,12-14]

Use

Cosmetic

According to information supplied to the FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP), kojic acid is used in a total of 16 products. In a survey of current use concentrations conducted by the Personal Care Products Council, kojic acid is used at concentrations ranging from 0.1% to 2%, with the maximum concentration used in face and neck creams, lotions, and powders.[15] The available data on uses and use concentration as a function of product type are presented in Table 3.

Gottschalck and Bailey described the current use of kojic acid as an antioxidant; however, trade names and trade name

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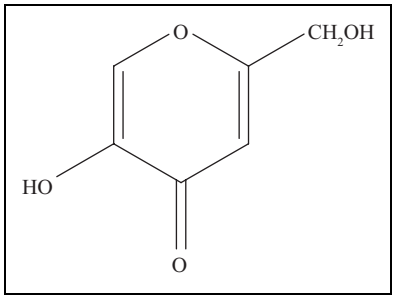

Figure 1. Kojic acid.

Table 1. Technical Names, Trade Names, and Trade Name Mixtures for Kojic Acid[3]

Technical Names	Trade Names	Trade Name Mixtures
Kojic acid		
4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-;	AEC Kojic acid	Botacenta SLC 175
5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one	Kojic acid	Dermawhite HS
	Kojic acid SL	Melarrest A
	Melanobleach-K	Melarrest L
	OriStar KA	Vegewhite
	Rita KA	
	Tonelite Kojic acid	

mixtures such as Melanobleach-K, Dermawhite HS, and Vegewhite suggest skin-whitening uses.[16] As noted earlier, the FDA has not approved kojic acid for use in over-the-counter pharmaceutical products. The Environmental Working Group (EWG) reports that there are 79 cosmetic products that contain kojic acid, of which approximately half of the products are described to have a skin fading/lightener effect.[18] One product reportedly contains 4% kojic acid.

Health Canada's Cosmetic Notification System reported that 148 products contain kojic acid, with all uses in skin care products (mostly moisturizers/antiwrinkle creams; L. K. Carter, Personal Communication, February 15, 2010).[19] The ranges of concentrations of use for kojic acid in Canada are 0.1% or less (37 products), 0.1% to 0.3% (11 products), 0.3% to 1% (34 products), 1% to 3% (45 products), 3% to 10% (14 products), and 10% to30% (3 products).

The European Commission's Scientific Committee on Consumer Products (SCCP) determined that, based on a margin of safety calculation, the use of kojic acid at a maximum concentration of 1.0% in skin care formulations poses a risk to human health due to potential systemic effects (thyroid effects). The SCCP also found kojic acid to be a potential skin sensitizer.[20]

Kojic acid is not included on the list of ingredients that must not be used in cosmetic products that are marketed in Japan.[21]

Table 2. Physical and Chemical Properties of Kojic Acid

Properties		Reference
Physical form	Crystalline; prismatic needles from acetone, ethanol + ether, or methanol + ethyl acetate	4,5
Molecular weight	142.11	4
Melting point	152°C-154°C	4,5
pKa	7.90, 8.03	4
Log K_{ow}	−1.25	6
Solubility	Soluble in water, ethanol, acetone; sparingly soluble in ether, ethyl acetate, chloroform, pyridine; insoluble in benzene	4,5
UV absorption peaks	215-216 nm and 268-269 nm in acidic or neutral solutions; 226-227nm and 309-312 nm in alkaline solution; 280 nm (pH of solution not reported)	7 (K. Kariya, H. Okamoto, H. Iwaki, A. Yamauchi, and Y. Higa, Unpublished data, 1979)

However, in Japan, products used as skin whiteners are regulated as quasi-drugs, and kojic acid is used as a skin-whitening product in Japan.[22-26] Quasi-drugs are defined as "having a mild effect on the body but are intended for neither the diagnosis, prevention, nor treatment of disease, nor to affect the structure or function of the body."[25]

Kojic acid may be used in cosmetic spray products, and effects on the lungs that may be induced by aerosolized products containing this ingredient are of concern.

The aerosol properties that determine deposition in the respiratory system are particle size and density. The parameter most closely associated with deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of $\leq$10 μm are respirable. Particles with a d_a from 0.1 to 10 μm settle in the upper respiratory tract and particles with a $d_a < 0.1$ μm settle in the lower respiratory tract.[28,29]

Particle diameters of 60 to 80 μm and $\geq$80 μm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[30] In practice, aerosols should have at least 99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as $\sim$38 μm.[31] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

Noncosmetic

Kojic acid is an antibiotic produced by many species of *Aspergillus* and *Penicillium* that has anti-inflammatory and pain

Table 3. Cosmetic Product Uses and Concentrations for Kojic Acid

Product Category	2009 Uses (Total Number of Products in Category)[14,15]	2008 Concentrations of Use (Personal Care Products Council, Unpublished Data, 2010), %
Kojic acid		
Bath products		
Soaps and detergents	1 (1665)	–
Other bath products	1 (234)	–
Eye makeup		
Eye lotions	−(254)	0.1-1
Skin care products		
Skin cleansing creams, lotions, liquids, and pads	2 (1446)	–
Face and neck creams, lotions, powders, and sprays	2 (1583)	2[a]
Body and hand creams, lotions, powders, and sprays	−(1744)	1[a]
Moisturizers	2 (2508)	–
Skin fresheners	2 (259)	–
Other skin products	6 (1308)	–
Total uses/ranges for Kojic acid	16	0.1-2

[a] Concentrations of use reported for kojic acid in this category were not in spray products.

relief properties, with skin whitening activity reportedly caused by the inhibition of tyrosinase.[32]

According to *The Merck Index*,[4] kojic acid is used in maltol and ethyl maltol synthesis and in flavor-enhancing additives in food.

Uses for kojic acid in *Hawley's Condensed Chemical Dictionary* include chemical intermediate, metal chelation, and insecticidal, antifungal, and antimicrobial agents.[5]

Kojic acid was reported to be used in a number of Japanese foods, including soybean paste, soy sauce, sake, and mirin.[33] Additional uses in foods include use as an antioxidant, a preservative, a food additive to inhibit tyrosinase, an inhibitor of nitrosopyrrolidine formation in fried food, and a reddening agent in unripe strawberries.

General Biology

Absorption, Distribution, Metabolism, Excretion

Sansho Seiyaku Co, Ltd described a 1978 rat absorption, distribution, metabolism, and excretion study (oral, subcutaneous, and dermal routes) that was revised in 2001.[34,35] [^{14}C] Kojic acid was biosynthesized by adding [^{14}C-U] glucose into a cultured broth of *Aspergillus candidus*, extracting with ethyl acetate, and purifying by recrystallization. The purity of the radiolabeled kojic acid was 99.9%. [^{14}C] Kojic acid was administered to groups of 3 male JCL-Wistar rats at a dose of 10 μCi/100 g body weight via a single oral, subcutaneous, or dermal administration. An additional group of rats received the same subcutaneous dose over a period of 7 days. Blood samples were collected from the tail tip 0.5, 1, 3, 6, 24, and 48 hours after administration for the rats that received a single dose. Urine and feces were collected from metabolic cages, and bile samples were collected from cannulation in the common bile duct at 0 to 10 minutes, 30 minutes to 1 hour, 1 to 3 hour, 3 to 6 hour, and 6 to 24 hour. In rats that received repeated doses of kojic acid, blood, urine, and feces samples were collected at 24 hours after each administration. Enterohepatic circulation was studied by connecting a cannula from a bile duct of a treated rat to an untreated rat's duodenum, from which the bile samples were collected. At the end of the experiment, the rats were killed 30 minutes, 1, 3, 6, 24, 48, or 72 hours after treatment and tissues were collected and cut into sections for autoradiograph examination.

Radiolabel from the single oral exposure was found in the intestine within 3 hours and in the cecum within 6 hours after administration. The radioactivity was distributed in tissues and organs very rapidly and maximum values were reached within 30 minutes of administration. Very high levels of radiolabel were measured in the liver, kidneys, and pancreas, and high levels were measured in the lungs, heart, and spleen. In the blood, radioactivity decreased to 20.63% and 25.05% of total radioactivity at 30 minutes and 1 hour, respectively, and decreased to background levels within 24 hours. The amount of ^{14}C in the bile within 24 hours was approximately 0.5 μCi/10 μCi administered dose. No radioactivity was detected in the bile samples from the enterohepatic circulation study. Approximately 70% of the administered radioactivity was excreted in the urine within 48 hours, while excretion in the feces over the same time period was only 0.82%.

Distribution of the radiolabel in the tissues and organs following a single subcutaneous exposure was slightly slower than that following the oral exposure. Distribution of radiolabel after a single dermal exposure was further slowed. High levels of radiolabel were measured in the kidney and liver 30 minutes and 1 hour after subcutaneous exposure, while no remarkable radioactivity was detected in the liver following dermal exposure. In the blood, radioactivity was 13.29% and 21.67% at 30 minutes and 1 hour, respectively, following subcutaneous exposure and 5% at 30 minutes following dermal exposure. The amount of ^{14}C in the bile within 24 hours was approximately 0.76 μCi/10 μCi and 0.5 μCi/10 μCi for the subcutaneous exposure and dermal exposure, respectively. No radioactivity was measured in the bile samples from the enterohepatic circulation study after either exposure type. Approximately 50% and 56% of the subcutaneous and dermal administered radioactivity, respectively, were excreted in the urine within 48 hours. Excretion in the feces over the same time period was 2.62% and 1.58% of the administered subcutaneous

and dermal doses, respectively. Recovery of radiolabel in expired air in the rats administered a single subcutaneous dose within 5 hours was 1.4%.

In the repeated subcutaneous dosed rats, radiolabel in blood and urine samples increased until the fourth dosing and reached an equilibrium state thereafter. Distribution of the radiolabel was measured 10 minutes, 1, 6, 24, and 48 hours after the last treatment. When compared to the single dose rats, radioactivity was several times higher in all organs and tissues in the repeated dose rats, especially in the intestinal tract 1-hour measurement and in the pancreas and adipose tissues.

For all portions of the study, the major metabolites in the urine and bile were glucuronide (6.4%-39.6% of total radioactivity) and sulfate conjugates of kojic acid (35.6%-93.7% of total radioactivity). Unmetabolized kojic acid was also detected in the urine.[34,35]

The transfer of [^{14}C] kojic acid (subcutaneous injection) to fetuses and milk in pregnant JCL-Wistar rats was also investigated.[34,35] Groups of 2 pregnant rats received 10 μCi/100 g body weight [^{14}C] kojic acid subcutaneously on day 11 or 20 of gestation. Ten minutes, 30 minutes, or 3 hours after treatment, fetuses were surgically extracted and prepared for autoradiograms, and the fluids, excreta, and tissues from the dams were evaluated for radioactivity content as described above for the male rats. For the milk transfer study, groups of 3 nursing dams received 10 μCi/100 g body weight [^{14}C] kojic acid subcutaneously on day 3 of lactation. The stomachs of nursing pups were extracted at 30 minutes, 1 hour, or 3 hours after treatment of the dams to determine the radiolabel concentration in milk.

In pregnant rats, the radioactivity was distributed rapidly in tissues and organs. Very high values were observed in the kidney and high values were observed in the liver, pancreas, spleen, salivary gland, lungs, and kidney immediately after administration. Radioactivity was also detected in the uterus, placenta, amniotic fluid, and the fetus 30 minutes after treatment. Fetal distribution of radiolabel was similar to that in the adults, with high amounts detected in the liver and gastrointestinal tract. In nursing pups, radioactivity was detected in the stomach wall and stomach content, with about 0.02% detected 3 hours after treatment. It was concluded that the radiolabel from kojic acid was transported freely to the fetus, uterus and other reproductive organs, and secreted into milk in this rat study.[34,35]

Dermal Penetration

In an in vitro percutaneous absorption and distribution study,[36] [^{14}C] kojic acid at 1.045% (w/w) in a formulation was applied to human dermatomed skin. The integrity of the skin was tested by measuring transepidermal water loss (TEWL) prior to test material application. The formulation was applied at 2 mg/cm^2 (20.61 ± 1.68 $\mu g_{eq}/cm^2$ of [^{14}C] kojic acid) on the skin surface. After 16 hours, the formulation was washed from the skin surface with sodium lauryl ether sulfate and distilled water. Liquid scintillation was employed to determine percutaneous absorption. Total recovery of the radiolabeled kojic acid was 96.41% ± 4.82% of the applied dose, with 75.55% ± 9.30% of the applied dose (15.52 ± 1.43 $\mu g_{eq}/cm^2$) in skin excess, 3.65% ± 2.22% of the applied dose (0.76 ± 0.48 $\mu g_{eq}/cm^2$) in the stratum corneum, 9.17% ± 4.31% of the applied dose (1.93 ± 1.07 $\mu g_{eq}/cm^2$) in the epidermis and dermis, and 7.81% ± 6.79% of the applied dose (1.65 ± 1.49 $\mu g_{eq}/cm^2$) in the receptor fluid. The total absorbed amount of [^{14}C]-kojic acid was 16.98% ± 10.28% of the applied dose (3.58 ± 2.38 $\mu g_{eq}/cm^2$).

In another study by Sansho Seiyaku Co, Ltd,[37] the in vivo percutaneous absorption of kojic acid was evaluated in human volunteers. The study was open and uncontrolled. Six healthy postmenopausal Japanese women received a single 500 mg application of a cream formulation containing 1% kojic acid. The test material was applied to the entire surface of the facial skin (left and right cheeks). The participants were examined the day before, immediately before, and 24 hours after application and samples were collected for hematology, blood chemistry, urinalysis, and immune serological tests. The amount of test material in plasma was measured before application and at 0.5, 1, 1.5, 3, 6, 12, and 24 hours after application.

Kojic acid was detected in the plasma of all the participants at one or more blood collection times. All the concentrations in plasma were only slightly above the quantitation limit of 1 ng/mL. The mean C_{max} was 1.54 ng/mL and the mean $AUC_{0\text{-}24\ h}$ was 19.4 h·ng/mL. There were no adverse effects observed in the participants. It was concluded that the potential dermal transfer of kojic acid into the blood was very low.[37]

Based on the pharmacokinetic studies in rats and in vitro percutaneous absorption values in human skin, a review by Nohynek et al calculated a systemic exposure dose (SED) range of 0.03 to 0.06 mg/kg per d in humans following a topical application.[38] This SED range was based on an application area of the hands and face (400 and 590 cm^2, respectively), a maximum application rate of 1.0 g of 1.0% kojic acid cream at 1 mg/cm^2 (total application of 10 mg kojic acid/d), and percutaneous absorption of 17% of the applied dose (3.6 μg/cm^2) in humans.

Tyrosinase Inhibition

Cabanes et al stated that kojic acid is a slow-binding inhibitor of catecholase activity of frog tyrosinase in a nonclassical manner.[39] In a study of several mammalian melanocyte tyrosinase inhibitors, kojic acid was considered a potent free enzyme inhibitor with an IC_{50} (50% inhibition concentration of tyrosinase activity) value of 6.2 ± 2 μg/mL.[40] In this study, however, Kojic acid did not reduce pigmentation in mammalian cells. Melanocyte toxicity IC_{50} was >200 μg/mL, which indicated that kojic acid was not considered cytotoxic.

Kojic acid was a reference sample in a study of the tyrosinase activity of a nitrogen analog of stilbene.[7] The IC_{50} value of kojic acid was 275.6 μmol/L (39.17 μg/mL). In the same study, kojic acid was a positive control for the evaluation of superoxide dismutase-like (SOD-like) activity and melanin production in the stilbene analog. Kojic acid inhibited 18.8%

and 21.9% SOD-like activity at concentrations of 10 (1.42 μg/mL) and 50 μmol/L (7.11 μg/mL), respectively. Kojic acid did not show inhibitory effects on melanin production at 10 (1.42 μg/mL) and 100 μmol/L (14.2 μg/mL) in cultured "melan-a" cells.

Kojic acid was a positive control in a study of the inhibitory effects of oxyresveratrol and hydroxystilbene compounds on mushroom and murine melanoma B-16 tyrosinase.[41] At 100 μmol/L (14.2 μg/mL), kojic acid had a 76.7% ± 1.1% inhibitory effect on mushroom tyrosinase and a 43.0% ± 2.5% inhibitory effect on murine tyrosinase. The IC_{50} values of kojic acid were 40.1 μmol/L (5.83 μg/mL) and >100 μmol/L (14.2 μg/mL) for mushroom and murine tyrosinases, respectively. Mushroom tyrosinase inhibitory effects were dose-dependent. Kojic acid was a competitive inhibitor of mushroom tyrosinase in the kinetic portion of the study. In comparison, the IC_{50} values of oxyresveratrol were 1.2 μmol/L (0.29 μg/mL) in mushroom tyrosinase and 52.7 μmol/L (12.9 μg/mL) in murine tyrosinase. The percentage inhibition for 100 μmol/L (24.4 μg/mL) of this compound was 97.3% ± 1.6% in mushroom tyrosinase and 63.3% ± 2.3% in murine tyrosinase.

Additional studies where kojic acid had been used as a positive control in mushroom tyrosinase inhibition studies have been identified.[42-45]

Animal Toxicity

Acute Oral Toxicity

Kynoch and Lloyd[46] reported the effects of acute doses of kojic acid in fasted CFLP mice. The mice were divided into groups of 2 males and 2 females and received 1, 4, or 16 g/kg kojic acid in a 40% w/v suspension with 0.5% methylcellulose by oral intubation. Dose volumes ranged from 10 to 40 mL/kg body weight. The control group received 40 mL/kg of the vehicle alone. Clinical signs of toxicity and mortalities were recorded during the 14-day observation period. Mice that died during the observation period and those that survived through day 14 were necropsied. Preliminary findings indicated the LD_{50} to be between 4 and 16 g/kg body weight. In order to pinpoint a more precise LD_{50}, dosing was extended to groups of 5 male and 5 female mice. The groups received 4, 6.4, 10, or 16 g/kg kojic acid.

Clinical signs observed shortly after dosing included lethargy, piloerection, hunched posture, ataxia, and depressed respiratory rate. Mice treated with 6.4 g/kg body weight also were observed gasping. One male and 2 females in the 4 g/kg, 4 males, and 3 females in the 6.4 g/kg, and all the males and females in the 10 and 16 g/kg dose groups died within 1 to 3 hours of dosing. Necropsy of these animals revealed congestion of the lungs and pallor of the liver, kidneys, and spleen. Survivors completely recovered by day 4. Body weight gains in females of the 4 g/kg dose group were slightly decreased during the first week of observation but were comparable to controls by the second week. No abnormalities were observed in the surviving mice at necropsy. No clinical signs or deaths were observed in the control group. The authors calculated the LD_{50} of kojic acid in mice to be 5.1 g/kg body weight (95% confidence limits = 3.9-6.7 g/kg body weight).[46]

A similar acute oral study of kojic acid was performed by Kynoch and Lloyd[47] using fasted CFY rats. The preliminary LD_{50} was determined to be between 1 and 4 g/kg body weight. To more precisely determine the LD_{50}, the dose groups were expanded to 5 males and 5 females and received 1, 1.6, 2.5, or 4 g/kg body weight kojic acid in a 40% w/v suspension of 1.0% methylcellulose via oral intubation.

Lethargy, piloerection, ataxia, depressed respiratory rate, and loss of righting reflex were observed shortly after treatment. Rats treated with doses above 1 g/kg also had increased salivation and body tremors. Increased lacrimation and diuresis were observed in the 1.6 g/kg dose group and convulsions prior to death were observed in the 2.5 and 4 g/kg dose groups. Two males and 1 female in the 1.6 g/kg dose group and all of the males and females in the 2.5 and 4 g/kg dose groups died within 3 to 67 hours after dosing. Necropsy of these rats revealed congestion in the lungs with no specific cause of death evident. Opacity of the right eye was observed in 1 female in the 4 g/kg dose group. Recovery of the survivors was complete within 7 days. Body weight increases were slightly decreased in the 1.6 g/kg dose group for the first week of observation but were comparable to controls by the second week. No abnormalities were observed in the surviving rats at necropsy. No clinical signs or deaths were observed in the control group. The authors calculated the LD_{50} of kojic acid in rats to be 1.8 g/kg body weight (95% confidence limits = 1.5-2.0 g/kg body weight).

The acute oral toxicity of kojic acid was evaluated by Manciaux[48] in 6-week-old Wistar rats. The test material, prepared in 0.5% methylcellulose, was administered at a dose of 2000 mg/kg (volume 10 mL/kg) by gavage to a group of 5 male and 5 female fasted rats. Another group of 5 males and 5 females received the vehicle alone. Clinical signs, mortality, and body weight gain were checked for 14 days following the single administration. At the end of the observation period, the animals were necropsied.

All animals in the treatment group were observed with sedation or hypoactivity, dyspnea, and lateral recumbency on day 1. One female rat was found dead 6 hours after treatment. The remaining animals fully recovered on day 2. No clinical signs or deaths were observed in the control group. Body weight gain in the surviving rats was similar to the control group. No abnormalities were observed at necropsy. It was concluded that the oral LD_{50} of kojic acid was greater than 2 g/kg in rats.[48]

Acute Subcutaneous Toxicity

The effects of acute subcutaneous doses of kojic acid in CFLP mice were studied.[49] Preliminary findings indicated the LD_{50} to be between 4 and 16 g/kg body weight. In order to pinpoint a more precise LD_{50}, dosing was extended to groups of 5 male and 5 female mice. The groups received 0, 1.6, 2.5, 4, 6.4, 10, or 16 g/kg kojic acid as a 40% w/v suspension with 0.5%

methylcellulose by injection. Clinical signs of toxicity and mortalities were recorded during a 14-day observation period.

Hemorrhage at the injection site was observed immediately after dosing in all mice receiving kojic acid. Clinical signs observed shortly after dosing included lethargy, piloerection, depressed respiratory rate, gasping, abnormal body carriage (hunched posture), and ataxia. Mice treated with 2.5 g/kg body weight also had coarse body tremors. In male mice, none from the 1.6 g/kg dose group, 3 from the 4 g/kg dose groups, and all in the remaining dose groups died. In female mice, 2 from the 1.6 g/kg dose group, 3 from the 2.5 dose groups, 4 in the 4, 6.4, and 10 g/kg dose groups, and all in the 16 g/kg dose groups died. Death occurred within 1 to 4 h after dosing. Necropsy of these animals revealed the presence of dose material in subcutaneous tissues near the injection site, pulmonary hemorrhage, and pallor of the liver. Opacities in the eyes were observed in 1 mouse each of the 1.6 g/kg and 10 g/kg dose groups. Survivors completely recovered by day 4. No abnormalities were observed in the surviving mice at necropsy. No clinical signs or deaths were observed in the control group. The authors calculated the LD_{50} of kojic acid in mice to be 2.7 g/kg body weight (95% confidence limits = 1.9-3.9 g/kg body weight.[49]

A similar acute subcutaneous study of kojic acid was done using CFY rats.[50] The preliminary LD_{50} was determined to be between 4 and 16 g/kg body weight. To more precisely determine the LD_{50}, the dose groups were expanded to 5 males and 5 females and received 1, 1.6, 2.5, 4, 6.4, or 10 g/kg body weight kojic acid in a 40% w/v suspension of 1.0% methylcellulose via injection.

Lethargy, piloerection, abnormal body carriage (hunched posture), diuresis, and depressed respiratory rate were observed shortly after treatment. Ataxia and convulsions accompanied these signs in rats in the 2.5 g/kg dose groups and above. Rats treated with 6.4 g/kg and above also had tremors. A total of 4 males and 3 females in the 2.5 g/kg dose group, 4 males and 4 females in the 4 g/kg dose group, all males and females in the 6.4 g/kg dose group, and all males and 3 females in the 10 g/kg dose group died within 2 to 21 hours after dosing. Necropsy of these rats revealed hemorrhage of the subcutaneous tissue at the injection site, pulmonary hemorrhage, and pallor of the liver. Opacity of one or both eyes was observed in about half of the mortalities. Recovery of the survivors was complete within 6 days. Body weight increases were slightly depressed in surviving males in the 2.5 and 4.0 g/kg dose groups and in the surviving female in the 4.0 g/kg dose group for the first week of observation but were comparable to controls by the second week. No abnormalities were observed in the surviving rats at necropsy. No clinical signs or deaths were observed in the control group.

An additional group of 5 male and 5 female rats were treated subcutaneously with 4 g/kg kojic acid to further investigate the opacities. Lenticular opacities were observed in both eyes of 2 male rats and drying and clouding of the cornea were observed in 5 rats along with swelling of the cornea in 1 male and 1 female rat. This last effect obscured observation of the lens in 2 rats. One male rat died before the reading 2.5 hours after dosing. The authors determined that these opacities were not inconsistent with those of acute reversible lens opacities that have been ascribed to changes in the osmolarity of the aqueous humor. The authors calculated the LD_{50} of kojic acid in rats to be 2.6 g/kg body weight (95% confidence limits = 2.0-3.2 g/kg body weight.[50]

Acute Intraperitoneal Toxicity

The effects of acute intraperitoneal injections of kojic acid in CFLP mice were studied.[7] Preliminary findings indicated the LD_{50} to be between 1 and 4 g/kg body weight. To pinpoint a more precise LD_{50}, dosing was extended to groups of 5 male and 5 female mice. The groups received 0, 1.6, 2.5, 4, 6.4, or 10 g/kg kojic acid in a 40% w/v suspension with 0.5% methylcellulose by injection. Clinical signs of toxicity and mortalities were recorded during a 14-day observation period.

Clinical signs observed shortly after dosing included lethargy, piloerection, depressed respiratory rate, and ataxia. Mice treated with 2.5 g/kg body weight were observed gasping. Three male and 2 female mice from the 1.6 g/kg dose group and all mice in the 4, 6.4, and 10 g/kg dose groups died. Death occurred within 1 to 3 hours after dosing. Necropsy of these animals revealed the pallor of the liver and kidneys, pulmonary hemorrhage, and injection of the blood vessels of the abdominal viscera. Survivors completely recovered within 2 days of dosing. Body weight gains were comparable to controls. No abnormalities were observed in the surviving mice at necropsy. No clinical signs or deaths were observed in the control group. The authors calculated the LD_{50} of kojic acid in mice to be 2.6 g/kg body weight (95% confidence limits = 2.2-3.0 g/kg body weight.[51]

A similar acute intraperitoneal study of kojic acid was done using CFY rats.[52] The preliminary LD_{50} was determined to be between 1 and 4 g/kg body weight. To more precisely determine the LD_{50}, the dose groups were expanded to 5 males and 5 females and received 1, 1.6, 2.5, or 4 g/kg body weight kojic acid in a 40% w/v suspension of 1.0% methylcellulose via intraperitoneal injection.

Lethargy, piloerection, abnormal body carriage (hunched posture), ataxia, and depressed respiratory rate were observed shortly after treatment. Coarse body tremors and convulsions were observed in rats in the 1 g/kg dose group. Rats treated with doses above 1 g/kg also had increased salivation, diuresis, gasping, coarse body tremors, and convulsions prior to death. One female rat in the 1 g/kg dose group had slight paralysis of the hind limbs on day 3 that was still apparent at study termination. No deaths occurred in any of the males or females in the 1 or 1.6 g/kg dose groups. All of the males and 3 females each in the 2.5 and 4.0 g/kg dose group died between 1 and 19 hours post dosing. Necropsy of these rats revealed congestion, pulmonary hemorrhage, pallor of the liver, and injection of the blood vessels of the abnormal viscera. Opacities of one or both eyes were observed in 7 of the 24 mortalities. Recovery of the survivors was complete within 5 days. Body weight

increases were slightly decreased in the male 1.6 g/kg dose group for the first week of observation but were comparable to controls by the second week. No abnormalities were observed in the surviving rats at necropsy. No clinical signs or deaths were observed in the control group. The authors calculated the LD_{50} of kojic acid in rats to be 2.4 g/kg body weight (95% confidence limits = 2.0-3.0 g/kg body weight.[52]

Acute Dermal Toxicity

The acute dermal toxicity of 100% kojic acid was evaluated in 8-week-old Wistar rats.[53] The test material, in its original powdered form, was applied to clipped skin on a gauze pad (premoistened with 2 mL of purified water) at a dose of 2000 mg/kg to a group of 5 male and 5 female rats. Another group of 5 males and 5 females were patched with just 2 mL of purified water. The patches were applied for 24 hours and any residual test material was removed with a moistened gauze pad. Clinical signs and mortality were observed daily for 14 days, and body weight gains were checked on days 1, 8, and 15. At the end of the observation period, the animals were necropsied.

No deaths or clinical signs or cutaneous reactions were observed during the study in either the test or control animals. Body weight gains were slightly decreased between day 1 and day 8 in 1/5 treated males and 3/5 treated females, when compared to control animals. No abnormalities were observed at necropsy. It was concluded that the dermal LD_{50} of kojic acid is greater than 2000 mg/kg in rats.[53]

Short-Term Oral Toxicity

In a preliminary study for an in vivo genotoxicity study, male mice received oral doses of kojic acid ranging from 0 to 2000 mg/kg for 5 days. The LD_{50} from the preliminary study was calculated to be 1031.2 mg/kg per d kojic acid.[54,55]

Short-Term Dermal Toxicity

The dermal toxicity potential of kojic acid was evaluated in a 4-week study in 104 Wistar Hannover rats.[56] The rats were randomly allocated to 3 treatment groups and 1 control group, which received 100, 300, 1000 mg/kg per d kojic acid, or the vehicle, 0.5% aqueous methyl cellulose solution (w/w), respectively. The high-dose group and the control group consisted of 16 male and 16 female rats each, while the remaining groups consisted of 10 male and 10 female rats each. The extra rats in the high-dose and control groups were kept for a 2-week treatment-free observation period. The rats received the treatment or the control solutions daily to clipped dorsal skin. The animals were checked daily for mortality and clinical signs of toxicity. Body weights and food consumption were measured once a week. Complete hematology and blood chemistry investigations and urinalysis were performed at the end of the treatment period in the first 10 males and females of the high-dose and control groups and in all of the remaining animals in the other treatment groups. White blood cell and lymphocyte counts were made in the reserved 6 males and 6 females of the high-dose and control groups. All animals were killed at the end of the treatment and treatment-free periods. Select organs were weighed and a complete gross examination was performed in all animals. Microscopic examinations were performed on select tissues from the high-dose and control groups.

No deaths occurred and no relevant clinical signs were observed during the treatment or treatment-free periods. Body weight gains and food consumption were comparable to the control group. Decreased lymphocyte counts were observed at the end of the treatment period in both males and females in the 300 and 1000 mg/kg per d dose groups. This effect had partially reversed at the end of the treatment-free period in males of the high-dose group. No treatment-related changes were observed in blood chemistry parameters or urinalysis. At necropsy, decreased absolute and relative spleen weights were observed in the high-dose females, but there were no treatment-related findings during the gross or microscopic examinations in any dose group. The study concluded that the no observable effect level (NOEL) was 100 mg/kg per d, although the author noted that observed changes in lymphocytes and white blood cell counts in the higher dose groups were minimal to mild in severity and the toxicological significance of this finding was uncertain.[56]

Subchronic Oral Toxicity. In a subchronic study,[8] male SD strain rats received daily oral (by stomach tube) doses of 0, 0.25, 0.5, 1.0, 2.0, or 3.0 g/kg kojic acid suspended in 1.0% carboxymethylcellulose for 13 weeks. The dose groups included 20 rats each. The administration period was followed by a 4-week recovery period. During treatment, the rats were weighed and observed for clinical signs of toxicity and mortality daily. Feed and water intake were measured weekly. Rats from each group were killed at 4, 13, and 17 weeks (5, 10, and 5 rats at each time period, respectively) for necropsy, hematological and serobiochemical examinations, and urinalysis. Animals with lowest weight gain in each treated group (except control) were selected for removal at each time point. In dose groups where the mortality exceeded the number of animals scheduled for termination, no animals were removed.

Rats that received 0.5 g/kg or more of kojic acid had dysbasia 20 to 30 minutes after treatment and developed a strong sedation followed by sleep. Animals in the 1.0, 2.0, and 3.0 g/kg dose groups during treatment bled from the eyes, and exhibited ablepsy, exophthalmos, hematuria, epistaxis, and vomiting. All animals in the 3.0 g/kg dose group died by week 3, while 11 animals in the 2.0 g/kg, 1 animal in the 1.0 g/kg, and 2 animals in the 0.5 g/kg died during the course of the study period. No clinical signs of toxicity or mortalities were observed in the 0.25 g/kg or control groups. Body weight gains were significantly decreased in the 0.5, 1.0, and 2.0 g/kg dose groups during treatment but became comparable to controls during the recovery period. No significant changes in feed or water intake were observed when compared to the control group. No significant changes were observed with regard to hematology or urinalysis in any treatment group when

compared to controls. When compared to control serum chemistry values, serum glutamic-oxaloacetic transaminase (SGOT) enzyme activity was increased in the 1.0 and 2.0 g/kg dose groups and glutamate and calcium levels were decreased in the 2.0 g/kg dose group. Necropsies of animals that died during the course of the study found pulmonary hemorrhage, congestion of the stomach and intestine, adrenal gland hypertrophy, ocular hemorrhage and opacity, and evidence of vomiting and clonic or tonic spasm. Pyoid substance was noted in the lung with partial sclerosis of pulmonary tissue in the rats from the 2.0 and 3.0 g/kg dose group. Necropsy at scheduled termination showed similar findings in a dose-dependent manner. At 13-week necropsy, weights of liver, kidneys, and testes increased in the 1.0 and 2.0 g/kg dose groups and the adrenal gland weights were increased in the 2.0 g/kg dose group. At 17-week necropsy, increases in testicular and thymic weights were noted in the higher dose groups. The observations of normalization during the recovery period suggested to the researchers that kojic acid and its metabolites were rapidly excreted and that toxicity occurred in a dose-dependent manner.[8]

In a 26-week toxicity study,[57] male SD strain rats received daily oral gavage doses of 0, 125, 250, 500, or 1000 mg/kg kojic acid in 1% carboxymethylcellulose. The dose groups consisted of 20 rats each except for the 125 mg/kg dose group. In each group that contained 20 rats, 10 rats were used for a 5-week recovery test following the treatment phase. Clinical signs of toxicity and mortality were observed daily and body weight, feed consumption, and water intake were measured twice a week for the first 13 weeks and then once a week for the remainder of the treatment phase. Urinalysis and hematology and biochemistry tests were performed prior to necropsy at study end. Tissues and organs were examined and weighed at necropsy.

No deaths were observed in any group. Rats in the 250 mg/kg dose group showed excitation followed by sedation, and some rats in the 500 mg/kg and 1000 mg/kg had these clinical signs accompanied by transient exophthalmos and salivation that disappeared 2 to 3 hours after the dosing. Rats that received 250 mg/kg or more of kojic acid had significant suppression of body weight gain when compared to the control group. Body weight gains seemed to recover during the 5-week nontreatment phase. Decreases in urine volume were observed in the 500 and 1000 mg/kg dose groups, with a decrease in the urinary pH also occurring in the 1000 mg/kg dose group. The 1000 mg/kg dose group also had a slight decrease in erythrocyte counts and decrease of hematocrit value and hemoglobin concentration. Increases of SGOT and glutamic-pyruvic transaminase (GPT) activities were observed in dose groups receiving 250, 500, and 1000 mg/kg. The 500 and 1000 mg/kg dose groups had increased alkaline phosphatase (ALP) activity, and slight increases in total cholesterol, bilirubin, and calcium were observed in the 1000 mg/kg dose group. During the nontreatment phase, the changes in urinalysis, hematology, and biochemistry were not observed. At necropsy, the absolute and relative weights of the adrenal glands were increased in the dose groups receiving 500 and 1000 mg/kg kojic acid; however, the absolute weights of the adrenal glands in the recovery groups were almost the same as that for the control group. In the 1000 mg/kg dose group, 2 rats had vacuolation of anterior cells of the pituitary gland, but the researchers of this study could not be certain this effect was treatment-related. No other treatment-related effects in the tissues were observed. It was concluded that the NOEL of kojic acid in this experiment was 125 mg/kg per d.[57]

Chronic Toxicity

Studies of chronic exposures have been summarized in the Carcinogenicity section of this safety assessment.

Ocular and Dermal Irritation

A 3% aqueous solution of kojic acid was tested for ocular irritation potential in rabbits (strain not reported).[58] In a preliminary study, 0.05 mL of the kojic acid solution was instilled in the right eye of 3 rabbits. The eyes were not rinsed. The rabbit eyes were observed at 1, 3, 6, and 48 hours posttreatment. No changes were observed. For the main study, the left eye of 5 rabbits was instilled with 0.05 mL of the kojic acid solution and not rinsed. The eyes were examined at 0.5, 1, 6, 24, 48, and 72 hours and 1 week posttreatment. Slight redness was observed only in 1 rabbit 0.5 hours after treatment. No other effects were observed. To determine the accuracy of this study, another laboratory performed a similar test in 4 Angola rabbits using the same sample of kojic acid.[59] Mild transient hyperemia was observed in 2 of the rabbits. No other effects were observed. A positive control, 3% Thesit Desitin in distilled water, yielded a 24-hour integrated edema value of 19, which was within the normal response range (15-30). A supplemental study of the 3% kojic acid solution in 1 eye of 9 Angola rabbits found no specific response and/or inflammatory response up to 72 hours.[59]

In a dermal irritation study,[60] 0.5 g of kojic acid was mixed with 0.5 mL distilled water and applied to clipped, abraded, and intact skin of 6 albino rabbits with gauze patches. The patches were removed after 24 hours and the skin was evaluated for reactions for a period of 72 hours. None of the animals had any observable skin responses. The primary irritation index (PII) was calculated to be 0 and kojic acid was not considered an irritant to rabbit skin.

Kojic acid at 1% and 3% was evaluated for primary skin irritation in a total of 12 male Japan white rabbits.[61] A solution of 10% sodium lauryl sulfate (SLS) was used as a positive control. The cream base at 0.25 g, the 1% or 3% kojic acid cream, or 0.1 mL of SLS were applied to clipped, abraded, and intact skin (patch sites were 2 cm^2 each). The patches were open. After 4 hours, the sites were wiped with warm water and assessed for reactions after 4, 28, 48, and 72 hours. Erythema was observed 2 to 4 hours after application of both 1% and 3% kojic acid. A score of 1 to 2 was apparent on almost all animals after 24 hours. Erythema gradually faded after 48 hours, with a few sites exhibiting local and very slight erythema after 72 hours.

No significant difference was observed between abraded and intact skin. No eschar formation or edema was observed in the cream base or kojic acid patches. The PII were 0.78, 0.93, 0.85, and 3.70 for the cream base, 1% kojic acid, 3% kojic acid, and SLS, respectively. In this study, 1% and 3% kojic acid was a mild skin irritant with a PII of no more than 1.

Dermal Sensitization

The potential of kojic acid to induce delayed contact hypersensitivity was evaluated in albino Dunkin-Hartley guinea pigs.[62] The control group and the treatment group consisted of 5 males and 5 females and 10 males and 10 females, respectively. The animals of the treatment group received 3 topical applications (0.5 mL) of 30% kojic acid (w/w) in corn oil on the shaved anterior flank on days 1, 8, and 15 of a 2-week induction phase. The application sites were occluded for 6 hours after each treatment. The animals in the control group received the 0.5 mL corn oil vehicle alone on application sites, which were also occluded. Following a 14-day rest period both groups of animals received a topical application of 30% kojic acid (w/w) in corn oil to the posterior right flank. The left flank was treated with only the corn oil and served as a negative control. Both application sites were occluded for 6 hours. The skin was evaluated for reactions 24 and 48 hours after patch removal. The animals were killed at the end of the study for skin sampling of the challenge application sites in all control animals and in animals that had cutaneous reactions in the treated group.

No clinical signs or deaths were observed during the study. In the induction phase, very slight or well-defined skin reactions were observed in a few of the animals that received kojic acid. Following the challenge phase, no cutaneous reactions were observed in the control group, while very slight erythema occurred in 1 animal and well-defined erythema was observed in another animal in the treatment group at the 24- and 48-hour readings. The latter animal had slight edema at the 48-hour observation. It was concluded that kojic acid should not be classified as sensitizing to the skin.[62]

Dermal Depigmentation

The depigmenting effects of kojic acid along with 5 other substances, including phenylhydroquinone and hydroquinone, were studied in a black guinea pig study.[63] Kojic acid at 0.1 mL was applied at concentrations of 1% and 4% (w/v) in a 1:4 mixture of dimethyl sulfoxide (DMSO) and ethanol to the shaved dorsal area (4 × 4 cm or 4 × 3 cm) of 4 JY-4 black guinea pigs. The vehicle alone was also tested. The test substance was applied once a day, 6 days a week, for 5 successive weeks. After the application period had ended, the animals were killed and skin samples were prepared for examination. The depigmentation action was evaluated by macroscopic observation and spectrophotometric colorimetry. Optical and electron microscopy of epidermal melanocytes were also performed for morphological examination. The mechanism for which skin whitening occurs was also investigated by measuring oxygen consumption and the relation of free radicals to melanin synthesizing enzyme tyrosinase.

The skin whitening action of kojic acid was very weak when compared to phenylhydroquinone: the results of the macroscopic evaluation of phenylhydroquinone at 1% and 4% were "+" and "++," respectively, while these results were "−" and "+ ∼ ±" in 1% and 4% kojic acid, respectively. The 4% kojic acid test group, however, showed no statistically significant difference from the vehicle group in the colorimetric value. A white substance that was thought to be crystals of the applied kojic acid may have been causing the whitening rather than an actual depigmenting action. With repeated application, the white substance on the skin surface of the 4% kojic acid group turned light brown. There was no difference in the melanocyte count nor were there any morphological differences between the kojic acid groups and the vehicle group. The number of melanocytes in the 1% and 4% kojic acid groups was comparable to the vehicle group. Kojic acid did not show oxygen consumption and free radical production, which indicated melanocytes were not damaged. The authors concluded that kojic acid showed almost no depigmenting action in black guinea pigs.[63]

Phototoxicity

The effect of UV light on skin treated with kojic acid was evaluated using 10 albino Dunkin-Hartley guinea pigs.[64] Kojic acid (5% w/v; pH not reported) in absolute alcohol (0.5 mL) were applied on 2 sites on clipped dorsal thoracic skin. One site was occluded while the other site was left unoccluded. The guinea pigs were irradiated with UV light (from 5 18 inch long Blacklite tubes of 15 W each; wavelengths not reported) at a distance of 6 inches from the dorsal skin for 30 minutes. After the UV exposure, the patch was removed and both sites were assessed for erythema and edema. The procedure was repeated daily for 5 consecutive days and the skin was assessed prior to each reexposure. On days 3, 4, and 5, the unoccluded site was cleaned with absolute alcohol after the UV exposure to remove a residual brown stain. On these days, the sites were scored 30 minutes after cleaning. No dermal reactions were observed at any of the occluded sites. Slight erythema was observed in 3 guinea pigs on isolated occasions on days 1, 2, and 3. An additional guinea pig developed erythema on day 3 that persisted to day 4. No reactions were observed in the remaining animals. It was concluded that kojic acid may produce slight skin reactions after UV irradiation in guinea pigs.

The photohypersensitization potential of 5% w/v kojic acid in absolute alcohol was studied in albino guinea pigs.[65] The test material (0.2 mL) was applied to the shaved dorsal neck region of 10 animals daily for 5 consecutive days. A control site on the mid-dorsal region was treated daily with 0.2 mL absolute alcohol. After each induction exposure, the animals were irradiated with UV light (from 5 18 inch long Blacklite tubes of 15 W each; peak wavelength ∼350 nm) held 12 inches away from the skin for 15 minutes and observed for the presence of erythema. After a 10-day rest period, a challenge application

of 1% w/v kojic acid in absolute alcohol was made to the induction sites on the neck region. The mid-dorsal region was again treated with absolute alcohol. The sites were exposed to UV irradiation for 15 minutes and then observed for the presence of erythema at 0, 24, 48, and 72 hours.

No dermal reactions were observed during the first and second induction exposures. Slight erythema was observed in 8 of the 10 animals at the third, fourth, or fifth induction exposures. No other dermal reactions were observed in any of the control animals during induction. During the challenge, no dermal reactions were observed in the test or control animals. The study concluded that kojic acid did not induce delayed contact photohypersensitization.[65]

The phototoxicity of 1% and 3% kojic acid in cream was evaluated in 3 groups of 10 male Hartley albino guinea pigs.[66] The positive control in this study was 10% anthracene ointment with white petrolatum. The groups of animals received either 0.25 g of 1% kojic acid cream: distilled water (1:5), 0.25 g of 3% kojic acid cream: distilled water (1:5), or the positive control on the right shaved dorsal thoracic region on patch sites 2 cm^2. The left dorsal regions of all animals served as vehicle controls. Half of the sites were irradiated with an irradiation device comprising 10 Blacklite lamps at a distance of 10 cm from the skin surface for 38 minutes. To keep the light to no more than 320 nm, a 3-mm thick glass filter was placed between the lamps and the animals. Nonirradiated sites were covered with a filter, aluminum foil, and tape. The irradiation treatments were repeated daily for 5 consecutive days. Reactions were evaluated 24 hours after irradiation. No dermal reactions were observed following irradiation in the 1% or 3% kojic acid groups. The positive controls yielded the expected results. It was concluded that kojic acid was not phototoxic.

Reproductive and Developmental Effects

The effect of kojic acid on fertility and pregnancy in CRL:COBS CD(SD)BR rats was studied.[67] Doses of 0, 25, 150, and 900 mg/kg per d were orally administered in methyl cellulose vehicle to groups of 20 rats of each gender. Male rats at least 6 weeks in age were treated daily for 9 weeks prior to mating and through mating in order for the effects of kojic acid on spermatogenesis to be observed. Sexually mature females were treated daily for 2 weeks prior to mating and through day 7 of gestation and were killed on day 20 of gestation.

The 900 mg/kg per d dose group had a transient increase in activity followed by lethargy accompanied by prone posture, lacrimation, dyspnea, unsteadiness, and catalepsy. This group also exhibited slight aggressiveness, increased salivation, and brown discoloration of saliva, urine, and coats. The 150 mg/kg per d dose group had slightly increased activity and salivation. One death in a 25 mg/kg per d dose group female was unrelated to treatment. No treatment-related effects were observed in the 25 mg/kg per d dose group. Body weight gains of both genders in the 900 mg/kg per d dose group were decreased and feed consumption of males at week 9 was significantly decreased. No body weight changes or feed and water consumption effects were observed in the 25 and 150 mg/kg per d dose groups.

Slight delayed mating was observed in the 900 mg/kg per d dose group and lower values of mean litter size and number of implantations per litter were observed in this group when compared to the control group. No other mating performance or pregnancy rate effects were observed in the other treatment groups. There were nonsignificant differences in respect to lower corpora lutea count and higher preimplantation loss in the 900 mg/kg per d dose group, which resulted in nonsignificant lower values for litter weights. No treatment-related effects were observed in any treatment group with regard to postimplantation loss, mean fetal weight, or embryonic or fetal development.[67]

In another study, pregnant New Zealand white rabbits received 0, 20, 100, or 500 mg/kg per d kojic acid in 1% methylcellulose through gavage on days 6 through 18 of gestation.[68] There were 13 rabbits in each dose group. The animals were observed daily for clinical signs of toxicity and mortality, and body weights were recorded. All animals were killed on day 29 of gestation, and litter parameters were measured and fetuses were examined for abnormalities.

The rabbits in the 500 mg/kg dose group had marginally lower body weight gains throughout treatment. Post-dosing reactions from day 12 of gestation included mydriasis, lethargy, and tachypnea. No effects on body weights were observed in the remaining dose groups when compared to control values. A sporadic occurrence of post-dosing reactions was observed in the 20 and 100 mg/kg dose groups but was not considered significant. No treatment-related effects on litter size, postimplantation loss, litter and mean fetal weights, or embryonic and fetal development were observed.[68]

The effect of oral administration of kojic acid on reproduction and development was studied on pregnant ddy-SLC mice.[69] Groups of 35 mice received 0, 25, 150, or 900 mg/kg per d kojic acid in 1% methylcellulose by gavage on days 6 through 15 of gestation. Clinical signs of toxicity and mortality were observed daily. On day 18 of gestation, 2/3 of the mice underwent Cesarean section to observe toxicity and teratogenicity in the fetuses. The remaining mice were allowed to deliver their litters naturally. From these litters, 4 male and female newborn mice per litter were chosen on day 4 after birth and 2 male and female pups per litter were chosen at weaning to observe growth and reproduction ability. The remaining weanlings underwent skeletal examination.

The maternal mice in the 900 mg/kg dose group exhibited mild calmness and ataxia, and in some cases, coma and dyspnea. In this dose group, there were no treatment-related body weight changes, feed consumption, water intake, course of gestation, or findings in delivery or lactation. Body weight gains in the 25 mg/kg maternal mice were significantly greater than the control values. An increase in body weight gain was also observed in the 150 mg/kg group, but it was not significant. No abnormal effects were observed in the 25 and 150 mg/kg maternal mice, but dams in the 900 mg/kg dose group had decreased heart weights compared to the controls.

No significant effects of treatment were noted in the 25 and 150 mg/kg dose groups, including numbers of corpus luteum verum, implantations, living fetuses, resorbed and dead embryos, survival rate, body weight, weight of placenta, or gender ratio. A slight but significant decrease in body weights of male fetuses in the 900 mg/kg dose group was observed. Male and female fetuses of this dose group also had slight but significant retardation of ossification. A significant dose-dependent decrease in the number of fetuses with ossified calcaneus was observed in the 150 and 900 mg/kg dose group fetuses. Hypoplasia of the lung and heart was observed in 5.1%, 4.8%, and 7.6% of the 25, 150, and 900 mg/kg dose group fetuses. A slight increase in body weights was observed at birth in the 25 mg/kg dose group pups. Pups in the 900 mg/kg dose group had significantly increased kidney weights at 3 weeks of age. No other effects were observed in the fetuses or weanlings in any dose group. F_1 dams from the 900 mg/kg dose group had significantly decreased heart weights on day 18 of gestation while 13-week males of the 25 and 900 mg/kg dose groups had decreased adrenal and prostate glands, respectively. No other abnormalities were observed in the reproduction of the F_1 mice or in the development of the F_2 fetuses. The no observable adverse effect level (NOAEL) for maternal toxicity and embryotoxicity in this study was 150 mg/kg per d.[69]

The effect of kojic acid was investigated on pregnant Slc:ddy mice and F_1 offspring.[70] The pregnant mice received once daily oral doses of 0, 30, 160, and 800 mg/kg on days 15 of gestation to day 21 postpartum. All dams were allowed spontaneous delivery of the pups and the second generation of mice were subjected to postnatal observations, with litter size adjusted to 4 males and 4 females per litter analyzed on day 4 postpartum and 2 males and 2 females per litter analyzed at weaning for growth and reproductive ability. The remaining weanlings were subjected to skeletal examination.

Dams in the 800 mg/kg per d dose group showed signs of calmness and ventral posture from days 15 of gestation to weaning. A significant decrease in feed consumption and water intake at the terminal stage of gestation accompanied by a significant decrease in body weight also were observed with this dose group. A significant decrease in body weights was also noted during the lactation period in the 800 mg/kg dose group, although no abnormalities were observed in lactation behavior. Gestation duration was significantly prolonged in this dose group. Significant decreases in the absolute and relative organ weights were observed in the kidney of the 160 mg/kg dose group, the thymus, and the spleen (absolute only) of the 800 mg/kg dose group, and the liver of both the 160 and 800 mg/kg dose groups. No significant adverse effects were noted in the dams in the 30 mg/kg dose group.

The number of live female pups at birth and total number of live pups were significantly decreased in the 800 mg/kg dose group when compared to the control values. One dam in this dose group had an entire stillborn litter. No other abnormal litter parameters, including numbers of implantations, total newborns, perinatal mortality, live male pups, gender ratio, or body weights of pups were observed at any dose level. There were no treatment-related effects on skeletal formation or motor responses in the F_1 mice. A significant decrease in body weight gain was observed in female weanlings of the 800 mg/kg dose group. Three-week-old F_1 mice had decreased relative organ weights in the liver (160 and 800 mg/kg dose groups), the brain, the kidney, and the adrenals (160 mg/kg dose group), and the testis (30 mg/kg dose group). Vaginal opening was delayed in the 30 and 160 mg/kg dose groups, and incisor eruption was retarded significantly and dose-dependently in the 160 and 800 mg/kg dose groups. No other developmental or reproductive abnormalities were observed in the F_1 mice and no changes were noted in the F_2 offspring. This study concluded that kojic acid was not teratogenic or a reproductive toxicant in the F_2 mice.[70]

The effect of oral administration of kojic acid, as well as 2 mycotoxins, on pregnant albino rats was studied.[71] The rats were divided into 4 groups, with 1 group of 7 receiving the vehicle (0.1 mL propylene glycol) and 1 group of 8 receiving 50 μg/d kojic acid dissolved in glycol on days 1 to 5 post coitum. The remaining 2 groups received either aflatoxin B_1 or patulin. The rats were laparotomized on day 8 of pregnancy to examine corpora lutea and implantation sites. Litter sizes were recorded at term as well as teratogenic defects, death of young, and behavior of the dams.

The rats that were given kojic acid had significant decreases in implantation sites and loss of viability 2 to 3 days after littering, when compared to the control group. A significant decrease in litter size was also observed in the females given kojic acid. No teratogenic effects were observed in any treatment groups; however, mortality of litter was significant in the kojic acid group. Mothers of these litters had cannibalistic behavior 2 days after delivery. In the kojic acid group, 1 rat died before litter delivery, and 2 other rats had acute nasal and mouth infections. Significant decreases in the number of implantations occurred, but no decline in the number of corpora lutea were observed. The authors concluded that kojic acid causes an anti-implantation effect, an abortifacient effect, and litter death in albino rats, which is mainly due to maternal toxicity.[71]

The potential of kojic acid to cause toxic effects on fertility and cannibalistic behavior was evaluated in another study of mycotoxins.[72] Eight male Sprague Dawley rats with proven fertility received oral doses of 50 μg/d kojic acid in propylene glycol for 21 days. A control group of 7 rats received propylene glycol alone for the same time period. Fertility performance was studied during days 16 through 21 of treatment when each male was caged separately with 2 females of proven fertility. In rats with confirmed pregnancies, a laparotomy was performed on day 8 of gestation to examine and record the number of corpora lutea and implantation sites in addition to litter size, teratogenic effects, and number of live and dead fetuses. The dams were observed for changes in behavior. The male rats were killed on day 22 and were necropsied. The fructose content in the coagulating gland and acid phosphatase activity in the ventral prostate was examined. Spermatozoa were collected from the caput, corpus, and cauda epididymis, and vas deferens and studied microscopically, and their number, morphology, and mortality were recorded.

Body weights were significantly decreased in males exposed to kojic acid and in females with which they were mated. Weights of the testis and epididymis in the males were also significantly decreased when compared to the control group. There were no treatment-related effects on the fructose content of the coagulating gland, acid phosphatase activity, or on spermatogenesis or sperm parameters. Of the 8 males treated with kojic acid, 6 bred successfully with a total of 8 females, as compared to 6 of the 7 control males. Implantations and litter sizes were significantly decreased in the treated group. Also noted was a loss of viability among the litter on the second or third day after delivery. Dams mated to males treated with kojic acid started to eat their litter 2 days after delivery; this was thought to be due to a disturbance in the chemical interaction of the mothers with the litters as there was no nutritional deficiency observed in the control group. The authors concluded that kojic acid caused anti-implantation and cannibalistic effects in females mated with treated males and decreased litter viability.[72]

The potential of kojic acid to cause toxic effects on embryonic and fetal development was studied in mated female Wistar Han rats.[73] Three groups of 6 female rats (10 weeks old) received kojic acid at doses of 100, 300, or 1000 mg/kg per d via oral gavage on days 6 through 17 of pregnancy. An additional group of 6 mated females received the 0.5% methylcellulose vehicle alone as the control. Clinical signs of toxicity, including evidence of abortion/resorption and mortality, were checked daily. Feed consumption and body weight gain were recorded on days 2, 6, 9, 12, 15, 18, and 20 post coitum. The rats were killed on day 20 of pregnancy and fetuses were removed. The dams were examined macroscopically and number of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses were recorded. The fetuses were weighed, sexed, and submitted for external examination.

In the dams, no clinical signs of toxicity, abortions/resorptions, or death were observed at any dose level. Body weight gains in the 300 and 1000 mg/kg dose groups were slightly lower than the control group on the first 3 days of treatment. The body weight gains of the 100 mg/kg dose group were similar to that of the control. Feed consumption in all dose groups was similar to the control group. No abnormal macroscopic findings were observed at any dose level, and there were no treatment-related effects on litter parameters nor external malformations or anomalies in fetuses in any dose group. The study concluded that aside from slight and transient maternal body weight decreases in the 300 and 1000 mg/kg dose groups, kojic acid caused no signs of maternal toxicity or fetal developmental effects in this study.[73]

Genotoxicity

Bacterial Assays

An Ames assay was performed on several 1,2-dicarbonyl compounds, including kojic acid, utilizing *Salmonella typhimurium* strains TA 98 and TA 100.[74] Kojic acid concentrations were 10 to 10 000 μg/plate, with and without S9 metabolic activation. Solvent controls were water or DMSO and positive controls were quercetin, sterigmatocystin, and benzo[α]pyrene. A dose-dependent increase in revertant colonies was observed in strain TA 100, but not in TA 98, with or without S9. The authors concluded that kojic acid was mutagenic in TA 100.

The mutagenic potential of kojic acid was studied in an Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537, with and without S9 metabolic activation.[54,75] The test concentrations were 500, 1000, 2000, or 4000 μg/plate. The positive controls were *N*-ethyl-N'-nitro-*N*-nitroguanidine (ENNG), furylfuramide (AF2), 9-aminoacridine, and 2-aminoanthracene. In the presence and absence of S9, dose-dependent increases in the number of mutant colonies were observed at doses of 1000 or 2000 μg/plate and above in all but the TA 1537 strain. The positive controls yielded expected results. Kojic acid was found to be a weak mutagen in this Ames test.

The mutagenic potential of kojic acid was studied in an Ames assay using *S typhimurium* strains TA 98 and TA 100, with and without S9, at concentrations ranging from 100 to 6000 μg/plate.[76] The negative control was the solvent, distilled water, and the positive controls were 2-aminofluorene (both strains with S9), methylmethane sulfonate (TA 100 without S9), and 2-nitrofluorene (TA 98 without S9). In TA 98, kojic acid was toxic at 1000 μg/plate and above without S9 and mutagenic at concentrations of 100 μg/plate and above without S9 and at 2000 μg/plate and above with S9. Mutagenicity was observed in the TA 100 at concentrations of 1000 μg/plate and above without S9 and at 2000 μg/plate and above with S9. Kojic acid was mutagenic in TA 98 and TA 100 in this Ames assay.

The mutagenicity of kojic acid was studied in *S typhimurium* strain TA 100, with and without S9.[77] To rule out the possibility that mutagenicity observed in earlier studies was due to contaminants in kojic acid samples, the researchers purified 3 samples of kojic acid (reagent, food additive, and cosmetic lots) by high-performance liquid chromatography (HPLC) and tested the resulting fractions. In the mutation assay, kojic acid was tested at 500, 1000, and 1500 μg/plate. Positive controls were 4-nitroquinoline 1-oxide (without S9) and benzo[α]pyrene (with S9) and these yielded expected results. The 3 samples of kojic acid were found to have similar mutagenic activities, before and after separation by HPLC and with and without S9, in a linear dose-dependent manner.

The mutagenicity of kojic acid was studied in an Ames test using *S typhimurium* strains TA 98, TA 100, TA 102, TA 1535, and TA 1537, with and without S9.[78] Doses of kojic acid per plate ranged from 0 to 5000 μg (diluted in distilled water). The positive controls for assays with S9 were 2-anthramine (for TA 98, TA 100, TA 1535, and TA 1537) and benzo[α]pyrene (for TA 102), and the positive controls for assays without S9 were sodium azide (for TA 100 and TA 1535), 9-aminoacridine (for TA 1537), 2-nitrofluorene (for TA 98), and mitomycin C (for TA 102). The positive controls yielded expected results. Kojic acid induced mutagenic activity in all 5 *Salmonella* strains, with and without metabolic activation.

The potential of kojic acid to induce gene mutation was studied in *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *Escherichia coli* strain WP2 uvrA using the reverse mutation assay.[79] The assay was performed with and without S9 metabolic activation, with the concentrations 0, 33, 100, 333, 1000, 2500, and 5000 μg/plate kojic acid (in DMSO). Positive controls for assays without metabolic activation were sodium azide (in TA 100 and TA 1535), 4-nitro-*o*-phenylenediamine (in TA 98 and TA 1537), and methyl methane sulfonate (in WP2 uvrA). The positive control in assays with metabolic activation was 2-aminoanthracene in all strains and species. In the first experiment, toxicity was observed in TA 1537 at 5000 μg/plate, with and without S9. In both experiments, a dose-dependent increase in revertant colony numbers was observed at higher concentrations in all strains treated with kojic acid, except in TA 1537, with and without S9. Positive controls yielded expected results. It was concluded that kojic acid induced gene mutations (through base pair changes and frame shifts) in *S typhimurium* strains TA 98, TA 100, TA 1535 and *E. coli* strain WP2 uvrA.

In another reverse mutation assay,[80] *S typhimurium* strains TA 98 and TA 100 received kojic acid at either concentrations ranging from 0 to 5000 μg/plate with S9 or 0 to 1000 μg/plate without S9. The solvent, DMSO, proved to be toxic to TA 98 without S9 and was replaced with deionized water. The positive control for both strains with S9, for TA 98 without S9, and for TA 100 without S9 were 2-aminoanthracene, 4-nitro-*o*-phenylenediamine, and sodium azide, respectively. "Erratic toxic effects" were observed in the first experiment; results for treatment with S9 only were reported. In both experiments, toxic effects were observed without S9 at concentrations of 333 μg/plate and greater in TA 98 and at concentrations of 100 μg/plate and greater in TA 100. No significant or reproducible increases in revertant colony numbers were observed in either test strain at any dose level, with or without S9. The positive controls yielded expected results. It was concluded that kojic acid was nonmutagenic to *S typhimurium* strains TA 98 and TA 100 in this assay.

Mammalian Cell Assays

The potential for kojic acid to induce sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells was studied.[76] The cells were incubated for 2 hours with kojic acid (with and without S9 metabolic activation) at concentrations of 3, 4.5, or 6 mg/mL, washed, and incubated for another 24 hours in fresh medium containing 5-bromodeoxyuridine. Cells were also incubated in the negative control, M-199 culture medium, or the positive controls, methylmethane sulfonate (without S9) and cyclophosphamide (with S9). Colchicine was added for the last 3 hours of culture. Cells were fixed and stained. At least 30 metaphases were scored for each dose per duplicate flask.

Cytotoxicity was tested in M-199 culture medium at concentrations of kojic acid ranging from 1.5 to 12 mg/mL. Kojic acid was cytotoxic at concentrations of 9 mg/mL and above. The TC_{50} (50% toxic concentration) was 10.86 ± 3.86 mg/mL based on loss of cellular proteins.

A dose-related and significant increase in SCE in CHO cells was observed after exposure with kojic acid, with and without metabolic activation. However, binding of kojic acid to constituents of the S9 mix may have resulted in reduction of SCE frequency in the groups that was treated with metabolic activation. The positive controls yielded expected results. It was concluded that kojic acid was genotoxic in this SCE study.

In the same study, the potential of kojic acid to induce chromosomal aberrations in CHO cells was studied.[76] The investigation was similar in methodology as the SCE study above except that the slides were stained with 4% Giemsa and that at least 100 metaphases were scored for each dose. Positive controls in this study were triethylenemelamine (without S9) and cyclophosphamide (with S9). A dose-related and significant increase in the percentage of aberrant CHO cells was observed after exposure with kojic acid, with and without metabolic activation. Except for ring aberrations, all categories of chromosomal aberrations increased with increased doses of kojic acid without S9. The authors concluded that kojic acid was clastogenic in this study.

Kojic acid was studied for cell mutation in mouse lymphoma L5178Y $TK^{+/-}$ cells at the *hprt* locus.[81] After a range-finding test to measure cytotoxicity, 2 independent experiments were performed. The concentrations for both experiments ranged from 300 to 1421 μg/mL, with and without S9 metabolic activation. The doses were selected to determine viability and 6-thioguanine resistance 7 days after treatment. Relative survival at the highest concentration was 79% with S9 and 95% without S9, respectively, in the first experiment, and 81% with S9 and 92% without S9 in the second experiment. The vehicle control was purified water. The positive controls were benzo[α]pyrene with S9 and 4-nitroquinoline 1-oxide without S9. A small, statistically significant increase in mutation frequency was observed at 300 μg/mL with S9 in the second experiment. There was no evidence of a dose-related response, however, and no other statistically significant increases in mutation frequency were observed at any dose level tested with or without S9 in either experiment. The controls yielded expected results. It was concluded that kojic acid was not mutagenic in the cell mutation assay.

The mutagenic activity of kojic acid was evaluated in guanidine-resistant Chinese hamster V79 cells.[55,82] The cells were assayed without S9 at concentrations of 0, 30, 100, 300, 1000, or 3000 μg/mL kojic acid in culture medium. The positive control was ethyl methanesulfonate (EMS). Cells were treated for 16 hours and then washed and successively cultured at 2-day intervals for 3 times. Cells were then plated for a culture period of 12 days with 10 μg/mL 6-thioguanine. No significant increase in mutation rate was observed at any dose level and there was no statistically significant difference between the treatment and the solvent control groups. The positive control produced expected results. In this study, kojic acid was not mutagenic in Chinese hamster V79 cells.

The potential of kojic acid to induce structural chromosome aberrations was assessed in vitro using V79 cells of Chinese

hamsters.[83] A range-finding experiment was used to determine the concentrations of the test material to be evaluated with and without S9 metabolic activation in 2 independent experiments. Toxic effects were observed only in the absence of S9. In experiment 1, the concentrations of kojic acid tested were 355, 710, or 1420 μg/mL, with and without S9, and in experiment 2, the concentrations tested with S9 were 355, 710, or 1420 μg/mL and those without S9 were 250, 500, or 1000 μg/mL. Each experiment had 2 parallel cultures. The culture medium and deionized water served as the negative and solvent controls while EMS (without S9) and cyclophosphamide (with S9) were the positive controls. The treatment period for experiment 1 was 4 hours with a 14-hour recovery in both the presence and absence of S9, while the treatment periods in experiment 2 were 4 hours with a 24-hour recovery in the presence of S9 and 18 or 28 hours with no recovery in the absence of S9. Cytogenetic analysis for chromosome aberrations was performed on 100 metaphases/culture.

In the range-finding assay, no toxicity occurred at any concentration after 4 hours, with or without S9, but toxic effects were observed at concentrations of 710 μg/mL and higher without S9. In experiment 1, no toxic effects were observed in cultures tested with S9, but a dose-dependent reduction in cell numbers were observed in both experiments 1 and 2 without S9 and with S9 in experiment 2. The number of cells did not fall below 50% of the solvent control, however. Weak clastogenic effects were observed in experiment 2 with number of cells with aberrations increased significantly after 18 hours (250 and 1000 μg/mL) and 28 hours (1000 μg/mL). No precipitation and no relevant influence of kojic acid on pH value or osmolarity were observed. No biologically relevant increase in polyploid cells was observed when compared to the controls. The positive controls yielded the expected results. It was concluded that in the absence of S9 metabolic activation and after 18 or 28 hours exposures, kojic acid was a weak clastogen, although the effects observed may be related to cytotoxicity.[83]

In Vivo Mammalian Tests

The genotoxic potential of kojic acid was evaluated using a micronucleus test.[84] The main study was preceded by range-finding studies. NMRI mice received 500, 750, 1000, or 2000 mg/kg body weight kojic acid. The test material was administered by a single intraperitoneal injection in 1% carboxyl methyl cellulose (CMC) at a volume of 10 mL/kg body weight. In the main study, mice received 187.5, 375, or 750 mg/kg body weight of the test material. Each treatment group consisted of 5 males and 5 females. There were also vehicle (1% CMC) and positive (cyclophosphamide) control groups. Mice in all dose groups were killed at 24 hours; an additional 750 mg/kg dose group was killed at 48 hours (the high-dose groups had 6 males and 6 females, each). Bone marrow was sampled upon death in all mice. Two thousand polychromatic erythrocytes (PCEs) per animal were studied for the presence of micronuclei. Normochromatic erythrocytes (NCEs) were also studied for micronuclei. The PCE/NCE ratio was measured in 2000 erythrocytes.

In the range-finding studies, deaths occurred within 1 hour of dosing in the 2000 mg/kg dose group. Toxic effects in the other dose groups included reduced spontaneous activity, abdominal position, eyelid closure, and apathy. In the main study, the 750 mg/kg dose group was also observed with the aforementioned clinical signs of toxicity. The mean number of NCEs was not increased after treatment with kojic acid when compared to vehicle control values, indicating that kojic acid was not cytotoxic in the bone marrow. In all dose groups, the number of micronucleated PCE was not statistically increased when compared to the vehicle control group. The positive control group yielded expected results. It was concluded that kojic acid was not genotoxic in this micronucleus assay.[84]

The genotoxic potential of kojic acid was studied in another micronucleus test using male ddY mice.[85] The main study was preceded by a range finding study in which groups of 2 mice received a single intraperitoneal injection of 125, 250, 500, 1000, 2000, or 4000 mg/kg body weight kojic acid in 0.9% physiological saline in a dose volume of 10 mL/kg. In the main study, groups of 6 mice received either 2 or 5 intraperitoneal injections at 24-hour intervals. The doses for the "2-repeated dose" mice were 125, 250, 500, or 1000 mg/kg body weight kojic acid, and the doses for the "5-repeated dose" mice were 125, 250, or 500 mg/kg body weight kojic acid. There were also vehicle (0.9% physiological saline) and positive (mitomycin C) control groups. All mice were killed 6 hours after the final dosing. Bone marrow was sampled upon death in all mice. One thousand PCEs per animal were studied for the presence of micronuclei. A single dose of 1000 mg/kg body weight kojic acid killed 5 of the 6 mice. In the surviving mouse of that dose group, no micronucleus was observed in the 1000 PCEs. The number of micronucleated PCEs was not increased in the 125, 250, or 500 mg/kg dose groups for the 2-day or 5-day exposures when compared to the vehicle control group. The positive control group yielded expected results. Kojic acid was not genotoxic in bone marrow cells of mice.

In a micronucleus assay, male ddY mice (3 and 9 weeks old) and male F344 rats (9 weeks old) in groups of 4 received 0, 500, or 1000 mg/kg kojic acid by gastric intubation.[77] Groups of 3 rodents received the positive control compounds, diethylnitrosamine or cyclophosphamide. At 24 hours after treatment, two-thirds partial hepatectomies were performed on the 9-week-old animals. After 4 days, all animals were killed and the livers were prepared for analysis. In the 3-week-old mice, partial hepatectomies were not performed and livers were removed for analysis at 72, 96, or 120 hours after treatment. The number of micronucleated hepatocytes among 1000 hepatocytes was recorded for each animal. Mean values of micronucleated hepatocytes in the 9-week-old mice were increased dose dependently. At 1000 mg/kg, the value was significantly increased over the negative control. No increases were observed in the rats or in the 3-week-old mice. Positive controls yielded expected results. The authors concluded that while genotoxicity was observed in the mouse liver following kojic acid exposure, it was not proved that this genotoxicity is involved in hepatic tumor development in mice.

A dominant lethal test of kojic acid in 1% sodium carboxymethylcellulose was conducted on groups of 30 BDF_1 mice.[54,55] Male mice received 0, 350, or 700 mg/kg kojic acid by oral gavage. At the end of the dosing period, each male mouse was mated with a single female. Mating continued for 56 days, with the male mating with an unmated female every 4 days. Thirteen days after mating, the females were killed, necropsied, and number of successful pregnancy, corpora lutea, implantations, and live and dead fetuses were recorded. The number of pregnant females in the treated groups was comparable to the negative control. Postimplantation losses were slight but decreased in a statistically significant manner in the 700 mg/kg per d dose group during mating days 37 to 40. No other induced dominant lethality was observed in either concentration. The positive control, 7,12-dimethylbenz(a)anthracene, induced the expected dominant lethal response. It was concluded that kojic acid did not induce dominant lethality in this test.[54,55]

An unscheduled DNA synthesis study of 100% kojic acid was conducted on Wistar HanIbm male rats.[86] The rats received a single oral gavage dose of 150 or 1500 mg/kg body weight of the test material. Each dose group included 4 rats, 3 of which were processed for the assay. A vehicle control group received 10 mL/kg body weight deionized water and a positive control group received 10 mg/kg body weight 2-acetylaminofluorene. At 2- and 16-hour postadministration, primary hepatocytes were isolated from the rats and incubated with tritiated methyl thymidine for 4 hours and then incubated overnight in medium containing unlabelled thymidine before processing for autoradiography.

The viability of the hepatocytes was not substantially affected in any dose group for either treatment period. Enhanced mean nuclear and cytoplasmic grain counts in addition to slight shifts of the percentage distribution of nuclear grain counts to higher values at the 2- and 16-hour treatment interval after dosing with 1500 mg/kg kojic acid were observed. The net grain values of all dose groups, however, were consistently negative and comparable to the vehicle control. The positive controls yielded expected results. This study concluded that kojic acid did not induce DNA damage leading to unscheduled DNA synthesis in rat hepatocytes and, thus, was not genotoxic to rats.[86]

Kojic acid (100.6% pure) was tested in an in vivo Comet assay in male Wistar rats.[87] Groups of 5 males received 2 oral doses of 0, 1000, or 2000 mg/kg body weight kojic acid in a 0.5% aqueous solution of cremophor. The 2 doses were 21 hours apart. The positive control was EMS (300 mg/kg body weight in a single oral dose). The animals were killed 24 hours after the last treatment (3 hours for positive controls) and the stomach, colon, and liver were examined. Slides were prepared with nuclei isolated from homogenized tissue samples for the Comet assay. Electrophoresis was performed in an ice bath for 40 minutes (30 minutes for stomach cells) at 25 V and at 300 mA.

During a pilot study for this assay, rats in both dose groups had roughened fur, strongly semianesthetized state, and strongly reduced motility. In the main study, rats in the 2000 mg/kg dose group showed signs of toxicity (no details provided). No treatment-related cytotoxicity was observed in the liver, stomach, or colon cells after isolation. No biologically significant increases in mean Comet tail length were observed in the cells from rats treated with kojic acid, but such increases occurred as expected in the positive controls. Kojic acid was considered not genotoxic in this Comet assay of rat liver, stomach, and colon cells.[87]

DNA adduct formation from kojic acid exposure was investigated in male F344/DuCrj rats.[88,89] Rats in groups of 3 received 100.3% kojic acid in the diet at concentrations of 0%, 0.5%, or 2.0% for 7 or 28 days. The positive control, 2-acetylaminofluorene, was administered by gavage once at 16 hours before necropsy. The rats were observed daily for clinical signs of toxicity and weighed weekly. The animals were killed 1 day after the last treatment, and organs were examined and livers weighed. The ^{32}P-postlabeling method was utilized in determining the DNA adducts. Chromatography was performed using 3 solvent systems for kojic acid analysis in order to determine unknown DNA adducts.

No treatment-related clinical signs of toxicity were observed during the treatment period and no abnormalities were observed during gross pathology. Rats in the 2% kojic acid treatment group had significantly decreased body weights after the day 7 treatment when compared to the control group. This treatment group also had slightly decreased food consumption. Liver weights in all treatment groups were comparable to the control group. An unclear autoradiograph pattern was observed in 2 of the solvent systems for the 2.0% treatment groups. A second experiment was performed and these results could not be reproduced. No distinct spots of DNA adducts were detected for the control or 0.5% treatment group. The positive control yielded expected spots of DNA adducts on the autoradiogram. It was concluded in this study that kojic acid has no potential to form DNA adducts in rat liver.[88,89]

The formation of DNA adducts and 8-hydroxydeoxyguanosine (8-OHdG) in rat thyroids was studied in rat thyroids after exposure to kojic acid.[24] Groups of 20 male F344 rats received food with either 0% or 2% kojic acid for 1 or 2 weeks. After the designated treatment period, the thyroids were removed from the rats and the DNA was extracted. Twenty thyroid lobes per animal from 10 animals per group were combined and 2 samples were achieved for the DNA adduct investigation; 6 lobes from 3 rats were combined as one sample for the 8-OHdG investigation; a total of 5 and 6 samples were created from the control animals for the 1 and 2 week exposures, respectively. ^{32}P-postlabeling analysis with HPLC coupled to an electrochemical detector was utilized in determining the DNA adducts and 8-OHdG. No spots indicating DNA adduct formation were detected in the thyroids of rats fed the diet containing 2% kojic acid for 2 weeks. The 8-OHdG values were slightly reduced at 1 week after administration of 2% kojic acid and became significantly decreased after 2 weeks when compared to the controls. The authors of this study concluded that kojic acid has no potential to form DNA adducts or 8-OHdG in rat thyroid.

Genotoxicity studies are summarized in Table 4.

Photogenotoxicity

The potential of 100% pure kojic acid to induce gene mutations in *E coli* strain WP2 during irradiation was investigated by Wollny.[90] The concentrations of kojic acid (dissolved in DMSO) for each experiment were 33, 100, 333, 1000, 2500, or 5000 μg/plate. The positive control was 8-methoxypsoralen (MOP) and the negative control was the solvent. Irradiation was performed with a metal halogenide light source. The UV doses were 10 mJ/cm^2 UVA and 0.5 mJ/cm^2 UVB and the duration was 10 seconds.

No relevant toxic effects were observed. In the first experiment, the 2500 μg/plate concentration had an increase in revertant colonies slightly exceeding the threshold when compared to the solvent control. The threshold was exceeded in the 2500 and 5000 μg/plate concentrations in the second experiment. However, irradiation did not further increase the number of revertant colonies when compared to the corresponding treated but nonirradiated controls. The positive control yielded expected results. The author concluded that irradiation had no influence on the mutagenic potential of kojic acid.[79]

A photo-reverse mutation assay of kojic acid in *S typhimurium* strains TA 98 (concentration ranges 0-2500 μg/mL) and TA 102 (concentration ranges 0-5000 μg/mL) and in *E coli* strain WP2/pKM101 (concentration ranges 0-5000 μg/mL) was done.[89] The bacteria were tested with the plate method with or without UV irradiation and in the absence of metabolic activation. Positive controls were mitomycin C or AF2 (without irradiation) and MOP or chlorpromazine hydrochloride (with irradiation). Revertant colonies were twice the negative control in TA 102 at 5000 μg/mL and in WP2/pKM101 at 2000 μg/mL and higher with UV irradiation. A dose-dependent response was observed. An increase of revertant colonies was also observed in UV irradiation groups as compared to groups without irradiation. An increase of more than twice that of the negative control was not observed in the TA 98 strain, with or without irradiation. The positive controls yielded expected results. The authors concluded that kojic acid was a weak photo-mutagen.

The potential of kojic acid to produce chromosome aberrations in Chinese hamster lung cells following UV irradiation was studied.[89] The cells were exposed to 0.35, 0.70, or 1.4 mg/mL kojic acid with and without light irradiation. The solvent control group was treated with DMSO and the positive control groups were treated with either *N*-methyl-N'-nitro-*N*-nitrosoguanidine ([MNNG] without irradiation) or MOP (with irradiation). A nontreated control group that received light irradiation was also prepared. No statistically significant increase of cells with structural chromosome aberrations or polyploidy cells was observed at any dose level without UV irradiation. Statistically significant increases of cells with structural chromosome aberrations (1.4 mg/mL dose) and polyploidy (0.70 and 1.4 mg/mL doses) were observed. It was concluded that kojic acid was a weak photo-mutagen.

A micronucleus study on male HR-1 mice to determine the photomutagenticity of kojic acid was also done.[89] The backs of the mice were treated with a cream containing 1.0% or 3.0% kojic acid or a positive control solution containing MOP dissolved in acetone:olive oil (2 groups of 3 mice for each substance plus an additional 2 groups of 3 mice that received a control cream that did not contain kojic acid). The materials were applied at 24-hour intervals and 1 group of mice from each treatment type was exposed to UVA irradiation. At 48 hours after the second irradiation, epidermal cells of mouse skin were prepared for micronucleus examination.

After the first irradiation, the skin of the mice treated with kojic acid became brown in tone. No clinical signs of toxicity of mortality were observed in any of the dose groups. Micronucleated cells in the kojic acid-treated groups, with or without UV irradiation, were comparable to the control values. Positive control values yielded expected results both with and without UV irradiation. It was concluded that kojic acid did not produce micronuclei in mouse epidermal cells, in the presence or absence of UV irradiation.[89]

Carcinogenicity

International Agency for Research on Cancer (IARC) determined that kojic acid is "not classifiable as to its carcinogenicity to humans (Group 3)".[91]

A 78 week carcinogenicity study of kojic acid in mice was done.[92] Male and female $B_6C_3F_1$ mice were fed diets containing 0%, 0.16%, 0.4%, or 1% kojic acid. The mice were observed daily for clinical signs of toxicity and mortality, while body weight and feed consumption were measured once a week for 13 weeks and then once every 4 weeks. The mice were killed and necropsied at the end of the treatment period.

A few deaths occurred in both male and female mice during the course of the study, but these occurrences were comparable with the control group. The cumulative survival rates were 92% and 100% for male and female mice, respectively. Gross external examination discovered preputial gland swelling, Harderian gland enlargement, and palpable masses in the fermoral subcutis in the treated male and control groups, but the authors determined that these findings were not related to kojic acid exposure. A slight decrease in body weight gain was observed in both males and females in the 1% dose group, starting at week 3 in males and week 11 in females. Slight body weight gain decreases were also noted in the 0.4% females and 0.16% males but were considered insignificant by the researchers due to the briefness of the occurrence and the fact that the opposite gender in each dose did not have similar results. There were no significant differences in feed consumption between the treated groups and the controls.

Females in the 0.16% dose groups and higher and males in the 0.4% dose groups and higher had a significant increase in both the absolute and relative thyroid weights. Statistically significant, but very slight (less than 1%) and nondose-dependent increases or decreases in absolute organ weights were observed in the prostate glands, adrenal glands of males and females, lungs of males, salivary glands of males, and kidneys of

Table 4. Genotoxicity Studies for Kojic Acid

Strain/Cells Tested	Concentrations Tested	Methodology	Results	Reference
Bacterial cell assays				
Salmonella typhimurium TA 98 and TA 100	10 to 10 000 μg/plate	Ames test with and without metabolic activation	Mutagenic in TA 100	43
S typhimurium TA 98, TA 100, TA 1535, and TA 1537	500 to 4000 μg/plate	Ames test with and without metabolic activation	Weakly mutagenic	39 (S. Iwahara and K. Sakamoto, Unpublished data, 1980)
S typhimurium TA 98 and TA 100	100 to 6000 μg/plate	Ames test with and without metabolic activation	Mutagenic	44
S typhimurium TA 98, TA 100, TA 102, TA 1535, and TA 1537	0 to 5000 μg/plate	Ames test with and without metabolic activation	Mutagenic	D. Marzin, Unpublished data, 1997
S typhimurium TA 98, TA 100, TA 1535, and TA 1537; *Escherichia coli* WP2 uvrA	0 to 5000 μg/plate	Reverse mutation assay with and without metabolic activation	Mutagenic	H. E. Wollny, Unpublished data, 1998
S typhimurium TA 98 and TA 100	0 to 5000 μg/plate with S9, 0 to 1000 μg/plate without S9	Reverse mutation assay with and without metabolic activation	Non-mutagenic	H. E. Wollny, Unpublished data, 2001
S typhimurium TA 100	500 to 1500 μg/plate	Reverse mutation assay with and without metabolic activation	Mutagenic	45
Mammalian cell assays				
CHO cells	3 to 6 mg/mL	SCE test with and without metabolic activation	Genotoxic	44
CHO cells	3 to 6 mg/mL	Chromosomal aberration study with and without metabolic activation	Clastogenic	44
Mouse lymphoma L5178Y $TK^{+/-}$ cells at the *hprt* locus	300 to 1421 μg/mL	Cell mutation assay with and without metabolic activation	Not mutagenic	M. Lloyd, Unpublished data, 2002
Guanidine-resistant Chinese hamster V79 cells	0 to 3000 μg/mL	Cell mutation assay without metabolic activation	Not mutagenic	39, S. Iwahara, Unpublished data, 1981
Chinese hamster V79 cells	355 to 1421 μg/mL with out and without S9 in first experiment, 355 to 1421 μg/mL with S9 and 250 to 1000 μg/mL without S9	Chromosomal aberration study with and without metabolic activation	Weakly clastogenic	M. Schulz, Unpublished data, 2002
In vivo mammalian tests				
NMRI mice	187.5 to 750 mg/kg	Micronucleus test	Not mutagenic	(N. Honarvar, Unpublished data, 2001)
Male ddY mice	125 to 1000 mg/kg	Micronucleus test	Not mutagenic	(H. Omura and M. Nonaka, Unpublished data, 1980)
3- and 9-week-old male ddY mice and 9-week-old F344 male rats	0 to 1000 mg/kg	Micronucleus test	Genotoxic only in 9 week old mice	45
BDF_1 mice	0 to 700 mg/kg	Dominant lethal test	Negative	39 (S. Iwahara, Unpublished data, 1981)
Male Wistar HanIbm rats	150 or 1500 mg/kg	Unscheduled DNA synthesis	Not genotoxic	(W. Volkner, Unpublished data, 1997)
Male Wistar rats	0 to 2000 mg/kg	Comet assay	Not genotoxic	(S. Brendler-Schwaab and B. Kramer-Bautz, Unpublished data, 2004)
Male F344/DuCrj rats	0% to 2.0%	DNA adduct assay	Negative	46 (M. Nakano, Unpublished data, 2005)
Male F344 rats	0% or 2.0%	DNA adduct assay	Negative	21
Photogenotoxicity				
E coli WP2	33 to 5000 μg/plate	Gene mutation study with and without light irradiation	Negative	(H. E. Wollny, Unpublished data, 1998)
S typhimurium TA 98 and TA 102; *E coli* WP2/pKM101	0 to 2500 μg/plate for TA 98, 0 to 5000 for TA 102 and *E coli*	Photo-reverse mutation assay	Weak photo-mutagen	46
Chinese hamster lung cells	0.35 to 1.4 mg/mL	Chromosomal aberration study with and without light irradiation	Weak photo-mutagen	46
Male HR-1 mice	1.0% or 3.0%	Micronucleus test with and without UV irradiation	Negative	46

Abbreviations: CHO, Chinese hamster ovary; SCEs, sister chromatid exchanges.

males and females. At necropsy, hepatic adenomas and hemangiomas, pulmonary adenomas, malignant lymphomas, leukemia, or pituitary adenomas were observed. These tumor incidences did not differ between the kojic acid treatment groups and the control group. Likewise, nodular hyperplasia in the liver, adrenal subcapsular spindle cell hyperplasia, and uterus cystic endometrial hyperplasia did not occur at significantly differing rates in the treatment groups versus the control groups. The researchers concluded that kojic acid was not tumorigenic to mice in this 78-week study.[92]

The tumorigenic potential of kojic acid was evaluated, using heterozygous *p*53-deficient CBA, *p*53(+/−), mice and wild type littermates, *p*53(+/+).[22] The mice were fed diet containing 0%, 1.5%, or 3.0% kojic acid for 26 weeks. The mice were observed daily for clinical signs of toxicity and were weighed weekly. All surviving mice were killed after blood sampling for hormone assays and necropsied. Livers and thyroid glands were removed and weighed. These organs along with the pituitary, spleen, lungs, and other organs and tissues with macroscopic lesions were fixed for histopathological examination. Additionally, tissue sections were immunohistochemically stained for proliferating cell nuclear antigen (PCNA). Five thousand hepatocellular nuclei in normal background parenchyma in each mouse were counted for PCNA determination.

One wild type male from the 3.0% dose group was found dead at week 13. Both *p*53(+/−) and *p*53(+/+) mice of the 3.0% dose group had decreased body weight gains compared to controls. Absolute thyroid gland weights were significantly ($P < .01$) increased in a dose-related fashion by 209% and 444% in the 1.5% and 3.0% kojic acid dose groups, respectively, in *p*53(+/−) mice and by 140% and 374% in *p*53(+/+) mice. Absolute and relative liver weights in the kojic acid-treated groups had somewhat higher values in both types of mice when compared to controls but was not significant except for the relative weight in the 3.0% *p*53(+/+) mice.

Diffuse hypertrophy and hyperplasia of thyroid follicular epithelial cells were observed along with decreased serum thyroxine (T_4) levels in both *p*53(+/−) and *p*53(+/+) mice treated with kojic acid. No thyroid tumors were observed, however. In the liver, the incidence of altered hepatocellular foci was significantly increased at 1.5% and 3.0% in *p*53(+/−) and at 1.5% in *p*53(+/+) mice. The authors concluded that there is tumorigenic potential of kojic acid in the liver but not in the thyroid follicular epithelial cells in CBA mice. The genotoxic potential of kojic acid on hepatocellular tumor development could not be ruled out.[22]

The above study was repeated using male CBA mice that received 0%, 0.5%, 1%, or 2% kojic acid in their diet for 26 weeks.[25] Incidences of hepatocellular adenomas were 5%, 17%, 10%, and 21%, respectively. Incidences of hepatocellular foci in these dose groups were 15%, 39%, 45%, and 47%, respectively, with a statistically significant difference ($P < .05$) only between the control group and the 2% dose group.

Male F344 rats were used in a 55-week toxicity dietary study of kojic acid.[93] The 7-week-old rats were divided into groups of 20 and received 0%, 0.5%, or 2.0% kojic acid (equivalent to 0, 227, or 968 mg/kg body weight/d, respectively). One week prior to treatment, rats received a single subcutaneous injection of 5 mL/kg saline. The rats were observed daily for clinical signs of toxicity and were weighed regularly. Feed consumption was recorded weekly. At the end of treatment, surviving rats were killed after blood sampling and necropsied. Major organs and tissues were weighed and/or fixed for histopathological examination. Additionally, liver sections were studied immunohistochemically for glutathione S-transferase-placental form (GST-P), PCNA, and single-strand DNA (ssDNA).

No mortality or obvious clinical signs of toxicity were observed during the treatment period. Body weight gains were decreased in the 2.0% group from week 6 until treatment end, when compared to the controls. No significant changes in feed consumption were observed. In both the 0.5% and 2.0% treatment groups, red blood cell counts and hematocrit values were decreased. Significant increases or a tendency for increase were observed in aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, γ-glutamyl transpeptidase (γ-GTP), blood urea nitrogen (BUN), and sodium values in both the 0.5% and 2.0% dose groups. In the 2.0% group, total protein, total bilirubin, and total cholesterol values were significantly increased and the albumin/globulin ratio was decreased.

Absolute and relative spleen and thyroid gland weights were increased or had a tendency for increase in both the 0.5% and 2.0% dose groups. In the 2.0% dose group, absolute and relative weights of heart, lungs, liver, adrenal glands, testes, and relative weights of brain and kidneys were significantly increased. Single-cell necrosis of hepatocytes and proliferation of small bile ducts or ductules were recorded in animals from both treatment groups, with the incidence of the proliferation of bile ducts significantly increased in the 2.0% dose group. All 2.0% dose group animals had diffuse hepatocellular hypertrophy and/or vacuolization and formation of microgranulomas containing crystals and/or brown pigment; the incidence of the granulomas was significantly increased. Areas of GST-P-positive foci were significantly increased in the liver of the 2.0% dose group. Incidences of hyaline casts and basophilic tubules were also significantly increased in the 2.0% dose group. Diffuse follicular cell hyperplasia was noted in the thyroid glands in both treatment groups, with focal follicular cell hyperplasia, and adenomas and/or carcinomas observed in the 2.0% group. The 2.0% dose group also had increased hypertrophy of cortical cells in zona fasciculate in the adrenal glands. The study concluded that the NOAEL of kojic acid was below 0.5% (227 mg/kg body weight/d).[93]

Carcinogenicity studies are summarized in Table 5.

Tumor Promotion

The carcinogenesis-modifying action of kojic acid in rat liver using a 2-stage model with initiation by diisopropanolnitrosamine (DHPN) was investigated.[94] Sixty male F344 rats received either a single subcutaneous injection of 2000 mg/kg DHPN or the vehicle and then were fed a diet containing

0%, 0.125%, 0.5%, or 2% kojic acid for 20 weeks. At the end of the treatment period, the rats were killed and necropsied. The liver was removed, weighed, and prepared for paraffin sectioning. H&E staining and immunostaining to GST-P and PCNA were performed and the sections were investigated histopathologically and cell-kinetically.

Rats treated with 2% kojic acid with DHPN initiation had significantly increased ($P < .01$) relative liver weights. Histopathology revealed an increased incidence of microgranuloma and vacuolation of centrilobular hepatocytes. The number and area of GST-P-positive foci per unit area of the liver in the DHPN and 2% kojic acid group were 22.30 foci and 3745 μm^2, respectively, which was a significant increase ($P < .01$) when compared the 8.48 foci and 531 μm^2 in the group treated with only DHPN. The incidence of GST-P-positive foci and the percentage of PCNA-positive cells were more prominent in animals with marked vacuolation of hepatocytes. In the group treated with 2% kojic acid without DHPN, the number and area of GST-P-positive foci were 1.39 foci and 109.5 μm^2, respectively, which was also a significant increase when compared to the control group values of 0.40 foci and 9.7 μm^2. No treatment-related effects were observed in the rats treated with 0.5% kojic acid or lower, with or without DHPN. The researchers concluded that kojic acid has a carcinogenesis-promoting action in the rat liver and may be carcinogenic without promotion.[94]

Further study on the tumor promotion potential of kojic acid was done.[95] Groups of 20 male F344 rats received 0%, 0.5%, or 2% kojic acid in feed for 20 weeks without DHPN initiation. At the end of the treatment period, the rats were killed and necropsied, and the livers were studied in the same manner as described above. Dose-related increases in absolute and relative liver weights were observed in both kojic acid treatment groups. Numbers and areas of GST-P-positive foci were significantly increased ($P < .01$) in the 2% kojic acid group when compared to the control group. Increased incidences of microgranuloma and vacuolation of hepatocytes were observed in the 2% kojic acid treatment group. PCNA expression was significantly increased ($P < .05$) in the 2% kojic acid dose group when compared to the control group, with PCNA-positive hepatocytes mainly localized around the vacuolated and granulomatous regions.

The authors also performed a medium-term liver bioassay of kojic acid in groups of 25 F344 male rats at concentrations of 0%, 0.125%, 0.5%, or 2% to determine kojic acid's promoting influence.[95] Two weeks prior to the start of the 6-week dietary exposure of kojic acid, the rats received a single intraperitoneal injection of 200 mg/kg *N*-diethylnitrosamine (DEN). At week 3, the rats were subjected to a two-third partial hepatectomy. At the end of the treatment period, the rats were killed and livers were prepared for analysis as above. A dose-related decrease in body weight gains and an increase in relative liver weights were observed, with statistical significance ($P < .01$) in the 2% dose group. Significant increases ($P < .01$) in number and areas of GST-P-positive foci were observed in the 2% dose group when compared to the control group. The authors concluded that kojic acid at 2% was tumor-promoting and had weak hepatocarcinogenic potential. The authors further opined that the enhanced replication of hepatocytes related to toxic changes may have been involved as an underlying mechanism.

Tumor Initiation

A study on the tumor-initiating potential of kojic acid in mouse liver was performed using male ICR mice.[23] The mice received a diet containing 0% or 3% kojic acid for 4 weeks, followed by distilled water containing 0 or 500 ppm phenobarbital (PB) for 14 weeks. Two weeks after the treatment with PB, a two-third partial hepatectomy was performed on all mice. At the end of the study, all mice were killed and liver slices were performed to evaluate γ-glutamyltransferase-positive foci as preneoplastic foci markers in the liver as well as PCNA.

No treatment-related deaths were observed and there were no significant changes in feed consumption or body weights during the course of the study. No proliferative lesions were observed in any dose groups during microscopic examinations. There were no differences in the number of γ-glutamyltransferase-positive cells between the kojic acid and distilled water and the kojic acid + PB groups. Significant increases in the labeling index of PCNA were observed in the control + PB and kojic acid + PB dose groups as compared to the control + distilled water group (1.28 ± 1.93); however, no significant difference in the positivity of PCNA was observed between the control + PB and the kojic acid + PB groups. The authors concluded that kojic acid has no tumor-initiating activity in mouse liver.[23] In reviewing this report, however, the SCCP concluded that the kojic acid effect on proliferation of liver cells cannot be excluded since kojic acid + distilled water PCNA values were increased compared to basal diet + distilled water.[20]

The initiation potential of kojic acid (99.5% pure) in rat liver was examined in a 2-part study.[26]

In the first experiment, groups of 5 male F344 rats were fed a diet containing 0% or 2% kojic acid for 3, 7, or 28 days. All rats were injected with 100 mg/kg body weight bromodeoxyuridine (BrdU) intraperitoneally once a day for the last 2 days of exposure and 2 hours prior to termination. Livers were removed and weighed at necropsy and slices were prepared for BrdU immunostaining. Labeling indices (LIs) were calculated as percentages of cells positive for BrdU incorporation divided by the total number of cells counted. In addition, 8-oxodeoxyguanosine (8-OxodG) was measured in nuclear DNA to examine the formation of oxidative DNA adduct by HPLC-ECD detection.

On day 28 of the experiment, body weight gains in the 2% kojic acid group were significantly decreased compared to the control group. In the 2% kojic acid dose group, absolute liver weights were significantly increased on day 7 but decreased on day 28. Relative liver weights were significantly increased at all time points. The LI values of hepatocytes of the 2% dose group were significantly increased as compared to the controls on days 3 and 7. All 8-OxodG levels in the liver DNA in the 2% dose group were slightly higher than the control values but were not statistically significant.

Table 5. Carcinogenicity Studies for Kojic Acid

Strains Tested	Concentrations of Kojic Acid Tested	Study Duration And Type	Results	References
General carcinogenicity				
$B_6C_3F_1$ mice	0.16% to 1%	78-week; dietary	Not tumorigenic	(Kudo Safety Research Institute, Unpublished data, 1981)
Heterozygous p53-deficient CBA, p53(+/−), mice and wild type littermates, p53(+/+)	1.5% or 3.0%	26-week; dietary	Tumorigenic potential in liver but not thyroid follicular epithelial cells	19
Male CBA mice	0.5% to 2%	26-week; dietary	Hepatocarcinogenic	22
Male F344 rats	0.5% or 2.0%	55-week; dietary	NOAEL below 0.5%	48
Tumor promotion				
Male F344 rats	0.125% to 2%	20-week; dietary 2-stage model with DHPN initiation	May be carcinogenic without promotion; carcinogenesis-promoting in rat liver	(T. Shibusawa, T. Imai, T. Tamura, et al, Unpublished data, 2002)
Male F344 rats	0.5% or 2.0%	20-week; dietary 2-stage model without DHPN initiation	Tumor-promoting	49
Male F344 rats	0.125 to 2.0%	Medium-term liver bioassay	Weak hepatocarcinogenic potential	49
Tumor initiation				
Male ICR mice	3%	Dietary; kojic acid exposure for 4 weeks and PB exposure for 14 weeks	No tumor-initiating activity in mouse liver	20
Male F344 rats	2%	28-day; dietary	Significantly increased LI vales in hepatocytes, nonsignificantly increased 8-OxodG levels in liver DNA	23
Male F344 rats	1000 or 2000 mg/kg	Single oral exposure with dietary administration of 2-AAF for 2 weeks	Tumor-promoting effects in liver	23
Male F344 rats	0.5% to 2%	4-week dietary exposure followed by 6 weeks of PB	No initiation potential in rat liver	46 (M. Kawabe, Unpublished data, 2003)
Dermal tumor promotion				
Female CD-1 (ICR) mice	0.3% or 3%	20-week; topical application with DMBA or kojic acid initiation and TPA or kojic acid promotion	No dermal promotion potential	46 (M. Kawabe, Unpublished data, 2003; M. Kawabe, Unpublished data, 2004
Thyroid Effects				
$B6C3F_1$ mice	1.5% or 3.0%	20-month; dietary	Thyroid adenomas observed likely due to decrease in serum T3 levels and increased TSH	50
Male F344 rats	0.008% to 2.0%	4-week; dietary	Tumor-promoting effects on development of thyroid proliferative lesions; iodide uptake and iodine organifcation in thyroid prohibited	51-53
Male F344 rats	0.008% to 2.0%	4-week; dietary	Diffuse hyperplasia in thyroid glands	54
Male F344 rats	2.0%	12-week; dietary with BHP initiation	Thyroid proliferative lesions observed	55
Male F344 rats	4 to 1000 mg/kg	4-week; gavage	Decreased blood T4 concentration with enhanced thyroid function	56
Male F344 rats	0.02% to 2.0%	31-week; dietary treatment of kojic acid for 8 weeks followed by 23 weeks of SDM treatment in drinking water	No tumor-initiation activity in thyroid	21

Abbreviations: PB, Phenobarbital; SDM, sulfadimethoxine; BHP, bis(2-hydroxypropyl)nitrosamine; TSH, thyroid-stimulating hormone; TPA, phorbol-12-myristate-13-acetate; DMBA, 9,10-dimethyl-1,2-benzanthracene; DHPN, diisopropanolnitrosamine; NOAEL, no observable adverse effect level; 2-AAF, 2-acetylaminofluorene; LI, labeling index.

In the second experiment of this study, 30 male F344 rats were subjected to a two-third partial hepatectomy on day 0. At 12-hour postsurgery, the rats were treated once orally with carboxymethylcellulose vehicle (8 rats), 1000 mg/kg kojic acid (12 rats), or 2000 mg/kg kojic acid (10 rats) at a dose volume of 10 mL/kg body weight. The rats were then fed basal diet for 2 weeks and then diet containing 0.015% 2-acetylaminofluorene (2-AAF) for another 2 weeks. At 3 weeks post kojic acid administration, rats received a single 0.8 mL/kg body weight dose of carbon ttetrachloride (CCl_4). Surviving rats were killed at the end of week 5 and slices of all liver lobes were stained immunohistochemically for GST-P. The mean area and number of GST-P-positive foci per unit area of all liver sections were calculated. During the course of the experiment, 1 rat in the control group died. Slight decreases were observed in the mean area and numbers of GST-P positive foci, but these differences were not statistically significant.

The researchers of this second experimental study concluded that kojic acid has neither liver initiation activity nor the capability of 8-OxodG formation; however, the findings suggest that kojic acid has liver tumor-promoting effects.[26]

The initiation potential of kojic acid (100.3% pure) in a liver carcinogenesis bioassay was performed on F344 male rats.[89,96] In one portion of the study, groups of 15 rats received 0%, 0.5%, 1%, or 2% kojic acid or the positive control 2-AAF at concentrations of 0.01% or 0.001% in their feed for 4 weeks. After the treatment period, all rats received basal diet for 1 week, and then a diet containing 0.5% phenobarbital sodium salt (SPB) for 6 weeks. In another portion of the study, groups of 9 rats received 0% or 2% kojic acid or 0.01% or 0.001% 2-AAF in feed for 4 weeks, and then all rats received basal diet for 7 weeks. At 6 weeks after the beginning of the study, all animals from both portions of the study underwent a two-third partial hepatectomy. Rats were checked twice daily for clinical signs of toxicity and mortality. Body weights were measured weekly and daily feed consumption and intake of kojic acid, 2-AAF, and SPB were calculated. All surviving rats were killed at study end, and organs were examined macroscopically. Liver weights were recorded and sections from 3 liver lobes were stained immunohistochemically for GST-P.

No treatment-related effects or deaths were observed during the study. Rats that received 2% kojic acid in both portions of the study had significant decreases in body weights during initiation period of the study, but body weights returned to control levels during the SPB or basal diet treatments. Decreases in feed consumption during the initiation period occurred in the 1.0% and 2.0% kojic acid groups, but increases in feed consumption during the SPB or basal diet treatment were marked with increases in body weight change. No treatment-related differences were observed in final body or liver weights, with or without SPB. Numbers of GST-P-positive foci in kojic acid-treated groups were similar to the control values, with or without SPB. No other treatment-related effects were observed. In the positive control groups, the numbers of GST-P-positive foci were statistically significantly increased in the 0.01% 2-AAF groups, with and without SPB. This study concluded that kojic acid did not possess initiation potential in the rat liver.[89,96]

Dermal Tumor Promotion

A skin carcinogenesis bioassay to determine the promotion potential of kojic acid (reported as 100.3% pure) in a cream formulation was performed using female CD-1 (ICR) mice.[89,96,97] The positive initiator control was 9,10-dimethyl-1,2-benzanthracene (DMBA) and the positive promoter control was phorbol-12-myristate-13-acetate (TPA). Groups of 10 or 15 mice were treated in the following manner: DMBA + vehicle, DMBA + 0.3% kojic acid, DMBA + 3% kojic acid, DMBA + TPA, acetone + 0.3% kojic acid, acetone + 3% kojic acid, vehicle + TPA, or 3% kojic acid + TPA. The control or test substances were applied to the shaved backs of the mice (4 cm^2). The mice receiving DMBA or acetone were treated once at the beginning of the experiment while the mice treated with vehicle + TPA or 3% kojic acid + TPA received 50 mg of the test substances daily for 1 week. A week after the study commencement, the treatment groups with DMBA or acetone received 50 mg of the test substances 5 times weekly for 19 weeks. The remaining groups received TPA twice weekly for 19 weeks 1 or 2 weeks after study commencement. Animals were checked for clinical signs of toxicity and mortality once daily and for skin nodules once weekly. All surviving animals were killed after the completion of the promoter treatment and examined macroscopically. A histological examination of the skin was performed and liver weights were recorded.

No treatment-related mortalities were observed. Body weight gain was significantly decreased in week 2 or weeks 3 and 4 in the DMBA + 0.3% kojic acid and acetone + 3% dose groups, respectively. Squamous cell papilloma was observed in 1 mouse from the DMBA + 3% kojic acid. The positive control group, DMBA + TPA, had significantly increased body weight gain (starting at week 3) and absolute and relative liver weights. The positive control group also had skin nodules, which were revealed to be squamous cell hyperplasia, squamous cell papilloma, or squamous cell carcinoma at necropsy. It was concluded that kojic acid did not possess promotion potential for skin carcinogenesis.[89,96]

Thyroid Effects

The tumorigenicity of kojic acid was studied in a 20-month study in $B6C3F_1$ mice.[98] Groups of 65 male and female mice received 0%, 1.5%, or 3.0% kojic acid in feed for 20 months. Subgroups of 5 animals were killed at 6 and 12 months after the beginning of treatment. Serum was collected for hormone assessment at 6, 12, and 20 months from 5 animals in each treatment group. Another subgroup of 10 to 14 animals in each treatment group was switched to normal diet at month 19. At the end of the treatment period, all surviving animals were killed and necropsied, with major organs and tissues weighed and fixed for histopathological examination.

Survival rates in mice in the treatment groups were comparable with the control groups during the course of the administration period. Thyroid weights were increased significantly in the kojic acid-treated groups of both genders, especially in the male groups; there were no significant differences in other major organ or tissue weights or hematological values or serum biochemical parameters in any of the treatment groups. Incidences of thyroid gland hyperplasia and follicular adenomas were significantly increased in all treatment groups. In mice that received normal feed 30 days prior to termination, incidences of thyroid gland adenomas were significantly decreased, although average thyroid weights were unchanged. The serum-free triiodothyronine (T_3) levels in the 3.0% dose groups of both genders were significantly lower than the control at month 6, while the thyroid-stimulating hormone (TSH) levels were increased. The decreases in the free T_3 levels continued at the later measurements, but changes in the TSH levels disappeared. It was concluded that chronic high doses of kojic acid induces thyroid adenomas in male and female $B6C3F_1$ mice. The authors proposed that the likely mechanism is the decrease in serum T_3 levels and increased TSH.[98]

A study was performed to determine the mechanisms of serum thyroid hormone reduction and thyroid tumor-promotion effects of kojic acid exposure in rats.[99] Groups of 8 male F344 rats received basal diet containing 0%, 0.008%, 0.03%, 0.125%, 0.5%, or 2.0% kojic acid for 4 weeks (doses equivalent to 0, 5.85, 23.8, 95.3, 393.6, and 1387.3 mg/kg body weight/d). At the end of treatment, blood was collected from 5 rats per group for hormone assays. The remaining animals were injected intraperitoneally with 0.4 mL of 0.1 mol/L $Na^{125}I$ in saline 24 hours before they were killed. Measurement of ^{125}I uptake was taken and the thyroid was examined for organification.

No significant changes in body weights were observed in the treated rats when compared to the control rats. Absolute and relative thyroid gland weights were increased in all groups treated with kojic acid in a dose-dependent manner, with significant increases occurring at 0.5% or more. The relative pituitary gland weights were significantly increased in the 2.0% kojic acid group and relative liver weights were significantly greater in all kojic acid groups except the 0.125% group. These last two observations were not dose-dependent or associated with significant changes in absolute weights, and thus were not biologically relevant. A statistically significant decrease in serum T_3 and T_4 levels was observed in the 2.0% kojic acid group when compared with the control group. The serum TSH in the 2% kojic acid group was significantly increased when compared to the controls. There were no other significant differences in these parameters in the other dose groups. Thyroid ^{125}I uptake was significantly decreased in a dose-dependent manner starting at 0.03% kojic acid. A significant reduction in organic formation of iodine was observed in the 2.0% kojic acid group.

Histopathologic examination revealed decreased colloid in the thyroid follicles and follicular cell hyperthrophy in the thyroid in high incidences in groups that received 0.03% kojic acid or more. All rats in the 2.0% kojic acid group had thyroid capsular fibrosis. In a quantitative morphometric analysis, the ratio of the area of follicular epithelial cells to the area of colloids was significantly increased in the 0.03% kojic acid dose group and higher. In this rat study, kojic acid inhibited iodide uptake and iodine organification in the thyroid, with tumor-promoting effects on the development of thyroid proliferative lesions. These effects were likely secondary to prolonged serum TSH stimulation resulting from negative-feedback through the pituitary–thyroid axis.[99] Additional studies found similar results.[100,101]

The mechanism of tumorigenesis in the thyroid from exposure to kojic acid was examined in a 3-part study.[102]

In the first experiment, groups of 9 male F344 rats received 0%, 0.008%, 0.03%, 0.125%, 0.5%, or 2.0% kojic acid in their diets for 4 weeks. Twenty-four hours prior to experiment end, 4 rats in each dose group received 0.2 mL/100 g body weight $Na^{125}I$ at 0.1 mol/L in saline. Rats were killed and the thyroid glands were weighed and examined for ^{125}I uptake. The remaining 5 animals were killed on the same day. Thyroid gland weights were increased in a dose-dependent manner in rats receiving 0.125% or more kojic acid in diet, with the thyroid gland weights from the 2.0% dose group 9 times that of the controls. ^{125}I uptake into the thyroid gland was more sensitive to kojic acid treatment, with significant suppression at 0.03%. Organic ^{125}I formation was interrupted only in the 2.0% dose group. Serum T_3, T_4, and TSH levels were affected only at 2.0%.

In the second experiment, male and female F344 rats were divided into 8 and 4 groups, respectively, with each group consisting of 8 animals. The groups received diet containing 0% or 2.0% kojic acid. Male groups were killed at weeks 1, 2, 3, and 4 and female groups were killed at weeks 2 and 4. Half of the rats were studied for ^{125}I uptake and the other half for hormonal and histopathological examination. In males, thyroid gland weights increased linearly from 11 to 98 mg in the 4 weeks of treatment with 2.0% kojic acid. A less prominent, but still significant, increase in thyroid gland weights was observed in females, from 7.5 to 40 mg. The suppression of ^{125}I uptake was also time dependent and in males, the decrease started at 1 week after kojic acid treatment and reached about 2% of control values by week 3, with organic ^{125}I formation significantly decreased by 50% compared to the controls. These effects were not as significant in females, with only 20% suppression of ^{125}I uptake at week 4. Serum T_3 and T_4 levels were decreased to minimum levels after 2 weeks of kojic acid treatment, but recovered thereafter although at lower than control values in both genders. Serum TSH started to increase at week 1 and reached a maximum at weeks 2 and 3.

For the final experiment in this study, 6 groups of 8 male F344 rats received 0% and 2.0% kojic acid in diet for 4 weeks. At the end of the treatment, kojic acid was replaced with basal diet for 0, 6, 12, 24, or 48 hours. The groups were killed and examined as in the first 2 experiments, except that ^{125}I was injected 12 hours before death. The organic ^{125}I formation returned to normal limits after 6 hours and ^{125}I uptake per unit of thyroid weight increased to 70% of the control values within

24 hours. Serum T_3 and T_4 were 47% and 34% of the control values after 4 weeks of the kojic acid diet. The levels increased to normal limits within 48 hours after return to basal diet and high levels of TSH decreased to normal within 24 hours.

The histopathological investigation on thyroid glands in these 3 experiments found a diffuse type of hyperplasia caused by the kojic acid diet. After 2 weeks of returning to basal diet, normal thyroid follicular structure was apparent in enlarged thyroid glands. The authors of this study suggest that the proliferative effect of kojic acid on the thyroid is not related to a genotoxic pathway.[102]

In a study to determine whether kojic acid causes a promoting effect on thyroid carcinogenesis, male F344 rats were initiated with *N*-bis(2-hydroxypropyl)nitrosamine (BHP) with a single subcutaneous injection (2800 mg/kg).[103] The dose groups included 10 rats each. One week later, the rats received basal diet containing 0% or 2% kojic acid for 12 weeks. An additional group of 8 rats received no BHP initiation or kojic acid and were fed basal diet for 13 weeks. Half of the rats were killed at week 4 and the remainder after the last week of exposure. In the second experiment of the same study, another 2 groups of 10 rats not initiated with BHP received diet containing 0% or 2% kojic acid for 20 weeks. Again, half of the rats were killed at week 4 and the remainder after week 20. Body weights were recorded and blood samples for hormone analysis were taken before death in all animals.

Body weights were decreased in the rats that received kojic acid at both week 4 and 12. Rats in both experiments exposed to kojic acid also had increased absolute and relative thyroid weights up to 25-fold greater than the control group, as well as increased relative liver weights at each time point. Absolute liver weights were significantly increased in rats exposed to kojic acid for 20 weeks. Serum T_3 and T_4 levels were significantly decreased (approximately one half to one third the values of the BHP alone group) and serum TSH was significantly increased (13-19 times higher than the BHP alone group) in the BHP + kojic acid group at both time periods. Similar changes in other serum thyroid-related hormones were observed in the 2% kojic acid alone group at week 4 but not at week 20.

At week 4, 4 of the 5 rats in the BHP + kojic acid group had focal thyroid follicular hyperplasias, while 3 of the 5 rats had focal thyroid follicular adenomas. These lesions were observed in all rats in the BHP + kojic acid group by week 12. Rats that only received kojic acid had marked diffuse hypertrophy of follicular epithelial cells at week 4 and 20. The BHP alone and the untreated control groups had no changes in thyroid-related hormone levels or histopathological lesions. There were no significant intergroup changes of the liver T_4-uridine diphosphate glucuronosyltransferase (UDP-GT) activity. The authors concluded that kojic acid induced thyroid proliferative lesions due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary–thyroid axis, with decreases of T_3 and T_4 caused by a mechanism independent of T_4-UDP-GT activity.[103]

In a study on the effect of kojic acid on thyroid function, 24 groups of 10 male F344/Du Crj rats received 0, 4, 15, 62.5, 250, or 1000 mg/kg kojic acid daily for 4 weeks.[104] Kojic acid was suspended in 0.5% carboxymethylcellulose and administered at a dosing volume of 5 mL/kg via gavage. At the end of each treatment week, a group of rats from each dose group were killed and necropsied (1 group of rats were necropsied prior to test material administration).

No abnormalities were observed in rats in the 0 to 250 mg/kg dose groups during treatment. Several rats in the 1000 mg/kg dose group had transient and slight decreases in motility 30 minutes to 1 hour after dosing on day 18 to 28 of treatment. Body weights and feed consumption in the 1000 mg/kg dose group were significantly inhibited when compared to the control group. The absolute and relative weights of the thyroid glands were nearly comparable to the control in the 4 to 250 mg/kg dose groups throughout the treatment period. Absolute and relative weights of the thyroid gland in the 1000 mg/kg dose groups were 1.2-fold and 1.3-fold greater than the control group, respectively. Serum T_3 concentration in the 250 mg/kg dose group had a significant decrease only at week 1 when compared to the control group, but the other dose groups showed no significant differences compared to the control at week 2 to 4. The serum T_4 concentration in the 1000 mg/kg dose group was significantly decreased at week 4, but no dosage of kojic acid affected the serum TSH concentration significantly. The 1000 mg/kg dose group had hypertrophy of epithelial cells in the thyroid gland at week 1 to 4; this was not observed in the 250 mg/kg dose group.

In this study, the uptake of iodine and iodination were determined prior to the beginning of treatment and at week 1, 2, 3, and 4 of treatment in 5 animals in each dose group. The rats received ^{125}I-NaI intraperitoneally 24 hours after the last treatment at the end of each week and blood was collected to measure radioactivity 24 hours after each administration of the radiolabel. Animals were killed and thyroid glands were excised and homogenized for radioactivity measurement. Radioactive iodine uptake in the 4 to 250 mg/kg dose groups was comparable to the control group at week 1 to 4. In the 1000 mg/kg dose group, the iodine uptake was about 2-fold greater than the control group in week 1; the uptake in this group continued to be constant and high through week 4. The TCA-precipitable radioactive iodine in the thyroid gland was also increased in the 1000 mg/kg dose group.

This study also determined the absorption of radioactive kojic acid in male Wistar rats dosed with a single-oral dose of 10 μCi/100 g body weight ^{14}C-U-kojic acid. Blood was collected 10 and 30 minutes and 1, 3, 6, and 24 hours after administration and radioactivity was measured with liquid scintillation. The absorption of kojic acid was rapid as manifested by the T_{max} of blood concentration of radioactivity, which was as short as 1.0 ± 0.0 hours and the $t_{1/2}$ was 4.8 ± 0.3 hours. Blood concentrations of radioactivity had nearly disappeared by 24 hours after treatment. The authors concluded that kojic acid may decrease blood T_4 concentration and that thyroid function may be enhanced compensatorily; however, the toxic effect observed on the thyroid gland from the 1000 mg/kg dose group may depend on a fast decrease

following a transient increase of concentration of kojic acid in the blood.[104]

The potential thyroid gland tumor initiation activity of kojic acid was evaluated in a 2-part study on rats.[24] Groups of 20 male F344 rats received a diet containing 0%, 0.02%, 0.2%, or 2% kojic acid for 8 weeks that was followed by treatment with 0.1% sulfadimethoxine (SDM) in drinking water for 23 weeks. A 13-week recovery period followed the SDM treatment. Controls included a group that received 4 subcutaneous injections of BHP during the initiation period followed by an administration of 0.1% SDM, a group that received diet containing 2% kojic acid for the initial 8 weeks alone, a group that received 2% kojic acid for the entire 31 weeks, and a group that received only basal diet. Body weights were measured weekly. At the end of 31 weeks of experimenting, blood was drawn for hormone analysis. Half of the rats in each group were killed prior to the recovery and the remaining rats were killed after. All rats were necropsied. Thyroid glands from the animals were weighed, fixed, and underwent histopathological examination.

During the treatment and recovery periods, deaths from tracheal obstruction from extremely hypertrophied thyroids were observed in the BHP control group (5 in total), the 31-week administration of kojic acid control group (3 in total), the 8-week kojic acid control group (1 in total), and the 2% kojic acid + SDM treatment group (1 in total). Significant suppression of body weight gains was observed in the BHP and 31-week kojic acid control groups during administration that continued until the end of the recovery period in the 31-week kojic acid control. All treated groups had significantly increased absolute and relative thyroid gland weights when compared to the untreated (basal diet) control group at the end of the administration period. These values, however, were decreased at the end of the recovery period, except in the BHP control group. When compared to the untreated controls, serum T_3 levels in the 0% kojic acid + SDM, 2% kojic acid + SDM, and BHP control group were significantly decreased at the end of the administration period, as were the serum T_4 levels in all treatment groups except the 8-week kojic acid control. The serum T_3 and T_4 levels in the 8-week kojic acid control were significantly increased compared to the untreated controls. Dose-dependent significant increases in the serum TSH levels occurred in all treatment groups, except the 8-week kojic acid control. These increases were also dependent on treatment duration in the groups that received kojic acid.

Thyroid carcinomas and adenomas were observed in all rats of the BHP control group while no histopathological lesions were observed in the untreated control group. One adenoma was observed in the 31-week kojic acid control group, but no other carcinomas or adenomas were observed in the remaining treatment groups. At the end of administration, focal follicular cell hyperplasias were significantly higher in rats in the 2% kojic acid + SDM, BHP control, and 31-week kojic acid control groups. This effect was observed in the latter 2 groups until the end of the recovery period. The mean percentage of PCNA-positive cells to 150 to 700 follicular cells counted per proliferative lesion was significantly increased in the BHP control and the 31-week kojic acid control group. The authors concluded that kojic acid had no tumor-initiation activity in the thyroid and observed thyroid tumorigenic activity in earlier studies was likely attributable to nongenotoxic mechanisms.[24]

In this safety assessment, the only thyroid carcinogenesis data available are those pertaining to rodents. A review by Capen reported that rodent thyroid glands, especially in male rats, have greater sensitivity to chemical substances and physiologic perturbations than human thyroid glands.[105] This difference is attributed to several factors, including shorter plasma half-life of T_4 in rodents and differences in transport and binding of proteins for thyroid hormones. Capen concluded that induction of neoplasia in humans from prolonged stimulation of the human thyroid by TSH would occur only in exceptional circumstances. In contrast, a review by Hill et al stated that the US Environmental Protection Agency (EPA) follows the position that chemically induced rodent thyroid tumors are presumed to be relevant to humans and that when interspecies information is lacking, the default is to assume comparable carcinogenic sensitivity in rodents and humans.[106] The SCCP noted that while thyroid tumor induction due to tumor-promoting effect from hormonal disruption occurs in rodents, the effect of kojic acid on human thyroid glands does not pose a significant carcinogenic risk.[20]

Clinical Assessment of Safety

Case Studies

A 30-year-old woman that developed hyperpigmentation following sclerotherapy for varicose veins was prescribed a cream containing 3% kojic acid, 10% urea, 2% hydroquinone, 4% lactic acid, 74% witch hazel, 5% castor oil, 1% citric acid, 1% cellulose, and 10% propylene glycol.[107] After 4 months of use, she saw no improvement of the hyperpigmentation and was prescribed another medication (a mixture of melilotus, alpha bisabolol, Ginko biloba extract, and ascorbic acid) to use along with the cream. A few weeks later, the patient presented with eczematous eruption on and around the hyperpigmentation. Patch tests with the Grupo Español de Investigación Dermatitis de Contacto (GEIDC) series were negative, while a patch test of the entire cream was ++ after 4 days. The individual components of the cream were tested, including kojic acid aqueous solutions of 0.1%, 0.5%, 1%, and 5%. All kojic acid patches were positive after 2 and 4 days, with a ++ reaction to concentrations of 1% and 5%. Patch tests of the other components were negative. Twenty controls tested with the same kojic acid concentrations were negative.

In another case study, a 54-year-old woman with actinic lentigines on her arms and forearms developed pigmented contact dermatitis on her arms.[108] The patient admitted to using a compound with a formulation similar to the one described above containing 3% kojic acid for 5 years. One year before presentation, she noticed progressive, asymptomatic erythematous and hyperpigmented areas on her arms but continued applying the skin lightening compound. Biopsy showed pigmentary

incontinence, melanophagia, and moderate lymphohistiocytic infiltrate without a spongiotic epidermis. Patch tests with GEIDC series, disperse dyes, and photopatch tests were negative. Patch tests with 1% aqueous kojic acid and the compound "as is" were negative on day 2, but hyperpigmentation was present at both sites on day 4 and 7. These lesions persisted for 1 month. Twenty controls tested with the same compound and 1% aqueous kojic acid were negative.

Clinical Testing and Therapeutic Use

A human repeat insult patch test (HRIPT) of the potential of kojic acid to induce primary or cumulative irritation and/or allergic contact sensitization was conducted using 54 participants.[109] The participants received applications of a cream product containing 1% kojic acid. Induction applications were made to the same, previously untreated site on the back 3 times per week for 3 successive weeks. An amount sufficient to cover the contact surface of kojic acid was applied to a ¾ inch square absorbent pad portion of an adhesive dressing. The test sites were occluded. The patches were removed after 24 hours. Following the 2-week nontreatment period, the challenge application was applied to a previously untreated site for 24 hours, and the site was scored 24 and 72 hours after patch removal. No responses were observed during either the induction or challenge tests.

In another HRIPT study, the potential of a formula containing 2% kojic acid to induce sensitization was evaluated using 218 participants. The induction phase consisted of 9 consecutive applications of 0.2 mg of the test material. The test material was applied on a 2 cm × 2 cm Webril pad, and the test sites were semioccluded. The patches were removed after 24 hours, and the test sites were evaluated after 48 or 72 hours. After a 2-week rest period, the participants received challenge applications on previously untreated sites for 24 hours, and the test sites were evaluated after 48 or 72 hours. During the induction phase, 11 minimal or doubtful ("?") responses and 4 definite erythema ("+") responses were observed. Only one minimal or doubtful response was observed at 48 hours but was resolved at 72 hours. The study concluded that there was no evidence of sensitization in a formula containing 2% kojic acid.

Of the 220 female patients patch tested for suspected cosmetic-related contact dermatitis, 5 reacted to kojic acid as well as products they owned that contained 1% kojic acid.[110] Reactions to 1% and 5% kojic acid in these patients were + and ++. The 5 patients had developed facial dermatitis within 1 to 12 months of using kojic acid-containing cosmetic products. The remaining 215 patients in the patch test group, including 3 that had previous exposures to the kojic acid, did not have any reactions to kojic acid.

The effectiveness of hydroquinone and kojic acid (concentration of 2%) formulations with glycolic acid for the treatment of melasma in 39 patients was compared.[111] The formulations were applied on each half of the face once daily (increasing to twice daily if well tolerated) for a month. Burning and desquamation were reported in all patients, with the kojic acid formulation being more irritating of the 2 formulations tested. None of the patients discontinued treatment, however.

The effectiveness of a gel containing 2% kojic acid, 10% glycolic acid, and 2% hydroquinone to treat melasma was determined in a 12-week study of 40 Chinese women.[112] One half of each woman's face was treated with the test gel and the other half was treated with a gel that did not contain kojic acid. All patients experienced redness, stinging, and mild exfoliation on both halves of the face, with symptoms settling by the third week of the study. Three patients had to withdraw from the study due to these side effects.

Prignano et al[32] described the use of kojic acid in treatment for melasma (cloasma). Kojic acid is normally used in 1% preparations for this skin condition at a frequency of 2 times daily for 2 months. A side effect of this treatment is contact allergy.

Summary

Kojic acid is used as an antioxidant in cosmetics and is derived from several fungal species.

The FDA reports that kojic acid is used in a total of 16 products. In an industry survey of current use concentrations, kojic acid is used at concentrations ranging from 0.1% to 2%. Health Canada and the EWG report 148 and 93 uses, respectively, with the uses in Canada reported as high as 10% to 30%. Kojic acid may be used in cosmetic spray products, but the particle sizes produced by such products are not respirable.

The European Commission's SCCP determined that, based on a margin of safety calculation, the use of kojic acid at 1.0% in skin care formulations poses a risk to human health due to potential systemic effects. The SCCP also found that kojic acid is a potential skin sensitizer. Kojic acid is not included on the list of ingredients that must not be used in cosmetic products that are marketed in Japan.

Noncosmetic uses reported for kojic acid include therapeutic uses for melasma, antioxidant and preservative in foods, antibiotic, chemical intermediate, metal chelate, pesticide, and antimicrobial agents.

In rats, kojic acid is rapidly absorbed and distributed to all organs in oral treatments. Kojic acid is not as rapidly absorbed or distributed in subcutaneous treatments, is slowly absorbed in dermal treatments, and can be transferred at low levels to milk. Kojic acid is mainly excreted in the urine; metabolites are sulfate and glucuronide conjugates of kojic acid.

Absorption of kojic acid through human dermatomed skin resulted in 17% of the applied dose being absorbed. A study of percutaneous absorption of kojic acid in human volunteers found the potential for dermal transfer into the blood to be very low. Based on application of a 1% kojic acid cream to the hands and face and percutaneous absorption of applied dose in human skin, a SED range of 0.03 to 0.06 mg/kg per d was calculated.

Because of its well-documented ability to inhibit tyrosinase activity, kojic acid has been used in numerous studies as a positive control.

In acute mouse studies with kojic acid, oral, subcutaneous, and intraperitoneal LD_{50} values were 5.1, 2.7, and 2.6 g/kg body weight, respectively. In rats, the LD_{50} values were greater than 2 g/kg body weight in oral and dermal studies, and 2.6 and 2.4 g/kg body weight in subcutaneous and intraperitoneal studies, respectively.

A short-term dermal study in rats found that exposure to kojic acid lowered lymphocyte counts at doses of 300 and 1000 mg/kg per d and decreased absolute and relative spleen weights at 1000 mg/kg per d. The NOEL for this study was 100 mg/kg per d.

The subchronic oral toxicity study in male rats concluded with a NOEL for kojic acid of 125 mg/kg per d. Rats that received 250 mg/kg or more of kojic acid had significant suppression of body weight gain when compared to the control group. The 1000 mg/kg dose group also had a slight decrease in erythrocyte counts and decreases of hematocrit value and hemoglobin concentration. Increases of GOT and GPT were observed in dose groups receiving 250, 500, and 1000 mg/kg. The 500 and 1000 mg/kg dose groups had increased ALP, and slight increases of total cholesterol, bilirubin, and calcium were observed in the 1000 mg/kg dose group. At necropsy, the absolute and relative weights of the adrenal glands were increased in the dose groups receiving 500 and 1000 mg/kg kojic acid.

Kojic acid was not an ocular irritant but was a mild dermal irritant in rabbits. In guinea pigs, this ingredient was not a dermal sensitizer but did produce slight skin reactions with UV light exposure in acidic conditions in human repeat insult patch tests, 1% and 2% kojic acid was not sensitizing. A study of 1% and 4% kojic acid in black guinea saw almost no skin-whitening effects.

Several studies of kojic acid, with doses tested up to 900 mg/kg per d in rodents, found the substance was not a reproductive or developmental toxicant.

Kojic acid was genotoxic in several bacterial assays, but the results in mammalian cell assays were mixed. In vivo mammalian tests of kojic acid were negative for genotoxicity. Kojic acid was a weak photo-mutagen in a photo-reverse mutation assay and a chromosomal aberration study with light irradiation.

International Agency for Research on Cancer has concluded that kojic acid is a group 3 carcinogen—not classifiable to human carcinogenicity. Several studies on mice and rat liver found kojic acid to have carcinogenesis-promoting potential but not an initiation potential. Kojic acid did not possess initiation or promotion potential for skin carcinogenesis in mice. Studies on the effect of kojic acid on rodent thyroids found the chemical inhibits iodine uptake and organification in the thyroid, which causes a proliferative effect.

Thyroid proliferative responses in rodent systems may be due to such factors as shorter plasma half-life of T_4 in rodents and differences in transport and binding of protein for thyroid hormones that do not occur in humans.

Case studies of contact dermatitis have been reported in patients that have used cosmetic products or medicinal creams containing 1% kojic acid. Kojic acid is reportedly used to treat melasma. An efficacy study in Chinese women reported that the patients experienced redness, itchiness, and exfoliation, although these results were also observed on skin that was not treated with kojic acid. Another therapeutic study reported that the side effect of the treatment of melasma with 1% kojic acid was contact allergy.

Discussion

Because kojic acid is not a toxicant in acute, chronic, reproductive, and genotoxicity studies, the Cosmetic Ingredient Review (CIR) Expert Panel considered that these data posed no safety issues. The Panel did note that some animal data suggest tumor promotion and weak carcinogenicity. Kojic acid, however, is slowly absorbed into the circulation from human skin, and likely would not reach the systemic level at which these effects were seen. The available human sensitization data support the safety of kojic acid at a concentration of 2% in leave-on cosmetics, suggesting that a limit of 2% might be appropriate. A depigmentation study of kojic acid in black guinea pigs, however, found that skin whitening was statistically significantly at a concentration of 4%. In the same study, a kojic acid concentration of 1% did not result skin whitening that was different from the vehicle control. Kojic acid did not appear to damage melanocytes, and the skin-whitening effect at 4% likely is attributed to tyrosinase inhibition. While reversible, the Panel considers tyrosinase inhibition to be an adverse effect with a NOEL of 1%. Therefore, the Expert Panel finds that kojic acid should only be used up to a concentration of 1% in cosmetic products.

The Panel recognizes that the EWG on its Web site and Health Canada in its product database have reported uses of kojic acid at concentrations greater than 1%. Because these data may include over-the-counter drug uses, it was not possible to determine the extent to which cosmetic products were being sold with concentrations greater than 1%, the limit established by the Panel.

The CIR Expert Panel noted the large number of studies on the effects of kojic acid on rodent thyroid glands. The weight of evidence indicates differing factors, such as shorter plasma half-life of T_4 in rodents and differences in transport and binding of protein for thyroid hormones between rodents and humans, allow the rodent thyroid system to be more likely to have a proliferative response to physical or chemical stimulation attributable to an indirect effect on thyroid hormone synthesis and secretion rather than a genotoxic mechanism. Recognizing that the rodent thyroid gland is sensitive to chemical substances and physiologic perturbations in ways different from that in humans, the Expert Panel concluded that kojic acid would not pose significant risk to human thyroid glands at the levels used in cosmetic products.

The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration, and the duration of the exposure and their site of deposition within the respiratory system. In practice, aerosols should have at least

99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 μm. Particles with an aerodynamic diameter of ≤10μm are respirable. In the absence of inhalation toxicity data, the Expert Panel determined that kojic acid can be used safely in cosmetic spray products, because the product particle size is not respirable.

Conclusion

The CIR Expert Panel concluded that kojic acid is safe for use in cosmetic products up to a concentration of 1%.

Author's Note

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, MD, FACP; Donald V. Belsito, MD; Ronald A. Hill, PhD; Curtis D. Klaassen, PhD; Daniel C. Liebler, PhD; James G. Marks Jr, MD, Ronald C. Shank, PhD; Thomas J. Slaga, PhD; and Paul W. Snyder, DVM, PhD.

The CIR Director is F. Alan Andersen, PhD. This report was prepared by Christina L. Burnett, CIR Scientific Analyst/Writer.

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Conflict of Interest

The author's declared no potential conflict of interest relevant to this article was reported. F. Alan Andersen and Christina L. Burnett are employed by the Cosmetic Ingredient Review.

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